The Effect of Variable Seed Rate Proportions on Agronomic Attributes, Dry Matter Production, Biological Potential and Economic Viability of Some Grass-Legume Mixed Pastures

Tessema Zewdu

Department of Animal Sciences, Haramaya Univesity, P O Box 188, Ethiopia
E-mail: tessemaz@yahoo.com

Abstract: An experiment was conducted to assess the agronomic attributes, dry matter (DM) yield, biological potential and economic viability of grass-legume mixtures at Haramaya University in Ethiopia during 2004 and 2005. *Chloris gayana, Panicum coloratum, Melilotus alba and Medicago sativa* were planted as pure stand and in mixtures using 50:50, 33: 67, 67: 33, 25: 75 and 75: 25 seed rate proportion to give 24 treatments in a randomised complete block design with 3 replications. There were significant ($P < 0.05$) differences in the number of seedlings per m^2 (SLM), row cover, number of tillers per m^2, days to 50% flowering (DF 50%) of the grass components among the grass/legume combinations due to seed rate proportions. Moreover, SLM, the number of branches per m^2, the height of the plant at harvest, DF 50% of the legume components were significantly ($P < 0.05$) affected among the mixtures due to seed rate proportions. There was a significant ($P < 0.05$) difference in the DM yield of pure stand grasses and legumes, and their mixtures due to seed rate proportion. Significant ($P < 0.05$) effects were also observed on the relative yield (RY), the relative total yield (RTY), the relative crowding coefficient (RCC) and the aggressivity index of both the grass and legume components and their mixtures due to different seed rate proportions throughout the study. The two years' mean RTY of all grass-legume mixtures were greater than one (range: 1.23-2.11) and *Chloris* mixed with *Melilotus and Medicago* with 67: 33 seed rate proportion had higher RTY than other mixtures. Both the grass and legume components in the mixture produced mean RCCs values in excess of unity and almost equal values in both components, indicating that all yielded better in the mixture than expected in pure stands at different seed rates. *Chloris gayana* mixed with *Melilotus alba* at 50:50 and 33:67 seed rate proportion gave higher DM yield, average net return/ha and average net return/ha/yr compared to pure stand grasses and legumes and their different mixtures during the study. Therefore, to alleviate the feed scarcity in the area; these grass-legume mixtures could be introduced to smallholder farms. In addition, further studies on animal performances should be conducted using feeding trials and under grazing conditions.

Keywords: Agronomic Attributes; Biological Potential; Dry Matter Yield; Economic Viability; Grass/Legume Mixture; Seed Rate Proportion

1. Introduction

Livestock plays a crucial role in the smallholder farming systems of Ethiopian agriculture. Currently, productivity per animal is very low, and the contribution of the livestock sector to the overall economy is much lower than expected. A major constraint to the livestock industry is feed inadequacy. Both under-nutrition and malnutrition are major problems for the greater part of the country and for most of the time (Lulseged, 1985). Livestock depends on natural pastures and crop residues and both the quantity and the quality of these feedstuffs are too low to sustain satisfactory levels of animal production.

The development of grass-legume pastures is one of the recognised strategies for enhancing both the quantity and quality of feed resources. Grass-legume mixtures are a means of providing protein rich feeds and improving soil fertility. The mixtures give better land-use efficiency than the respected monocultures without additional investment (Prasad *et al.*, 1991). Forage quality and seasonal distribution of the biomass of grass-legume pastures have proved to be superior to those of grasses or legumes grown alone (Daniel, 1990; Minson, 1990). Forage legumes have long been lauded for their ability to fix atmospheric nitrogen and contribute to the sustainability of agricultural production systems (Thomas, 1995). Since the emphasis should be on low-input production systems, such a forage strategy could play an important role in improving both the quality and yield of forage without the addition of organic and/or inorganic fertiliser. In addition, grass-legume mixtures provide advantages over pure stands by reducing the incidence of bloat from legumes, the effects of diseases and insect pests and the level of soil erosion (Lulseged, 1985; Minson, 1990).

The adaptability and agronomic performances of some of the most promising tropical perennial grass and legume mixtures in the eastern parts of Ethiopia have been evaluated in recent years (Berhan, 2005; 2006; Yisehak, 2005). However, the performance of grass-legume mixtures depends on the compatibility of the species mixed and the different seed rate proportions. A low seed rate may result in a poor stand while higher seed rates may not be economical due to the high cost of forage seeds, especially the legumes (Berhan, 2006). Moreover, Diriba (2002) reported a progressive increase in the contribution of the legume component resulting from the agronomic attributes of the mixtures as the seed rate of the legume increases. Therefore, the objective of this study was to assess the agronomic attributes, dry matter (DM) yield, biological potential, and economic viability of some tropical grass-legume mixtures at different seed rate proportions.

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2. Materials and Methods

2.1. Experimental Site

A grass-legume mixture experiment was conducted during 2004 and 2005 at Haramaya University Research Centre (9° 26' N, 42° 03' E; 2240 m a s l), 511 km from Addis Ababa on alluvial-Vertisols (Tamire, 1982). The 0-40cm layer of the soil before sowing and fertiliser application had a pH of 6.34, total nitrogen (N) of 0.16, available phosphorus of 0.66 ppm, organic mater of 2.28% and organic carbon of 1.33%. The twenty years' mean annual rainfall of the area was 625 mm and the average annual air temperature was 20.15°C. The rainy season extended from May to October with a peak during July-September during the study periods. The monthly rainfall, number of rainy days, and the minimum and maximum air temperatures during the study period are presented in Table 1.

Table 1. Monthly total rainfall, number of rainy days, and minimum and maximum air temperatures during 2004 and 2005 at Haramaya Research Centre, Ethiopia.

Months	Rainfall (mm)		Temperature			
			Mean maximum (^{0}C)		Mean minimum (^{0}C)	
	2004	2005	2004	2005	2004	2005
January	38.2	0.5	24.2	21.4	9.3	9.7
February	0.0	2.0	24.0	24.3	5.95	6.35
March	25.4	39.9	25.7	24.5	8.9	8.8
April	163.5	119.5	23.9	25.7	14.2	13.5
May	39.5	198.3	26.5	23.7	11.7	12.5
June	25.5	19.2	24.8	25.0	14.1	14.4
July	71.3	68.1	24.0	23.0	13.3	13.2
August	116.4	126.2	24.5	24.0	13.6	13.5
September	126.7	156.4	24.0	23.0	12.1	12.5
October	43.8	17.0	25.1	23.1	6.5	11.2
November	38.6	33.6	24.7	23.7	5.5	7.9
December	4.5	0.0	21.4	22.4	4.0	6.1
Total	693.4	780.7	-	-	-	-

2.2. Experimental Design, Treatment and Management of the Experiment

The study was conducted using a randomised complete block design with 3 replications. A total of 24 treatments: 2 grasses, 2 legumes, and 20 grass-legume mixtures were used. The grass species were: *Chloris gayana* cv. Masaba and *Panicum coloratum*; and the forage legumes were: *Melilotus alba* and *Medicago sativa* cv. Hairy Peruvian. The 20 grass-legume treatments included all combinations of the 2 legumes and 2 grasses using 50: 50, 33: 67, 67: 33, 25: 75 and 75: 25 seed rate proportion of grasses and legumes respectively. An overall seeding rate of 10 kg/ha was used for pure stand grasses and legumes and their mixtures, according to the recommendation of IAR (1988). In the mixtures, the seeding rate was calculated according to the seed rate proportion of both pure stand grasses and legumes. The inclusion of pure grass and legume stands allowed the effect of competitions in mixtures to be assessed and comparisons with each mixture to be made. Seeds of grasses and legumes were weighed, then thoroughly mixed and row planted at a spacing of 20 cm interval on 2 m x 4.5 m plots. Spacing between replications and plots were 2 and 1 m respectively. Diammonium phosphate (DAP) fertiliser was applied at planting at 100 kg/ha on all treatments and nitrogen fertiliser (50 kg/ha N) was applied as urea after establishment of the pure stand grass swards according to the recommendation of IAR (1988).

2.3. Data Collection and Analytical Procedure

Agronomic attributes, such as the number of seedlings per m^2 (SLM), soil or row cover (RC), the number of tillers per plant (NTPP), the number of branches per plant (NBP), and the number of tillers m^2 (NTM), the number of branches per m^2 (NBM), the plant height at harvest (HT), the days to 10 (DF 10%) and 50% flowering (DF 50%), the leaf to stem ratio (LSR) as well as grass and legume proportions (%) of the treatments were recorded.

The relative DM yields (RY) of the components in the mixtures were calculated using the equations of De Wit (1960): $RY_{GL} = DMY_{GL}/DMY_{GG}$ ---------- (1)
$RY_{LG} = DMY_{LG}/DMY_{LL}$ ---------- (2)
where DMY_{GG} is the dry matter yield of any perennial grass 'G' as a monoculture; DMY_{LL} is the dry matter yield of any perennial legume 'L' as a monoculture; DMY_{GL} is the dry matter yield of any perennial grass component 'G' grown in mixture with any perennial legume 'L' and DMY_{LG} is the dry matter yield of any perennial legume component 'L' grown in mixture with any perennial grass 'G'.

Relative total yield (RYT) was calculated according to the formula of De Wit (1960): $RYT_{GL} = (DMY_{GL}/DMY_{GG}) + (DMY_{LG}/DMY_{LL})$ ---------- (3)

The relative crowding coefficient (RCC) of the perennial grass-legume mixtures was calculated to determine the competitive ability of the grass and legume in the mixture and to measure whether that component has produced less or more dry matter yield than expected in a 50:50

perennial grass-legume mixture, according to De Wit (1960):

$RCC_{GL} = DMY_{GL} / (DMY_{GG} - DMY_{GL})$ ---------- (4)

$RCC_{LG} = DMY_{LG} / (DMY_{LL} - DMY_{LG})$ ---------- (5)

For mixtures different from 50:50, RCC was calculated as:

$RCC_{GL} = DMY_{GL} \times Zba / (DMY_{GG} - DMY_{GL} \times Zab)$--(6)

$RCC_{LG} = DMY_{LG} \times Zab / (DMY_{LL} - DMY_{LG} \times Zba)$--(7)

Where Zab is the seed rate proportion of the grass component and Zba is the seed rate proportion of the legume component in grass/legume mixture.

The dominance or aggressive ability of the perennial grasses against the perennial legumes in different seed rate mixtures was described by calculating the aggressivity index (AI) as indicated by Mc Gilchrist (1965) and Mc Gilchrist and Trenbath (1971):

$AI_{GL} = (DMY_{GL}/DMY_{GG}) - (DMY_{LG}/DMY_{LL})$ -------- (8)

For any replacement treatment other than 50:50, AI was calculated as:

$AI_{GL} = (DMY_{GL}/DMY_{GG} \times Zab) - (DMY_{LG}/DMY_{LL} \times Zba)$ ---------- (9)

$AI_{LG} = (DMY_{LG}/DMY_{LL} \times Zba) - (DMY_{GL}/DMY_{GG} \times Zab)$ ---------- (10)

Sole grasses and legumes were harvested at about 5 cm above ground level at 50 and 100% flowering respectively, while the grass-legume mixtures were harvested when at least one component of the mixture had reached 50% flowering based on continuous visual observation of each component in the mixture. Immediately after harvesting, the whole sample was thoroughly mixed and 250 g samples from each plot were taken for DM yield determination by drying at 65°C for 72 h (constant weight). In addition, each grass-legume mixed whole sample was sorted into grass and legume botanical composition immediately after harvesting to determine the proportion of grasses and legumes in the mixed sward. The yields of treatments were also assessed for their economical viability, monetary advantages, and net returns per ha and per year (Willey and Rao, 1980), using the cost incurred for pasture production and the current market prices of hay in the area.

2.4. Statistical Analyses

Analysis of variance (ANOVA) was carried out using the General Linear Model procedures of Statistical Analysis System (SAS, 1998) applied to a randomised complete block design and mean separation was done using the Duncan's Multiple Range Test (DMRT).

3. Results

3.1. Agronomic Attributes

There were significant ($P < 0.05$) differences in SLM, RC, NTM, and DF to 10 and 50% of the grass components among the grass/legume combinations due to different seed rate proportions (Table 2).

Moreover, SLM, NBM, HT, and DF 10 and 50% were significantly ($P < 0.05$) affected on the legume component among the grass/legume combinations due to seed rate proportions (Table 3).

Table 2. Agronomic attributes of *Chloris gayana* and *Panicum coloratum* mixed with *Medicago sativa* and *Melilotus alba* at different seed rate proportions.

	Agronomic attributes							
Combinations	SLM	RC	NTM	HT	NTPP	DF (10%)	DF (50%)	LSR
Chloris (R)	23.33^{abcde}	4.70^{ab}	235.70^{bc}	100.70	61.33	79.33^{bc}	89.00^{ab}	1.45
Panicum (P)	29.00^{abc}	4.83^{a}	395.70^{a}	107.33	32.33	68.70^{de}	79.33^{cd}	1.40
R: M (50: 50)	16.70^{cdefg}	4.5^{abc}	197.70^{bcd}	122.70	33.70	87.70^{a}	94.00^{a}	1.43
R: A (50: 50)	7.00^{g}	3.70^{bcde}	79.33^{bfg}	105.00	31.00	85.70^{ab}	94.00^{a}	1.30
P: M (50: 50)	26.00^{abc}	4.33^{abc}	209.33^{bcd}	105.00	36.00	66.00^{e}	77.33^{d}	1.50
P: A (50: 50)	16.70^{cdefg}	4.00^{abcde}	179.00^{bcde}	104.70	56.00	66.00^{de}	77.33^{d}	1.33
R: M (67: 33)	23.33^{abcde}	4.00^{abcde}	103.33^{defg}	107.70	37.33	83.33^{ab}	94.00^{a}	0.87
R: M (33: 67)	24.33^{abcd}	3.70^{bcde}	123.00^{defg}	105.00	37.00	85.00^{ab}	94.00^{a}	1.53
P: M (67: 33)	25.70^{abcd}	4.50^{abc}	198.70^{bcd}	110.70	34.70	75.00^{cd}	84.33^{bc}	1.20
P: M (33: 67)	16.70^{cdefg}	3.83^{abcde}	143.70^{bcdef}	107.70	34.00	69.33^{de}	79.00^{cd}	1.23
P: A (25: 75)	10.33^{fg}	3.50^{cde}	31.67^{g}	95.33	15.33	70.00^{de}	79.00^{cd}	3.30
P: A (75: 25)	2.00^{bcdef}	3.70^{bcde}	244.00^{bc}	107.00	29.33	69.70^{de}	79.00^{cd}	1.40
P: M (25: 75)	10.33^{fg}	3.70^{bcde}	79.33^{efg}	100.00	54.70	69.33^{de}	79.00^{cd}	0.96
P: M (75: 25)	24.33^{abcd}	3.20^{de}	252.33^{b}	97.33	34.70	66.33^{e}	79.00^{cd}	1.31
R: M (25: 75)	13.33^{defg}	3.20^{de}	65.00^{fg}	103.00	24.70	82.70^{abc}	94.00^{a}	3.70
R: M (75: 25)	21.33^{bcdef}	3.83^{abcde}	158.00^{bcdef}	113.33	24.33	84.00^{ab}	94.00^{a}	1.30
R: A (25: 75)	5.33^{g}	3.00^{e}	102.70^{defg}	94.00	22.70	83.33^{ab}	94.00^{a}	1.42
R: A (75: 25)	11.70^{efg}	4.00^{abcde}	150.00^{bcdef}	109.70	34.33	83.33^{ab}	94.00^{a}	1.20
R: A (33: 67)	34.00^{a}	3.60^{bcde}	184.00^{bcde}	102.00	43.00	88.00^{a}	94.00^{a}	1.63
R: A (67: 33)	34.33^{ab}	4.20^{abcd}	143.70^{bcdef}	108.33	42.70	87.00^{ab}	94.33^{a}	1.70
P: A (33: 67)	26.86^{abc}	4.03^{abcde}	135.70^{cdefg}	109.33	39.00	81.33^{abc}	89.33^{ab}	1.30
P: A (67: 33)	25.00^{abcd}	4.50^{abc}	119.33^{defg}	114.00	37.00	80.70^{abc}	89.70	1.30
Mean	20.20	3.93	160.50	105.90	36.14	77.90	87.35	1.52
SEM	4.35	0.40	38.63	7.00	10.20	2.74	2.31	0.91
DMRT	12.40	1.13	110.1	NS	NS	7.82	6.60	NS

Within columns, means followed by the same letter are not significantly different at P = 0.05.

A = Medicago sativa; DF = days to flowering; HT = height at harvest; LSR = leaf stem ratio;

M = Melilotus alba; NTM = number of tiller per m^2; NTPP = number of tiller per plant;

NS = Non-significant at P < 0.05; RC = row cover; SLC = seedling count per m^2.

Table 3. Agronomic attributes of *Melilotus alba* and *Medicago Sativa* mixed with *Chloris gayana* and *Panicum coloratum* at different seed rate proportions.

Combinations	Agronomic attributes							
	SLM	RC	NBM	HT	NBPP	DF (10%)	DF (50%)	LSR
Melilotus (R)	48.3^{bcd}	4.7	386.7^{abc}	102.7^{a}	8.0	69.7^{de}	81.7^{cd}	1.2^{abc}
Medicago (A)	67.7^{a}	5.0	463.0^{ab}	71.0^{b}	7.2	70.0^{de}	79.0^{cd}	1.4^{a}
R: M (50: 50)	38.0^{cdef}	4.2	207.0^{defg}	85.7^{ab}	6.6	87.7^{a}	94.0^{a}	1.0^{a}
R: A (50: 50)	34.3^{defg}	4.7	254.7^{cdef}	71.3^{ab}	8.4	85.7^{a}	94.0^{a}	1.1^{bcde}
P: M (50: 50)	21.3^{fg}	3.3	79.3^{g}	87.3^{ab}	3.8	66.0^{e}	77.3^{d}	0.9^{e}
P: A (50: 50)	54.7^{abc}	3.7	317.7^{bcd}	81.7^{ab}	7.0	83.3^{a}	77.3^{d}	1.0^{cde}
R: M (67: 33)	33.3^{defg}	3.8	92.0^{g}	80.7^{b}	3.3	85.0^{a}	94.0^{a}	1.0^{cde}
R: M (33: 67)	35.0^{defg}	4.7	187.3^{defg}	86.3^{ab}	5.7	75.0^{cd}	94.0^{a}	1.0^{cde}
P: M (67: 33)	27.3^{eg}	3.7	93.3^{g}	75.3^{b}	3.5	69.3^{de}	84.3^{bc}	1.0^{cde}
P: M (33: 67)	26.3^{efg}	3.5	158.3^{efg}	88.0^{ab}	5.9	70.0^{de}	79.0^{cd}	1.0^{cde}
P: A (25: 75)	72.0^{a}	4.5	474.7^{a}	69.7^{ab}	6.5	69.7^{de}	79.0^{cd}	1.2^{abcd}
P: A (75: 25)	32.0^{defg}	4.3	179.0^{defg}	70.0^{bc}	6.1	69.3^{d}e	79.0^{cd}	0.95^{de}
P: M (25: 75)	41.0^{cde}	4.3	172.3^{defg}	84.3^{ab}	4.2	66.3^{e}	79.0^{cd}	1.04^{bcde}
P: M (75: 25)	23.7^{cdefg}	3.2	221.3^{defg}	87.0^{ab}	8.7	82.7^{ab}	79.0^{cd}	1.00^{cde}
R: M (25: 75)	36.3^{g}	4.0	274.3^{cde}	79.0^{b}	7.9	84.0^{a}	94.0^{a}	1.02^{cde}
R: M (75: 25)	27.70^{efg}	3.8	123.7^{efg}	49.0^{c}	7.2	83.3^{a}	94.0^{a}	0.95^{de}
R: A (25: 75)	59.8^{b}	4.7	394.3^{abc}	73.7^{b}	6.6	83.3^{a}	94.0^{a}	1.14^{abcd}e
R: A (75: 25)	28.3^{efg}	4.3	294.3^{cdef}	74.0^{b}	8.8	81.3^{abc}	94.0^{a}	1.02^{cde}
R: A (33: 67)	23.3^{efg}	4.0	152.0^{efg}	79.3^{b}	6.5	87.0^{a}	89.0^{ab}	1.1^{bcde}
R: A (67: 33)	30.0^{defg}	4.0	140.0^{efg}	77.3^{b}	4.8	87.0^{a}	94.3^{a}	1.1^{bcde}
P: A (33: 67)	28.7^{efg}	3.7	111.0^{fg}	78.0^{b}	3.7	86.70^{a}	94.7^{a}	1.1^{bcde}
P: A (67: 33)	32.0^{defg}	4.3	167.0^{defg}	89.3^{ab}	5.6	76.0^{bcd}	84.0^{bc}	1.3^{a}
Mean	36.90	4.1	222.7	79.1	6.2	77.24	86.8	1.1
SEM	6.43	0.5	53.00	7.40	1.8	2.41	2.10	0.10
DMRT	18.40	NS	151.2	21.10	NS	6.90	5.92	0.30

Within columns, means followed by the same letter are not significantly different at P = 0.05.
A = Medicago sativa; DF = days to flowering; HT = height at harvest; LSR = leaf stem ratio;
M = Melilotus alba; NTM = number of tiller per m^2; NTPP = number of tiller per plant;
NS = Non-significant at P < 0.05; RC = row cover; SLC = seedling count per m^2.

The higher NTM was obtained from *Panicum* as pure stand and *Panicum* mixed with *Melilotus* with 50: 50 ratio with 395.7 and 252.3 respectively. The maximum days to 50% flowering (94 days) was obtained from *Chloris* mixed with *Medicago* and *Melilotus*. In the case of the legume components, *Medicago* mixed with *Panicum* at 75: 25 seed rates, and pure stand *Medicago* and *Melilotus* had a higher NBM of 474.7, 463.0 and 386.7 respectively compared to other treatments. In all grass/legume mixtures, the LSR value of the grass and legume components was greater than one (Table 2 and 3). All grasses and legumes as pure stand and in the mixture grew well from the time of establishment to anthesis. *Melilotus* and *Medicago* showed fast regrowth ability after harvest while *Chloris* and *Panicum* were slow.

3.2. Grass/Legume Proportion

Eighty percent of the grass/legume mixtures produced more than 50% grass component while 20% of the mixture produced more than 50% legume component throughout the two years. Grass/legume mixtures with higher proportions of grass seed rate produced a high grass component in the mixture and vice versa for legumes (Table 4), indicating that the grass and legume component in the mixture is directly related to the seed rate proportion.

3.3. Biological potential

There was no significant (P > 0.05) effect on RY and RTY of both the grass and legume components and their mixtures due to different seed rate proportions throughout the study (Table 5). However, a significant (P < 0.05) effect was observed on RCC and AI of both the grass and legume components and their mixtures due to different seed rate proportions (Table 6). The mean RYs of both the legume and grass components were greater than unity (1.04). The 2 years' mean RTY of all grass-legume mixtures in the experimental period were greater than one (range: 1.23-2.11). Moreover, *Chloris* mixed with *Melilotus and Medicago* with 67: 33 seed rate proportion had a higher RTY than other treatments (Table 5).

Table 4. Percentage (%) of grasses and legumes in grass/legume mixture at different seed rate proportions.

Combinations	2004		2005		Mean (2004-2005)	
	Grass	Legume	Grass	Legume	Grass	Legume
Chloris gayana (R)	100	-	100	-	100	-
Panicum coloratum (P)	100	-	100	-	100	-
Melilotus alba (M)	-	100	-	100	-	100
Medicago sativa (A)	-	100	-	100	-	100
R: M (50: 50)	58	42	60	40	59	41
R: A (50: 50)	45	55	66	34	56	44
P: M (50: 50)	72	28	56	44	64	36
P: A (50: 50)	53	47	59	41	56	44
R: M (67: 33)	55	45	65	35	60	40
R: M (33: 67)	48	52	52	48	50	50
P: M (67: 33)	68	32	58	42	63	37
P: M (33: 67)	60	40	48	52	54	46
P: A (25: 75)	49	51	44	66	46	54
P: A (75: 25)	63	37	69	29	66	34
P: M (25: 75)	45	55	45	55	45	55
P: M (75: 25)	81	19	68	32	74	26
R: M (25: 75)	48	52	50	50	49	51
R: M (75: 25)	75	25	66	44	70	30
R: A (25: 75)	33	67	50	50	41	59
R: A (75: 25)	66	37	66	44	66	44
R: A (33: 67)	55	45	52	48	54	46
R: A (67: 33)	54	46	70	30	62	38
P: A (33: 67)	60	40	60	40	60	40
P: A (67: 33)	53	47	58	42	56	44
Mean	57	43	58	42	58	42

Table 5. Relative yields of both grasses and legumes and their relative total yield as influenced by different seed rate proportions in grass/legume mixtures.

Combinations	Relative yields of grasses			Relative yields of legumes			Relative total yield		
	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
R: M (50: 50)	0.70	0.71	0.70	0.49	0.57	0.53	1.18	1.28	1.23
R: A (50: 50)	0.60	0.68	0.64	0.54	0.73	0.64	1.14	1.41	1.28
P: M (50: 50)	1.38	1.14	1.26	0.69	0.58	0.64	2.07	1.73	1.90
P: A (50: 50)	0.89	0.84	0.86	1.66	0.24	0.95	2.55	1.08	1.81
R: M (33: 67)	1.81	0.66	1.23	0.82	0.60	0.71	2.63	1.26	1.95
R: M (67: 33)	1.99	0.62	1.30	1.17	1.00	1.08	3.15	1.62	2.39
P: M (67: 33)	1.03	0.82	0.93	0.53	0.60	0.56	1.56	1.42	1.49
P: M (33: 67)	1.25	0.83	1.00	0.81	0.85	0.83	1.96	1.69	1.82
P: A (25: 75)	0.76	0.51	0.63	1.44	0.73	1.09	2.20	1.24	1.72
P: A (75: 25)	0.61	0.92	0.77	0.66	0.62	0.64	1.27	1.55	1.41
P: M (25: 75)	0.76	0.64	0.70	0.74	0.76	0.75	1.50	1.39	1.44
P: M (75: 25)	1.11	1.06	1.08	0.33	0.37	0.35	1.43	1.43	1.43
R: M (25: 75)	0.84	0.44	0.64	0.92	0.76	0.84	1.77	1.20	1.48
R: M (75: 25)	1.21	0.63	0.92	0.65	0.54	0.60	1.86	1.17	1.52
R: A (25: 75)	0.49	0.36	0.42	1.36	0.55	0.96	1.85	0.91	1.38
R: A (75: 25)	0.99	0.46	0.73	1.02	0.49	0.76	2.01	0.96	1.49
R: A (33: 67)	0.27	0.48	0.87	1.36	0.79	1.08	2.63	1.26	1.95
R: A (67: 33)	0.73	0.61	0.67	2.34	0.55	1.45	3.07	1.15	2.11
P: A (33: 67)	1.72	0.86	1.29	0.75	0.65	0.70	2.48	1.50	1.99
P: A (67: 33)	0.80	0.77	0.78	0.95	0.61	0.78	1.74	1.38	1.56
Mean	1.04	0.70	0.87	0.96	0.63	0.80	2.00	1.33	1.67
SEM	0.49	0.11	0.25	0.37	0.14	0.20	0.66	0.19	0.35
P Level	NS	***	NS	NS	NS	NS	NS	NS	NS

*A = Medicago sativa; M = Melilotus alba; NS = Non-significant at P < 0.05; P = Panicum coloratum; R = Chloris gayana; * = P < 0.05; ** = P < 0.01; *** = P < 0.001*

Both the grass and legume components in the mixture produced mean RCCs values in excess of unity and almost equal values in both components (Table 6). *Chloris* mixed with *Medicago* (50: 50) and *Panicum* mixed with *Melilotus* at 50: 50 and 25: 75 seed rates produced high RCC values of the grass component while *Chloris* and *Panicum* mixed with *Melilotus* at 50: 50 and 25: 75 seed rates respectively produced higher RCC values of the legume component compared to other grass-legume combinations. More than 75% of the grass/legume mixtures produced mean AI values of less than unity and closer to zero (range: -0.06 to +0.88) and the mean AI value of both the grass and legume components was low (0.29) (Table 6).

3.4. Dry Matter Production

There was a significant ($P < 0.05$) difference in DM yield among pure stand grasses, legumes and their mixtures due to seed rate proportions in 2004 and 2005. A significant ($P < 0.05$) effect was also observed in the DM production of the grass and legume components among the grass/legume mixtures due to seed rate proportions (Table 7). There was variation in the DM yield between the pure stand grasses and legumes maintained for comparison. Moreover, the total DM yields of all grass-legume mixed pastures were different in the first and second years and DM yields increased progressively as the pasture sward advanced. Higher DM yields were obtained from *Chloris* mixed with *Melilotus* at 50: 50 and 33: 67 seed rate proportions with 26.7 and 26.1 t/ha respectively (Table 7), compared to other mixtures and pure stands of grasses and legumes throughout the study. Moreover, 75 and 100% of the grass/legume mixtures exceeded the DM yield of pure stand grasses and legumes respectively.

3.5. Economic Viability

The economic assessment of the dry matter yields showed that improved pasture production using different seed rate proportions is economical compared to pure stand grasses and legumes. *Chloris gayana* mixed with *Melilotus alba* at 50:50 and 33:67 percent seed rate proportion gave a higher average net return of 25,471.58 and 24,921.58 Ethiopian Birr/ha/yr respectively, compared to pure stand grasses and legumes as well as other mixtures in this study (Table 8).

4. Discussion

4.1. Agronomic Attributes

There were significant ($P < 0.05$) effects on SLM, RC, NTM, and DF to 10 and 50% of the grass component and on SLM, NBM, HT, and DF to 10 and 50% of the legume component due to the seed rate proportion among the different mixtures throughout the study. The result of the current study is in agreement with Yisehak (2005) and Diriba (2002). The NTM and NTPP in grasses, and the NTM and NBM in legumes showed significant ($P < 0.05$) differences due to varying seed rate proportions in a grass/legume mixture study in eastern Ethiopia as reported by Yisehak (2005). Higher NTM was obtained from *Panicum* as pure stand and *Panicum* mixed with *Melilotus* with 50: 50 with 395.7 and 252.3, respectively. Lowe *et al.* (1995) and Larbi *et al.* (1995) reported an increased number of tillers with increased seed rate proportions of Rhodes grass mixed with tropical legumes.

The maximum days (94 days) to 50% flowering of both the grass and legume component was obtained from *Chloris gayana* mixed both with *Medicago sativa* and *Melilotus alba*. Grasses mixed with higher seed rates of legumes may extend the growth period of the mixed pasture during the dry season and provide N for the companion grasses (Crowder and Chheda, 1982; Daniel, 1990). There was a similar report by Yisehak (2005) that higher seed rate proportion of *Melilotus* mixed with lower seed rate of Rhodes grass delayed the physiological maturity of the grass component and the mixture stayed in green condition compared to pure stand grasses. This may be one of the advantages of grass/legume mixed pasture contributed by the legume component. The Agronomic attributes of all mixed pastures were different in the first and second years of the sward. This could be due to the fact that all the grasses and legumes as pure stand and in mixture grew vigorously from the time of establishment to anthesis. In addition, the legumes showed fast regrowth after harvest compared to grasses. This might have contributed to the higher DM production of the grass-legume mixtures compared to pure stand grasses. Hence, as the growth pattern of legumes and grasses is variable over drier and wetter parts of the year, selection of the correct grass-legume combination could provide sustained availability of animal feed all year round.

Table 6. Relative crowding coefficient of both grasses and legumes and their aggressivity indices as influenced by varying seed rate proportions in grass/legume mixtures.

	RCCg			RCCl			Aggressivity index of grasses			Aggressivity index of legumes		
Combinations	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
R: M (50: 50)	3.14	3.76	3.46	0.76	4.4	2.58	0.24	-0.12	0.06	-0.24	0.12	-0.06
R: A (50: 50)	0.47	3.02	1.74	0.60	2.89	1.15	-0.09	-0.06	-0.07	0.09	0.06	0.07
P: M (50: 50)	3.40	7.09	6.84	0.30	4.95	2.63	0.75	0.28	0.52	-0.75	-0.28	-0.52
P: A (50: 50)	4.41	0.34	2.03	0.72	1.60	1.16	0.23	0.77	0.50	-0.23	-0.77	-0.50
R: M (67: 33)	0.24	1.21	0.73	0.13	3.58	1.86	-0.33	-0.84	-0.59	0.33	0.84	0.59
R: M (33: 67)	0.29	3.69	1.99	0.14	5.87	3.01	0.87	-0.06	0.41	-0.87	0.06	-0.41
P: M (67: 33)	0.36	3.23	1.79	0.09	3.48	1.79	0.40	-0.58	-0.09	-0.40	0.58	0.09
P: M (33: 67)	0.83	1.70	0.44	0.12	3.07	1.59	1.83	1.29	1.56	-1.83	-1.29	-1.56
P: A (25: 75)	0.17	3.45	1.81	0.18	3.74	1.96	1.51	1.04	1.28	-1.51	-1.04	-1.28
P: A (75: 25)	0.77	0.15	0.46	0.09	2.64	1.37	-0.23	-0.61	-0.42	0.23	0.61	0.42
P: M (25: 75)	0.11	6.96	3.54	0.09	2.79	5.20	1.12	1.54	1.33	-1.12	-1.54	-1.33
P: M (75: 25)	1.64	0.29	0.68	0.03	3.02	1.53	0.75	-0.56	0.09	-0.75	0.56	-0.09
R: M (25: 75)	0.20	2.71	1.25	0.19	2.72	1.45	1.79	0.75	1.27	-1.79	-0.75	-1.27
R: M (75: 25)	0.48	1.72	1.09	0.04	3.75	1.89	0.15	-1.33	-0.59	-0.15	1.33	0.59
R: A (25: 75)	0.05	1.75	0.90	0.14	1.17	0.67	0.23	0.45	0.34	-0.23	-0.45	-0.34
R: A (75: 25)	0.16	0.29	0.22	0.14	2.70	1.42	-0.39	-1.36	-0.88	0.39	1.36	0.88
R: A (33: 67)	0.29	3.22	1.75	0.20	2.68	1.44	0.98	0.27	0.63	-0.98	-0.27	-0.63
R: A (67: 33)	0.27	0.77	0.52	0.18	2.71	1.44	-0.12	-0.75	-0.44	0.12	0.75	0.44
P: A (33: 67)	0.62	1.95	1.29	0.14	1.08	0.61	1.09	1.94	1.52	-1.09	-1.94	-1.52
P: A (67: 33)	0.68	1.70	1.19	0.20	3.31	1.76	-0.39	-0.70	-0.55	0.39	0.70	0.55
Mean	0.91	2.45	1.70	0.22	3.25	1.71	0.52	0.07	0.29	-0.52	-0.07	-0.29
SEM	1.65	3.58	1.97	0.09	2.01	0.84	0.44	0.15	0.30	0.44	0.15	0.30
P Level	*	*	***	***	**	**	**	***	***	**	***	***

A = Medicago sativa; g = grass component; l = legume component; M = Melilotus alba; NS = Non-significant at P < 0.05; P = Panicum coloratum; R = *Chloris gayana; * = P < 0.05; ** = P < 0.01; *** = P < 0.001; RCC = relative crowding coefficient; NS = Non-significant at P < 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001*

Table 7. Dry matter yield (t/ha) of *Chloris gayana* and *Panicum coloratum* mixed with *Meliotus alba* and *Medicago sativa* at different seed rate proportions in grass/legume mixtures.

	2004			2005			Overall mean		
Combinations	Grass	Legume	Total	Grass	Legume	Total	Grass	Legume	Total
Rhodes (R)	12.0a	-	12.0abc	24.0ab	-	24.0cdef	18.0a	-	18.0bcdef
Panicum (P)	7.3bc	-	9.8abcdefgh	16.5bcdefg	-	20.1def	14.9ab	-	14.9ef
Melilotus (M)	-	10.8a	10.8abcdefg	-	18.3ab	18.3ef	-	14.6a	14.6ef
Medicago (A)	-	8.6ab	8.6abcdefgh	-	14.8abcde	14.8f	-	11.7abc	12.6f
R: M (50: 50)	7.4bc	5.5bcd	12.9ab	24.3a	16.1abcd	40.4a	15.9ab	10.8bcd	26.7a
R: A (50: 50)	3.5cde	4.1cde	7.6fgh	23.5abc	12.4bcdef	35.9abc	13.5abcd	8.3cdefg	21.8abcde
P: M (50: 50)	8.3ab	3.1cde	11.4abcde	21.7abcde	16.9abc	38.6ab	15.0ab	10.0bcde	25.0abc
P: A (50: 50)	5.5bcde	4.6cde	10.1abcdefgh	16.0cdefg	10.3def	26.3bcdef	10.7bcde	7.5defg	18.2bcdef
R: M (67: 33)	5.6bcde	5.5bcd	11.0abcdefg	23.1abcd	12.6bcdef	35.7abc	14.3abcd	9.0bcdefg	23.4abcd
R: M (33: 67)	5.7bcde	5.4bcd	11.1abcdefg	21.5abcde	19.6a	41.1a	13.6abcd	12.5ab	26.1ab
P: M (67: 33)	5.6bcde	2.6cde	8.2cdefgh	15.6defg	11.7cdef	27.4bcdef	10.6bcde	7.2efg	17.8cdef
P: M (33: 67)	6.5bcde	4.4cde	10.8abcdefg	16.0cdefg	16.6abc	32.6abcd	11.2abcde	10.5bcde	21.7abcde
P: A (25: 75)	3.6cde	4.7cde	8.3cdefgh	9.6g	12.5bcdef	22.1def	6.6e	8.6cdefg	15.2def
P: A (75: 25)	6.1bcde	3.5cde	9.7abcdefgh	17.5abcdef	7.8f	25.3cdef	11.8abcde	5.7g	17.5cdef
P: M (25: 75)	3.2de	4.2cde	7.4gh	12.1fg	14.8abcde	26.9bcdef	7.7cde	9.5bcdef	17.2cdef
P: M (75: 25)	7.8b	1.8e	9.6abcdefgh	20.2abcde	9.7ef	29.8abcde	14.0abcd	5.7g	19.7abcdef
R: M (25: 75)	7.2bcd	6.0bc	13.3a	15.3efg	14.8abcde	30.1abcde	11.3abcde	10.4bcde	21.7abcde
R: M (75: 25)	7.0bcd	2.2de	9.3bcdefgh	21.8abcde	10.6def	32.4abcd	14.4abc	6.4fg	20.8abcdef
R: A (25: 75)	2.7e	4.1cde	6.8h	12.2fg	11.6cdef	23.8cdef	7.4de	7.9defg	15.3def
R: A (75: 25)	7.6bc	2.7cde	7.8efgh	19.7abcdef	8.4f	24.6cdef	10.7bcde	5.6g	16.2def
R: A (33: 67)	6.5bcde	5.3bcd	11.7abcd	16.5bcdefg	13.4bcdef	29.9abcde	11.5abcde	0.3bcdef	20.8abcdef
R: A (67: 33)	5.8bcde	4.8cde	10.6abcdefg	21.1abcde	9.3ef	30.4abcde	13.5abcde	7.0efg	20.5abcdef
P: A (33: 67)	5.8bcde	4.0cde	9.8abcdefgh	18.2abcdef	11.0cdef	29.2abcde	12.0abcde	7.5defg	19.5abcdef
P: A (67: 33)	6.1bcde	5.3bcd	11.3abcdef	14.6efg	10.3def	24.9cdef	10.3bcde	7.8defg	18.1bcdef
Mean	6.2	4.7	10.1	18.2	12.9	28.5	12.2	8.8	19.3
SEM	1.42	0.90	1.58	3.24	1.55	3.38	1.74	0.90	1.86
DMRT	4.04	3.42	3.74	7.71	5.91	12.84	6.94	3.56	6.93

Within columns, means followed by the same letter are not significantly different at P = 0.05

Table 8. Cost benefit analysis of grass/legume mixtures as affected by different seed rate proportions.

	Total cost/ha		Total revenue/ha		Net return/ha		Average net return/ha/yr
	2004	2005	2004	2005	2004	2005	
Chloris (R)	2090.45	266.40	12,000.00	24,000.00	9,909.55	23,733.60	16,821.58
Panicum (P)	2090.45	266.40	9,800.00	20,100.00	7,709.55	19,833.60	13,771.58
Melilotus (M)	2090.45	266.40	10,800.00	18,300.00	8,709.55	18,033.60	13,371.58
Medicago (A)	2090.45	266.40	8,600.00	14,800.00	6,449.55	14,533.60	10,491.58
R: M (50: 50)	2090.45	266.40	12,900.00	40, 400.00	10,809.55	40,133.60	25,471.58
R: A (50: 50)	2,120.45	266.40	7,600.00	35,900.00	5,479.55	35,636.40	20,557.98
P: M (50: 50)	2090.45	266.40	11,400.00	38,600.00	9,309.55	38,333.60	23,821.58
P: A (50: 50)	2,120.45	266.40	10,100.00	26,300.00	7,979.55	26,033.60	17,006.58
R: M (67: 33)	2090.45	266.40	11,000.00	35,700.00	8,909.55	35,433.60	22,171.58
R: M (33: 67)	2090.45	266.40	11,100.00	41,100.00	9,009.55	40,833.60	24,921.58
P: M (67: 33)	2090.45	266.40	8,200.00	27,400.00	6,109.55	27,133.60	16,621.58
P: M (33: 67)	2090.45	266.40	10,800.00	32,600.00	8,709.55	32,333.60	20,521.58
P: A (25: 75)	2135.45	266.40	8,300.00	22,100.00	6,164.55	21,833.60	13,999.08
P: A (75: 25)	2,105.45	266.40	9,700.00	25,300.00	7,594.55	25,033.60	16,314.08
P: M (25: 75)	2090.45	266.40	7,400.00	26,900.00	5,309.55	26,633.60	15,971.58
P: M (75: 25)	2090.45	266.40	9,600.00	29,800.00	7,509.55	29,533.60	18,521.58
R: M (25: 75)	2090.45	266.40	13,300.00	30,100.00	11,209.55	29,833.60	20,521.58
R: M (75: 25)	2090.45	266.40	9,300.00	32,400.00	7,209.55	32,133.60	19,671.58
R: A (25: 75)	2,135.45	266.40	6,800.00	23,800.00	4,664.55	23,533.60	14,099.08
R: A (75: 25)	2,105.45	266.40	7,800.00	24,600.00	5,694.55	24,333.60	15,014.08
R: A (33: 67)	2,130.45	266.40	11,700.00	29,900.00	9,569.55	29,633.60	19,601.58
R: A (67: 33)	2,110.45	266.40	10,600.00	30,400.00	8,489.55	30,133.60	19,311.58
P: A (33: 67)	2,130.45	266.40	9,800.00	29,200.00	7,669.55	28,933.60	18,301.58
P: A (67: 33)	2,110.45	266.40	11,300.00	24,900.00	9,189.55	24,633.60	16,911.58

4.2. Biological Potential

The 2 years' mean RTY of all grass-legume mixtures in the experimental periods were greater than one (range: 1.23-2.11) reflecting that there was a yield advantage of 23-111 percent over the pure stands of grasses and legumes. This could suggest the occurrence of biological nitrogen fixation in the root nodules of the legumes and its transfer from the legume component to the grasses that might have supported the growth of grasses and their mixture in the pasture sward. This would also imply that the grass-legume mixtures were at least partly complementary in resource use. This may happen when the growth periods of the mixtures are partly overlapping or when they are using plant growth resources from varying soil depths. The results of the RY and RTY in the current study were in agreement with Daniel (1990) and Diriba (2002) for the *Chloris-Medicago* and *Panicum-Stylosanthes* mixtures, respectively. Tessema and Baars (2006) also reported RTY values to have ranged from 1.29 to 1.48 in a 50:50% perennial grass/legume mixture in the north-western part of Ethiopia. This situation could be attributed to the efficient utilization of plant growth factors by species in the mixture due to either temporal or spatial differences of their demands.

Both grasses and legumes in the mixture produced RCCs in excess of unity indicating that all produced a higher yield in the mixture than expected in pure stands at different seed rates. Moreover, the mean RCCs for both the grass and legume components were almost equal, indicating that they perform similarly in DM production in the mixture. This may indicate that the grasses benefited highly from the legume components, which could have contributed to the increased DM productions of the mixtures. Forage legumes benefit, in terms of increased herbage and animal production, smallholder agricultural production systems by their ability to fix atmospheric nitrogen (Thomas, 1995). Morrison (1984) also suggested that legumes such as *Medicago* and clovers increase the yield of the grasses when grown in combination with them. More than 75% of the grass/legume mixtures produced mean AI values of less than unity and closer to zero (range: -0.06 to +0.88) and the mean AI value of both the grass and legume components was low (+0.29 and -0.29, respectively), indicating that there was low dominance in both components and they were almost equally competitive and both contributed more in DM production of the entire mixture throughout the study. The AI values of the present study is similar to the result of Tessema and Baars (2006) who reported AI values for *Chloris* mixed with *Desmodium* and *Chloris* mixed with *Medicago* of +0.07 and +0.23, respectively.

4.3. Dry Matter Production

In the current study, *Chloris* and *Panicum* mixed with *Melilotus* at 50: 50 seed rate and *Chloris* mixed with *Melilotus* at both 67: 33 and 33: 67 seed rate proportions provided higher DM productions compared to other mixtures throughout the study. Moreover, 75 and 100% of the grass/legume mixtures exceeded the DM yield of

pure stand grasses and legumes, respectively and this indicates the contribution of different seed rate proportions of grasses and legumes towards the yield mixtures. This is in agreement with the findings of past research works (Tessema 1996; Diriba 2002; Tessema and Baars 2006). However, Lemma *et al.* (1991) reported that pure stand *Chloris gayana* produced a higher DM yield than *Chloris*/legume mixtures in the second and third year of establishment in the western part of Ethiopia. The variation could be due to the difference in location, soil fertility, season, climatic, and other biotic and abiotic environmental factors.

Total DM yields of all mixed pastures were different in the first and second years and DM production increased as the pasture sward advanced. This could be due to the fact that all the grasses and legumes as pure stand and in the mixture grew well and vigorously from the time of establishment to anthesis, enabling the grasses and legumes to produce more tillers and branches respectively that could contribute to the observed high DM production of the mixtures. In addition, legumes showed fast regrowth ability after harvest compared to grasses, which might have contributed to the higher DM production of mixtures compared to pure stand grasses (personal observation). Hence, as the growth pattern of legumes and grasses is variable over drier and wetter parts of the year, selection of the correct grass-legume combination could make sustained availability of animal feed all year round. This indicates that higher DM yields of grass-legume combinations could be obtained as the pasture advances in establishment and sward consolidation. Results of research works elsewhere have indicated that grass and legume mixtures significantly contribute to a high total herbage yield (Daniel, 1990; Tessema, 1996; Tessema and Baars, 2006). The DM production potential of the grass-legume mixture in the present study was similar to the reports of other research works (Morrison, 1984; Lemma *et al*., 1991; Diriba, 2002).

4.4. Economic Viability

Chloris gayana mixed with *Melilotus alba* at 50:50 and 33:67 seed rate proportion gave a higher net return/ha and average net return /ha/yr compared to pure stand grasses and legumes as well as other mixtures in the present study. However, Berhan (2005) reported that association of the grass *Panicum coloratum* and the legume *Stylosanthes guianensis* at a seed rate of 25:75% was the most economically-viable system and provided the best monetary advantage and net return in the eastern highlands of Ethiopia. The variation in the present findings compared to previous reports might be due to the forage species used, season, environmental and other edaphic factors during the growth of the mixtures.

5. Conclusions

A significant ($P < 0.05$) effect was observed regarding RY, RTY, RCC and AI of both the grass and legume components and their mixtures due to different seed rate proportions throughout the study. The mean RYs of both legume and grass components were greater than unity (1.04). The 2 years' mean RTY of all grass-legume mixtures in the experimental periods were greater than one (range: 1.23-2.11) and *Chloris* mixed with *Melilotus and Medicago* with 67: 33 seed rate proportion had a higher RTY than other mixtures. Both the grass and legume components in the mixture produced mean RCCs values in excess of unity and almost equal values in both components. *Chloris* and *Panicum* mixed with *Melilotus* at 50: 50 seed rate and *Chloris* mixed with *Melilotus* at both 67: 33 and 33: 67 seed rate proportions provided higher DM productions compared to other mixtures throughout the study. Therefore, to alleviate the feed scarcity in the area; these grass-legume mixtures could be introduced to smallholder farms. In addition, the result of the current study should be assessed in relation to animal performances using feeding trials and under grazing conditions to see how these mixtures perform in practical situations.

6. Acknowledgment

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7. References

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The Effects of Supplementation of Grass Hay with Different Levels of Brewer's Dried Grain on Feed Intake Digestibility and Body weight Gain in Intact Wogera Lambs

Mullu Moges[1], Berhan Tamir[1]* and Alemu Yami[2]

[1]Department of Animal Production, Faculty of Veterinary Medicine, Addis Ababa University, P O Box 34, Debre-Zeit, Ethiopia
[2]Ethiopian Agricultural Research Institute, P O Box 32, Debre-Zeit, Ethiopia

Abstract: The effects of supplementation of different levels of brewer's dried grain on feed intake, digestibility and body weight change were studied using 20 yearling male local ('Wogera') lambs weighing 16.96 ± 2.4 kg (Mean ± SD). Lambs were blocked based on their initial body weight into five blocks of four animals each, and randomly allocated to four dietary treatments, that consisted of feeding of lambs in T1 (control) with grass hay ad libitum and lambs in T2, T3, and T4 with grass hay ad libitum plus 100, 200 and 300 g DM brewer's dried grain (BDG) per lamb per day, respectively. Treatment rations were offered in two equal portions at 0800 and 1600 h and lambs had free access to water and mineral licks at all times. The results showed that the daily total dry matter intake (DMI) and crude protein intake (CPI) increased significantly ($P < 0.05$) with increasing levels of BDG, which correspondingly resulted in significantly higher ($P< 0.05$) final body weight (23.16 kg) and daily body weight gain (93.99 g/d) than the control group. Supplementation with 200 and 300 g DM BDG resulted in improved ($P< 0.05$) daily weight gain (70.44 and 93.99 g/d), respectively compared to supplementing with 100 g DM BDG (44.40 g/d). The un-supplemented group lost weight (3.0 g/d), even though their hay DMI was significantly higher ($P < 0.05$) than that of the lambs supplemented with 200 and 300 g DM BDG. The feed conversion efficiency was also significantly ($P < 0.05$) higher for the BDG supplementation. The apparent digestibility coefficient of CP was higher ($P < 0.05$) for lambs supplemented with 200 and 300 g DM BDG compared to those supplemented with 100 g DM BDG and the un-supplemented group, but no significant effect of BDG supplementation was observed for the apparent digestibility coefficients of NDF, ADF, OM and DMD. Based on DM and nutrient intakes, improved body weight gain and nutrient digestibility, it could be concluded that a daily supplementation of 300 g DM BDG might improve feed intake and nutrient utilization, body weight gain and feed conversion efficiency of yearling Wogera ram lambs.

Keywords: Body Weight Gain; Brewer's Dried Grain; Digestibility; Feed Intake; Supplementation; Wogera Ram Lambs

1. Introduction

Small ruminant production is an important agricultural activity in Ethiopia. There are approximately 38.8 million small ruminants, of which 21.9 million are sheep (FAO, 1996). The production potential of different indigenous sheep breeds has not been properly studied. Available data indicates that small body size, low and inferior quality of wool production, low lamb growth rate and quite high mortality rates characterize the indigenous sheep breeds. The annual off-take rate is estimated to be 40% with an average carcass weight of about 10 kg, which is the second lowest in sub-Saharan Africa (FAO, 1996). Despite the low productivity of indigenous breeds compared to temperate breeds, their ability to survive and produce in the harsh and mostly unpredictable tropical environment is remarkable. Small ruminants derive most of their feed from natural pasture, which forms a major feed resource, providing more than 90% of the livestock feed, either in the form of grazing or forage conserved in the form of hay for dry season use (Lulseged, 1985). However, the available natural grazing lands are either being encroached by arable farming or are seriously overloaded with livestock beyond their optimum carrying capacity leading to overgrazing, low productivity and the poor nutritional quality of natural pasture. Consequently, grazing animals suffer from under-nutrition, resulting in loss of the body weight and body condition, especially during the dry season.

Dietary nutrients, especially energy and protein, are the major factors affecting the productivity of sheep. The lowest energy density at which sheep do not lose weight is between 8 and 10 MJ ME/kg DM and the minimum protein level required for maintenance is about 8% of DM, while productive animals such as rapidly growing lambs and lactating ewes need about 11%, which are considerably higher than the average values found in natural pastures and crop residues (CTA, 1991).

Animal performance could be improved by the supplementation of protein sources. The protein requirement for maintenance could be met entirely by microbial protein from the rumen. In contrast, for fast-growing lambs, protein synthesis in the rumen is not sufficient to meet the animal's requirement, indicating the need for supplementation with additional sources of undegradable food protein. It has been established that, for higher production, the most effective way of providing the required amounts of amino acids and glucogenic compounds is by intestinal digestion. Therefore, the feeding strategies should always be designed in such a way that the parts of the dietary nutrients that could by-pass and be digested post ruminally are present in the diet (McDonald *et al.,* 1995).

*Corresponding author. E-mail: berhantamir@yahoo.com

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The need to improve indigenous ruminant livestock productivity has been emphasized, based on the demand for animal protein in the diet of the rapidly growing population. The consumption of animal protein in developing countries is below the recommended levels, due mainly to an inadequate supply of quality feeds. There are several complementary and alternative strategies that could be pursued in tropical regions with the objective of making low quality feeds more useful for the production of meat and milk. Livestock productivity in the tropical regions largely depends on the efficiency of the utilisation of locally available protein sources. Supplementation with protein and energy sources is one strategy which could be utilized to increase feed intake, nutrient supply and digestibility.

Concentrated feed sources, especially grains, are expensive and highly valued as human food and by-products like noug seed (*Guizotia abyssinica*) cake are expensive and frequently not readily available to smallholder farmers. Therefore, it is necessary to look for alternative, cheap feedstuffs to sustain and improve the ruminant husbandry. Such alternatives should not compete with feedstuffs suitable for monogastric animals and man (Older *et al.,* 1991), but should exhibit moderate nutrient concentration, support growth, and result in the production of edible food for human beings.

Among such feedstuffs, brewers' dried grain, the by-product resulting from brewing plants, could be a source of protein, energy and fibre in the diet of lambs. Recently, a new brewing plant has been built around the study area. It is, therefore, important to generate information related to the utilization of this important by-product feed ingredient as a source of a supplement for feeding ruminants. Thus, this study was designed to evaluate the effects of supplementation at different levels of brewer's dried grain from the 'Dashen' brewing plant on feed intake, digestibility and body weight change of local yearling 'Wogera' ram lambs fed a grass hay basal diet.

2. Materials and Methods

2.1. Study Site and Animal Management

The experiment was conducted in Gondar town, northern Ethiopia, located at an altitude of 2050 m.a.s.l and receiving a mean annual rainfall of 710–1138 mm. The mean annual minimum and maximum temperatures of the area are 16 and 25^{0}C, respectively (DOA, 1998). Twenty yearling ram lambs of local 'Wogera' breed sheep, weighing 16.96 ± 2.4 kg (mean ± SD), were used. The lambs were dewormed using albendazol bolus and ivermectin injection and sprayed using diazinol external parasites. Lambs were penned individually and adapted to the experimental conditions and feeds for fifteen days before the commencement of the feeding trial that lasted for 90 days.

2.2. Feeding, Experimental Design and Treatments

Grass hay mainly dominated by *Digetaria* species was used as a basal diet for the experiment. The supplement used was brewers' dried grain and was offered in a separate trough twice a day at 0800 and 1600 h prior to hay. The grass hay was offered *ad libtum* as a feed basis in the morning hours. Refusals of grass hay were collected and weighed before the next feeding. Samples of feed offers were taken per batch for each feed and treatment, and that of refusals daily per lamb and bulked over the experimental period for chemical analysis. Fresh water and salt lick were available at all times.

The design of the experiment was randomized complete block design. Lambs were blocked based on initial body weight into five blocks of four animals and randomly distributed to four experimental diets.

Treatments consisted of feeding of lambs in T (control) with *ad libitum* grass hay, and lambs in T2, T3 and T4 were fed with grass hay *ad libitum,* and supplemented with 100, 200 and 300 g DM brewer's dried grain per day, respectively. Feeds were offered daily on DM basis by multiplying the amount of daily feed offer by the respective DM content.

2.3. Measurements and Observations

Feeds offered and refused were recorded daily and feed intake was determined by subtracting the amount refused from the amount offered on a DM basis. The initial body weight of the lambs was recorded on the first day of the experiment and, thereafter, the body weight was taken at weekly intervals after overnight fasting using a spring balance. The daily body weight gain of each lamb was calculated as the difference between the initial body weight and final body weight divided by number of experimental days. Feed conversion efficiency was determined by dividing the daily weight gain by the amount of daily feed intake of each animal.

2.4. Faeces Collection

All lambs used the feeding trial were adapted to carrying faecal collection bags for 3 days, which was followed by a total faeces collection for a period of 7 successive days for each animal. Total faeces voided was collected and weighed every morning before feeding and 20% of faeces were sampled, composite samples stored in airtight plastic bags in a deep freezer at -20°C. On the last day of the collection period, faecal samples were thoroughly mixed for each animal from which DM, OM, N, NDF and ADF were determined. While feed offers and refusals were weighed daily, the body weight of each animal was taken at the start and end of the collection period.

2.5. Laboratory Analysis

Samples of feed offered and refused, and faeces were dried in an oven at 60°C for 72 h and ground using a Wiley mill, UK, passed through a 1mm sieve screen and kept in airtight plastic bags pending analysis. The analysis of the contents of DM, N, OM and ash, acid detergent fibre (ADF) was done according to AOAC (1990). Neutral detergent fibre (NDF) was determined by the method of Van Soest *et al.* (1991). The apparent digestibility coefficients for DM, OM, CP, NDF and ADF were determined as the proportion of DM or nutrient intakes not recovered in the faeces (McDonald *et al.*, 1995). The entire chemical analysis was carried out in the Nutrition Laboratory of Holetta Research Centre.

2.6. Statistical Analysis

The data collected from the experiment was analyzed using the General Linear Model (GLM) procedure of SAS (2002). Treatment means were separated using Duncan's Multiple Range Test. Data was subjected to analysis of variance using the following model:

$Y_{ij} = \mu + a_i + \beta_j + e_{ij}$

Where Y_{ij} = the observation in i^{th} treatment and j^{th} block, μ = Over all mean, α_i = the i^{th} treatment effect, β_j = the j^{th} block effect and e_{ij} = the random error.

3. Results and Discussion

3.1. Chemical Composition of Feeds

The grass hay used in this experiment could be characterized by its low CP (42 g/kg DM), and high NDF (768 g/kg DM) and ADF (520 g/kg DM) contents. This was, indeed, expected because the grass hay consisted of predominantly mature Digitaria species, which is rich in cell wall constituents. The mean chemical composition of the hay and supplements is shown in Table 1. The contents of NDF and ADF of the BDG were within the range reported values of 499 to 753 and 216 to 315 g/kg DM, respectively (MAFF, 1990). The crude protein content of the BDG in the present study was comparable to that reported by Anigbogu (2003). Differences in the initial raw material used and processing in the brewing plant attribute to variations in the CP content of BDG. McDonald *et al.* (1995) reported that the chemical composition of brewery grain is a function of mainly the quality of the barley and malt used in the process and the processing method used to elaborate the juice, and the filtration techniques.

Table 1. Chemical composition of hay and brewery dried grain.

Feeds	Feed nutrients					
	DM (g/kg)	g/kg DM				
		Ash	OM	CP	NDF	ADF
Hay	933	114	819	42	768	520
Brewery dried grain	937	35	902	198	765	284

3.2. Feed and Nutrient Intake

The average daily intakes of hay, BDG and total dry matter are presented in Table 2. The mean daily total of dry matter intakes of growing Wogera lambs increased significantly ($P < 0.05$) with increasing levels of BDG supplementation. The hay dry matter intake was lower ($P < 0.05$) for the lambs supplemented with 200 and 300 g DM per day than the control, however, no difference was observed between T1 and T2, and T2 and T3, and T3 and T4. The intake of the supplement significantly ($P < 0.05$) increased with the increasing level of the supplement. Higher total dry matter intake per unit of metabolic body weight was reported for Menz (75.5 g/kg) and Horro (78.3 g/kg) rams, respectively, when they were supplemented with 300 g of concentrate feed per day (Ewenetu, 1999). Compared to this study, a lower mean total dry matter intake of 398.1 g/d was reported for Horro lambs, when fed on grass hay and concentrate supplements (Melese *et al.*, 2001).

Dietary protein supplementation is known to improve intake by increasing the supply of nitrogen to the rumen microbes, which increases the microbial population and efficiency, enabling an increased rate of breakdown of the digesta, which in turn increases feed intake. A better supply of escape protein in feed could also have contributed to the higher feed intake, as a supply of escape protein was suggested to improve feed intake (McDonald *et al.*, 1995). The improved response in growing animals might be partly due to the escape protein that provides amino acids in which microbial protein is deficient and increasing total protein reaching into the small intestine, thus increasing growth rate, which in turn increases feed intake in addition to effects it might have to improve absorbed nutrient balance (McDonald *et al.*, 1995). The increased DM intake in lambs with supplementation resulted in increased CP intake, which increased body weight gain in sheep (Kabir *et al.,* 2004). On the other hand, the increase in total dry matter intake might be due to the consequence of the lower rumen fill of the supplement compared to the grass hay.

A higher dry matter intake as the proportion of brewers' dried grain increased might be due to increasing levels of protein. Consistent with the results in this study, improved dry matter intake as a result of improved rumen function was reported when low quality roughage was supplemented with a protein source like brewers' grain (Nguyen, 2003). Similarly, an increase in dry matter intake as the level of crude protein increased in the diet of goats has been reported (Tegene *et al.*, 2000). Contrary to the results of this study, no appreciable change in the daily dry matter intake per kg $W^{0.75}$ with increasing level of supplementation in goats has been reported (Sahlu *et al.*, 1993).

The reduction of hay intake with an increasing level of the supplement in this study might be due to the substitution of a basal diet with concentrate. Dixon and Egan (1999) reported a substitution effect of supplementation of mixtures of cereals and/or protein supplement to the grass hay based feeding. The hay intake was higher for lambs receiving low level of supplement than those receiving a high level of supplement. Similarly, voluntary intake of forage was lower ($P < 0.05$) in lambs offered barley and oat supplements than those offered the basal diet (Ponnampalam *et al.,* 2004). Nsahliai and Ummuna (1996) reported that the response to supplementation depends on feed and animal factors, the former including the quality of basal roughage and supplement feed. Supplement feeds that ferment rapidly replace the basal roughage to a lower extent than those that ferment slowly.

However, the effect of protein supplementation was reflected not only in increased roughage intake, but also in an improvement in nutrient supply. Lack of significant response up to four weeks (Figure 1) in DMI in the present study could be due to the fact that the amount of CP in grass hay might have been adequate to meet the minimum microbial N requirement.

Table 2. Feed and nutrient intake of Wogera yearling ram lambs fed a grass hay basal diet supplemented with different levels of brewery dried grain.

Feed intake	Treatments				
	T1	T2	T3	T4	SEM
Hay intake (g/d/head)	477.11^{a}	465.18^{ab}	420.18^{bc}	411.73^{c}	16.57
BDG intake (g/d/head)	-	100.00^{c}	200.00^{b}	300.00^{a}	0.09
Total DM intake (g/d/head)	477.11^{d}	565.18^{c}	620.18^{b}	711.73^{a}	16.6
TDM intake (g/kg $W^{0.75}$/d)	55.00^{b}	59.10^{b}	59.64^{b}	67.31^{a}	1.11
Hay CP intake (g/d/head)	20.10^{a}	19.58^{a}	17.68^{a}	17.33^{a}	1.05
BDG CP intake (g/d/head)	-	19.79^{c}	39.58 ^{b}	59.37^{a}	0.06
Total CP intake (g/d/head)	20.10^{d}	39.37^{c}	57.26^{b}	76.70^{a}	1.05

^{a,b,c,d} *Means in the same row with different superscripts differ significantly, ($P < 0.05$).*

T1=Control (grass hay alone), T2, T3 and T4= grass hay supplemented with 100, 200 and 300 g of DM brewers dried grain per day, respectively.

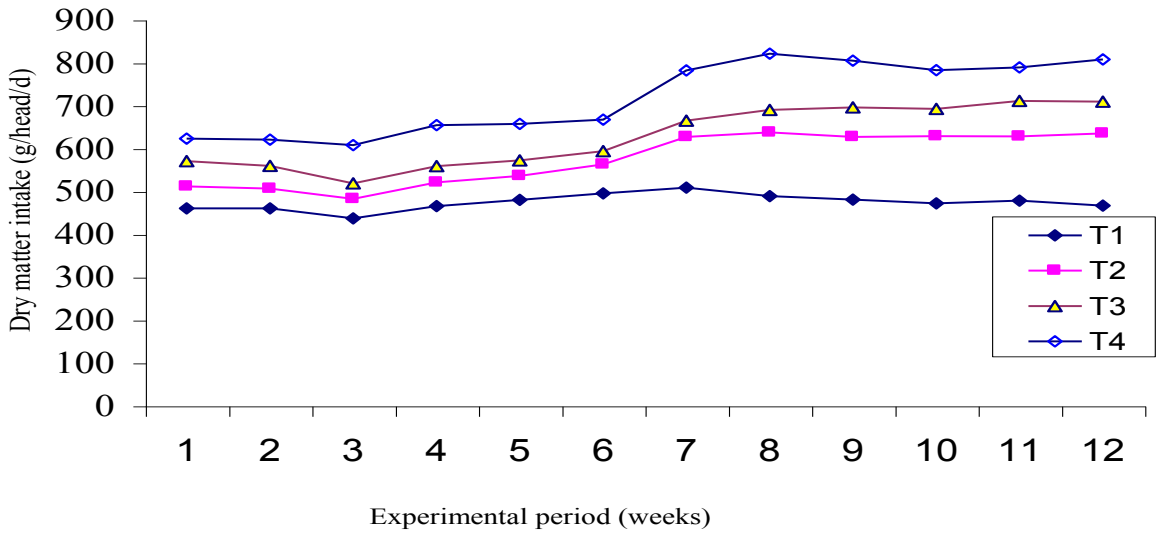

Figure 1. Dry matter intake (DMI) of Wogera yearling ram lambs as affected by levels of brewer's dried grain supplementation. T1=Control (grass hay alone), T2, T3 and T4= grass hay supplemented with 100, 200 and 300 g of DM brewers dried grain per day, respectively.

3.3. Body Weight Gain

The effect of different levels of brewer's dried grain supplementation on body weight gain of Wogera lambs is shown in Table 3. As the level of BDG increased in the diet, so did the final body weight and average daily weight gain (ADWG). Higher ($P < 0.05$) body weight gain (93.99 g/head/d) was recorded for lambs that received 300 g BDG, compared to 200 g, and 100 g, which gained 70.4, and 44.4 g/d, respectively. Weight loss of -3.0 g/d/head was recorded for lambs offered grass hay alone (control), indicating that nutrients provided by hay barely even meet the maintenance requirements. The loss in the body weight of lambs in the control treatment could be associated with low nitrogen and high cell wall and poor digestibility of the hay.

Table 3. Mean daily body weight gain of Wogera yearling ram lambs fed a grass hay basal diet supplemented with different levels of brewery dried grain.

Parameters	Treatment				SEM
	T1	T2	T3	T4	
Initial body weight (kg)	17.92^{a}	17.12^{a}	16.82^{a}	16.82^{a}	0.50
Final body weight (kg)	17.64^{d}	21.12^{c}	23.16^{b}	23.16^{b}	0.43
Daily body weight gain (g/head)	-3.00	44.4^{c}	70.44^{b}	93.99^{a}	5.68
Feed conversion efficiency	-0.006^{c}	0.08^{b}	0.11^{ab}	0.13^{ab}	0.14

^{a,b,c,d} *Means in the same row with different superscripts differ significantly ($P < 0.05$).*

T1=Control (grass hay alone), T2, T3 and T4= grass hay supplemented with 100, 200 and 300 g of DM brewers dried grain per day, respectively.

The growth rate observed on lambs taking different supplements during the feeding trial (Figure 2) indicated that both CP and energy were adequate in the diets, and the highest level were recorded for 300 g DM BDG inclusion level. The increase in body weight gain with the increasing level of supplementation agree with Anigbogu (2003), who reported the best growth rates with diets containing 312.2 and 516.8 g DM BDG per day, which supported daily gains of 152.5 and 168.5 g, respectively, when BDG was supplemented to a lower quality forage diet for growing lambs. The intake of CP increased as the level of inclusion of dried brewer's grain in the diet rose and weight gain of lambs was 83 g/d when 80% of the feed constituted BDG (Bovolenta *et al.*, 1998). Comparable to the present results (93.9 g/d) at 300 g BDG supplementation), body weight gain of 96 g/d for growing Ethiopian Highland sheep and 90 g/d for Horro rams when 35% and 50% of the hay was replaced with concentrate, respectively, has been reported (Galal *et al.*, 1979).

The higher daily weight gain with increasing levels of BDG could be associated with a higher CP intake. The improved growth rate of supplemented groups could be further explained by the fact that BDG degrades at a slower rate in the rumen, resulting in more efficient utilisation of BDG in the lower tract for growth (Aregheore *et al.*, 1991).

The feed conversion efficiency of lambs in this experiment the increased with increasing level of BDG supplementation (Table 3). Solomon and Solomon (1995) reported an increased feed conversion ratio with an increasing level of supplement. It was demonstrated that ram lambs on BDG gained more weight per unit of feed intake compared to others (Aregheore *et al.*, 1991). The reduction in the feed conversion efficiency of the control lambs in this study could be associated with low energy and CP intake and the high fibre content of the grass hay.

3.4. Feed and Nutrient Digestibility

Brewer's dried grain supplementation improved ($P < 0.05$) the digestibility of CP. However, DM, OM, NDF and ADF digestibility of treatment feeds were similar ($P > 0.05$) between supplemented and un-supplemented groups. The results agree with the findings that feeding of goats on a high protein diet significantly improves N digestibility compared to a low protein diet. However, dietary protein concentration had no effect on the digestibility of ADF and NDF. In contrast, improvements in fiber digestion have been reported in sheep supplemented with escape protein (Garett, 1989). Apparent digestibility of CP increased with the proportion of BDG in growing lambs, which demonstrated its high CP digestibility with increasing proportions of BDG. Apparent ruminal digestibility of dietary N tended to be influenced by both protein concentration and source, where the ruminal digestibility of dietary N tended to decrease as ruminal degradation of the protein supplement increased (Garett, 1989). The decrease in digestibility of DM, OM, ADF and NDF at higher levels of supplementation in this study could be due to the bulky nature of the feed at higher level of supplementation as it swells in the stomach and causes digestive disorders, thereby reducing digestibility (McDonald *et al.*, 1995).

Table 4. Apparent digestibility coefficient of feed nutrients in Wogera yearling ram lambs fed a grass hay basal diet supplemented with different levels of brewery dried grain.

Apparent digestibility coefficient	Treatment				SEM
	T1	T2	T3	T4	
DM	0.698	0.736	0.778	0.734	0.028
OM	0.710	0.736	0.790	0.748	0.028
CP	0.612[b]	0.696[ab]	0.746[a]	0.761[a]	0.097
NDF	0.763	0.776	0.797	0.711	0.032
ADF	0.674	0.733	0.771	0.706	0.032

[a,b] Means in the same column with different superscripts differ significantly ($P < 0.05$).
T1=Control (grass hay alone), T2, T3 and T4= grass hay supplemented with 100, 200 and 300 g of DM brewers dried grain per day, respectively.

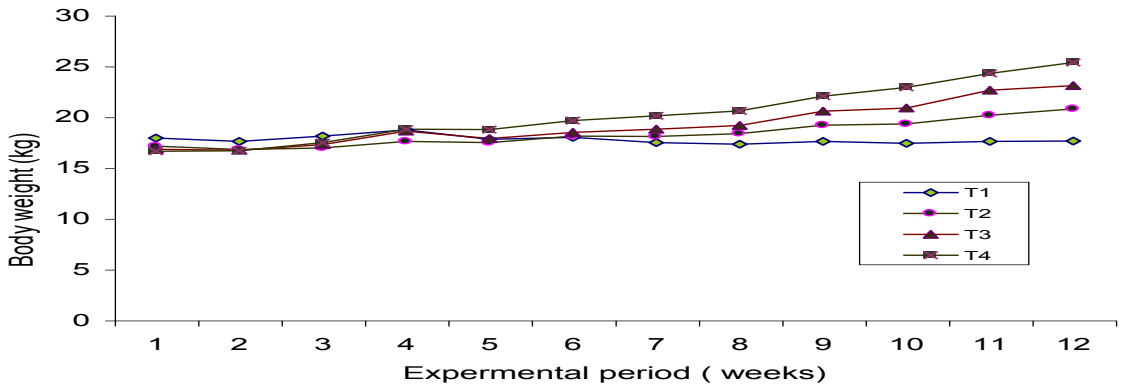

Figure 2. Body weight change of Wogera yearling ram lambs as affected by levels of brewer's dried grain supplementation.T1=Control (grass hay alone), T2, T3 and T4= grass hay supplemented with 100, 200 and 300 g of DM brewers dried grain per day, respectively.

4. Conclusions

The results of the chemical analysis indicated that brewer's dried grain had a higher CP content than the grass hay. The results obtained from the feeding and digestion trials revealed also that supplementation of brewer's dried grain at 300 g DM/d/head resulted in higher ($P < 0.05$) total DM and CP intakes and improved ($P < 0.05$) the daily body weight gain compared to the non supplemented and supplementation at 100 and 200 g DM/d/head. Supplementation also increased feed conversion efficiency compared to the control group. The digestibility of CP increased due to supplementation of brewer's dried grain.

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The Effect of Inclusion Rate of Cooked and Sun-dried Fish Offal Meal on Feed Intake, Growth and Feed Efficiency of Rhode Island Red Chicks

Asrat Tera[1], Tegene Neggese[2]*, Aberra Melesse[2] and Yosef Teklegiorgis[2]

[1]Wolaita Soddo Agricultural Technical Vocational Education Training College, P O Box 120, Wolaita Soddo, Ethiopia
[2]Hawassa University, Awassa College of Agriculture, P O Box 05, Awassa, Ethiopia

Abstract: The effects of feeding cooked and sun-dried fish offal meal (fishmeal) on feed intake, weight gain and feed conversion efficiency of RIR chicks were assessed at Wolayta Soddo, southern Ethiopia. Unsexed day-old RIR chicks (300) were brooded uniformly for 14 days and then vaccinated against Gumboro and Newcastle diseases. A feeding trial with 6 dietary groups, 5 replicates each and 10 chicks per replicate was run for 11 weeks and daily feed intake and weekly individual body weights were recorded. The control diet (T_1) consisted of Fishmeal (0%), Maize (34.1%), wheat short+bran (21.0%), limestone (1.20%), salt (0.5%), premix (0.1%), lysine (0.05%), methioinine (0.05%), roasted soybean (27.0%) and noug cake (16.0%); the rest of the diets contained ingredients in the control plus fishmeal included at rates of 3.32%(T_2), 6.64%(T_3), 9.96%(T_4), 13.28%(T_5), and 16.6%(T_6) of the diet replacing 7.6, 15.3, 22.9, 30.5 and 38.2% of the protein of the control diet. T_1, T_2, T_3, T_4, T_5 and T_6 had 19.76, 18.89, 19.82, 18.44, 18.96 and 19.20 % CP, respectively. Chicks fed T_1 had the lowest ($p<0.001$) daily intakes (68.5 g DM, 13.3 g CP, 0.54 g Ca, 0.35 g P and 231kcal ME head^{-1}) but those on T_6 had the highest daily intakes (77 g DM, 14.8 g CP, 1.81 g Ca, 0.58 g P and 243 kcal ME head^{-1}). Other groups fell in between these ranges. Higher ($p<0.01$) mean daily body weight gains (MDBWG) were observed in fishmeal groups compared to the control. The MDBWG of T_1, T_2, T_3, T_4, T_5, and T_6 were 10.65 g, 13.14 g, 12.82 g, 13.46 g, 13.2 g, and 12.78 g head^{-1}. MDBWG was significantly ($p<0.01$) and positively affected by the age of the chicken up to 2.5 to 3 months but it declined from then onwards. Fishmeal groups utilized feed [6.03(T_2), 6.12(T_3), 5.83(T_4), 6.03(T_5) and 6.35(T_6) g feed/g MDBWG] more efficiently ($p<0.01$) than the control group (6.79 g feed/g MDBWG, T_1). The feed conversion ratio decreased with advance in age. Differences in protein efficiency ratios (PER) were highly significant ($p<0.001$) between fishmeal groups (1.025, 0.973, 1.098, 1.013 and 0.957 for T_2, T_3, T_4, T_5 and T_6, respectively) and the control (T_1, 0.901). Mortality of chicks was not encountered during the trial. Cooked and sun dried fish offal can be incorporated in up to 16.6% of the diets of growing RIR chickens without affecting intake and growth, however, best results were obtained at 9.96% inclusion level.

Keywords: Chicks; Fishmeal; Growth; Intake

1. Introduction

Protein is an essential and key ingredient in poultry feed and is necessary for growth, body tissue maintenance and egg production. Cost of feed accounts for about 60-65% of the total cost of poultry production and protein shares about 13% of the total feed cost (Hassan *et al*., 2003).

A feasible approach that seems suitable is to incorporate agricultural and aquatic by-products which are not directly consumed by man. To this effect, investigation in to the potential of some feed resources that are cheaper, locally available and have comparative nutritional value as the conventional protein sources is necessary. These are valuable feed for poultry; they reduce feed cost, the problem of waste disposal and competition for food with humans (Boushy and Vander, 1994).

There is a possibility of producing more than 5700 tons of fresh fish offal (1900 tons of DM) per annum from fresh water bodies in Ethiopia (personal communications). The availability of major nutrients and unidentified growth factors, a well-balanced amino acid profile and the presence of omega fatty acids in fishmeal increases its importance in the feeding of simple stomached animals (Donald and William, 2002)

Although a substantial amount of work has been carried out teleported on using fishmeal in animal diet elsewhere, fewer studies have been completed on the nutritive value of locally-made fishmeal in Ethiopia. The levels of utilization need to be re-established as most of original works done elsewhere are old.

The limitations of fishmeal in animal feed include the "fishy" taste and odor that can be transferred to the poultry meat and eggs when the diet contains more than 6 to 10% fishmeal (Donald and William, 2002); danger of transmission of diseases; gizzerosine contents found in overheated fishmeal (Karimi, 2006) that can induce gastric acid secretion in young chicks, resulting in poor performance and gizzard erosion.

The current study was conducted to evaluate the impact of the dietary level of fishmeal prepared out of fish offal by cooking and sun drying on feed intake, weight gain and the feed conversion ratio of growing RIR chicks.

2. Materials and Methods

2.1. The Study Area

Fishmeal was prepared from fish offal collected from Lake Awassa that is located in Southern Nations, Nationalities and Peoples Regional State (SNNPRS) at 7:0 N latitude and 38:2 E longitude with an altitude of 1708 m. a. s. l, a surface area of 129 million square meters and depths of 11 to 22 m (Tudorancea *et al*, 1999).

The feeding trial was conducted at Wolayta Soddo Poultry Husbandry Center located 400 km South-west of Addis Ababa. It is situated at 1884 m. a. s. l and has bimodal rainfall with an annual rainfall ranging between 1000 and 1200 mm; and temperatures ranging between 22 and 24 ^{0}C (FAO, 1984).

*Corresponding author. E-mail: ntegene@yahoo.com

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2.2. Feeding Trial

2.2.1. Experimental Design

The chicks were assigned to the treatment diets in a completely randomized design (Table 1).

As shown in Table 3, the diets were iso-nitrogenous (from 18.44 to 19.82 % CP) and iso-caloric (from 2813 to 3335 kcal/kg DM). The fishmeal used was prepared according to the procedure described below. The control diet (T_1) did not contain fishmeal. The test diets, T_2, T_3, T_4, T_5 and T_6, contain fishmeal at a rate of 3.32, 6.64, 9.96, 13.28 and 16.6% of the DM, respectively, to replace 7.6, 15.3, 22.9, 30.5 and 38.2% of the CP of the control diet. Raw soybean was roasted for 5 minutes until brown to deactivate trypsin inhibitor. The coarse feed ingredients were first ground and mixed with a special mixer fitted to a miller.

Table 1. Experimental design of the feeding trial with Rhode Island Red chicks.

Treatment		Number of	Number of chicks	
acronym	Fishmeal inclusion rate (%)	replicates	per replicate	Total
T_1	0.00	5	10	50
T_2	3.32	5	10	50
T_3	6.64	5	10	50
T_4	9.96	5	10	50
T_5	13.28	5	10	50
T_6	16.60	5	10	50

T_1= Maize (34.10%) + Wheat bran and short (21.00%) + Soyabeans, roasted (27.00%) + Noug cake (16.00%) + Limestone (1.20%) + Salt (0.50%) + Vitamin-mineral premix (0.10%) + Lysine (0.05%) + Methionine (0.05%); T_2= Fishmeal (3.32%) + T_1 but with increased wheat bran and short (24.00%) and reduced soybeans (20.88%) and noug cake (15.80%); T_3= Fishmeal (6.64%) + T_1 but with increased wheat bran and short (27.16%) and reduced soybeans (15.20%) and noug cake (15.00%); T_4= Fishmeal (9.96%) + T_1 but with increased wheat bran and short (29.74%) and reduced soybeans (10.30%) and noug cake (14.00%); T_5= Fishmeal (13.28%) + T_1 but with increased wheat bran and short (33.72%) and reduced soybeans (5.00%) and noug cake (12.00%); T_6= Fishmeal (16.60%) + T_1 but with increased wheat bran and short (37.90%) and reduced soybeans (1.50%) and noug cake (8.00%);

2.2.2. Fishmeal Preparation

A rectangular cooking vessel made of sheet metal, 65cm×65cm×85cm; open at the top; with a 40 cm stand to allow heating of the vessel with fire wood; and with two outlets positioned at 5 cm and 50 cm from the bottom of the cooking vessel for removing liquid and allowing reading of temperature was used. Twenty liters of water were added to the bowl and heated with fire wood. When the water bailed, the offal was chopped with a knife, and transferred to the bowl, cooked for 17 minutes and stirred gently at intervals of 3 minutes. The cooked mass was left in the bowl undisturbed for 20 minutes and the floating oil was gently removed with a ladle. The water was discharged through the outlets to a beaker. The cooked offal was then thinly and evenly spread on a blue plastic sheet, exposed to sunlight for 10 to 12 days and stirred three times daily. It was covered with fine mesh wire to prevent flies from landing on it. Drying was stopped when a constant weight was obtained after consecutive weighings. After fine particles were screened using a 2mm sieve size, oversize particles were ground using a wooden mortar (Mukecha).

Table 2. The proportion of feed ingredients (% as fed basis) of the experimental diets.

	Treatments					
Ingredients (%)	T_1	T_2	T_3	T_4	T_5	T_6
Maize	34.10	34.10	34.10	34.10	34.10	34.10
Wheat bran + short	21.00	24.00	27.16	29.74	33.72	37.90
Soybeans, roasted	27.00	20.88	15.20	10.30	5.00	1.50
Noug cake	16.00	15.80	15.00	14.00	12.00	8.00
Fishmeal, cooked & dried	0.00	3.32	6.64	9.96	13.28	16.60
Lime stone	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.05	0.05	0.05	0.05	0.05	0.05
Methionine	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100

2.2.3. Chicks and Their Management

Chick houses were thoroughly cleaned, disinfected with 37% formalin, left empty for 14 days and then aerated for 5 days. A batch of 400 day-old RIR chicks were purchased from the Awassa Poultry Husbandry Center and uniformly brooded at the Soddo Poultry Husbandry Center for two weeks. They were vaccinated against New castle and infectious bursal diseases (Gumboro) on the 7th and 12th days, respectively. At the age of 14 days, they were weighed and 10 chicks were randomly assigned to each of the 30 replicates of the 6 treatment groups. The overall mean initial weight of the chicks was 114.6 ± 1.7

g. The average initial weights of T_1 (114.54 g), T_2 (114.12 g), T_3 (114.04 g), T_4 (115.66 g), T_5 (114.82 g) and T_6 (114.64 g) were similar. Male: female ratios of chicks (33:17, 33:17, 34:16, 33:17, 32:18 and 34:16 for T_1, T_2, T_3, T_4, T_5 and T_6, respectively) determined at the end of the experimental period were by chance fairly similar across the treatments by chance.

The chicks were reared in 30 separate pens with a floor space of 2250 cm^2/bird. Wood shavings were used as litter at a depth of 5 cm. All birds were exposed to continuous artificial light during the acclimatization period but, later on, it was reduced to 21 hours per day.

Feed and water was provided *ad-libitum*. Each day a known amount of feed was offered to each replicate. Refusals were collected and weighed in the morning before feed was offered. About 5% of the daily refusals were taken from each replicate and pooled for the replicates of each treatment diet. Samples of the diets were also taken daily while weighing the daily offer. Samples of diets and refusals were used for chemical analysis. Live weights of individual birds were taken at the beginning and end of the experiment and weekly during the experimental period. Birds were weighed in the morning before feed was offered.

2. 3. Chemical Analysis

The nutrient composition of feed ingredients, diets and feed refusals were analyzed at National Veterinary Institute laboratory, Debre Zeit. Dry matter, CF; mineral matter and EE were determined according to AOAC (1990). Nitrogen was determined by kjeldhal procedure and CP was calculated by multiplying N content by 6.25. Calcium was determined by atomic absorption spectrometer and phosphorus by spectrophotometer after dry ashing. Metabolizable energy values of feeds were calculated using the formula (Wiseman, 1987):

ME (kcal/kg DM) = 3951 + 54.4 fat - 88.7 crude fiber - 40.80 ash

2. 4. Statistical Analysis

Statistical differences in average daily live weight gains were analyzed using repeated measure ANOVA procedure (Zar, 1996; Morris, 1999); where the six feeds constituted levels of between subject factor (main treatment factor, A_i), the eleven weeks of experimental period composed the levels of the repeated measure factor (within subject factor B_j) and the five replicates under each feeding group were considered as subjects within main factor (replicates/feed or S_k/A_i). The specific ANOVA model is as follows:

$$Y_{ijkl} = \mu + A_i + B_j + S_k/A_i + A_i*B_j + B_j* S_k/A_i + e_{ijkl,}$$

Where, Y_{ijkl} = individual values of the dependent variable (mean daily gain);

μ = grand mean of the response variable;

A_i = the effect of the i^{th} feed (A) on the dependant variable (i= 1, 2, 3, 4, 5, 6);

B_j = the effect of the j^{th} week (repeated measure factor B) on the dependant variable (j = 1, 2, 3… 11);

S_k/A_i = the effect of the k^{th} replicate trial under the i^{th} feeding group (k= 1, 2, 3, 4, 5)

A_i*B_j = interaction effect of between subject (main treatment) factor (feed) and within subject (repeated measure) factor (age in week of chicks after the start of the experiment);

$B_j* S_k/A_i$ = interaction effect of with in subject (repeated measure) factor and replicate trials within each feeding group (reps/feed);

$e_{ijkl,}$ = random variation in the response of individual chick.

The effect of the diets on DM and nutrient intake as well as on DM and the protein efficiency ratio of the chicks was analyzed using single factor ANOVA. Day-to-day variations in intake and conversion efficiency of chicks could mask the effect of the main factor, thus the different days of the experimental period were considered as a covariate. Similarly, replicates within each feeding group were considered as a nested factor within feed to account for the variation in responses of chicks assigned under different replicates of the same feeds. Treatment means were tested for significance using Tukey HSD (for weight gain, DM and protein conversion ratios), Bonferroni (for intake) as a correction for multiple comparison. Differences between treatment groups were considered statistically significant at $p \leq 0.05$.

3. Results and Discussion

3. 1. Chemical Composition of Fishmeal and Diets

The chemical composition of the treatment diets used in the feeding trial is presented in Table 3. The ME content of T_1 was slightly higher than the ME (3200 kcal ME/kg DM) recommended for broilers (Scanes *et al.,* 2004). The ME values in the rest of the treatment diets were similar. The CP contents of the diets varied between 18.44 to 19.76%. T_1 with high ME and CP had low performance, possibly due to the poor quality of the CP in the diet as they were plant proteins.

Treatment 2 contained a slightly higher percentage of CF than the rest of the diets due to a higher amount of noug cake. Crude fiber in T_1 is lower than in T_2 because T_1 has a low proportion of wheat bran and a high amount of soybean. The composition of mineral matter increased with fishmeal inclusion levels as might be expected. Calcium and phosphorus compositions followed a similar pattern, and were within the recommended range for broilers (Scanes *et al.,* 2004).

3. 2. Nutrients and Energy Intakes

Table 4 shows the mean daily nutrient (DM, CP, OM, Ca and P) and ME (kcal) intakes of RIR chicks fed with different levels of fishmeal. Replacement of soybean with various levels of fishmeal significantly increased ($p<0.001$) nutrient intakes. Except for CP intake in T_4 and CF in T_6, which did not differ significantly from the control diet, the daily nutrient intakes were significantly ($p<0.001$) higher for the fishmeal groups than the control, possibly because the CP in the control diet was of poor quality as it was of plant origin. Significantly lowest ($p<0.001$) DM, CP, OM, Ca and P intakes were recorded in the control diet than in most of the groups with fishmeal. However, the birds did not gain more efficiently, the addition of fishmeal perhaps reduced digestibility of the diet.

Table 3. Nutrient content and energy value of the fishmeal and the experimental diets (on % DM basis).

Diets/meal	DM %	Ash % of DM	CF	CP	NFE*	Fat	Ca	P	ME kcal/kg DM
T_1	92.17	6.92	10.27	19.76	52.40	10.65	0.85	0.52	3335
T_2	91.81	8.69	13.72	18.89	50.74	7.96	1.30	0.63	2813
T_3	91.86	8.93	10.53	19.82	53.22	7.50	1.45	0.61	3061
T_4	91.65	8.69	11.99	18.44	53.22	7.66	1.45	0.83	2950
T_5	91.45	9.94	10.06	18.96	52.91	8.13	1.89	0.85	3095
T_6	91.45	11.17	9.07	19.20	51.94	8.62	2.36	0.76	3160
Fishmeal	94.16	24.76	2.56	44.70	6.36	21.62	6.08	1.00	3160

** NFE = 100-(Ash +CF + CP + Fat)*

T_1= Maize (34.10%) + Wheat bran and short (21.00%) + Soyabeans, roasted (27.00%) + Noug cake (16.00%) + Limestone (1.20%) + Salt (0.50%) + Vitamin-mineral premix (0.10%) + Lysine (0.05%) + Methionine (0.05%); T_2= Fishmeal (3.32%) + T_1 but with increased wheat bran and short (24.00%) and reduced soybeans (20.88%) and noug cake (15.80%); T_3= Fishmeal (6.64%) + T_1 but with increased wheat bran and short (27.16%) and reduced soybeans (15.20%) and noug cake (15.00%); T_4= Fishmeal (9.96%) + T_1 but with increased wheat bran and short (29.74%) and reduced soybeans (10.30%) and noug cake (14.00%); T_5= Fishmeal (13.28%) + T_1 but with increased wheat bran and short (33.72%) and reduced soybeans (5.00%) and noug cake (12.00%); T_6= Fishmeal (16.60%) + T_1 but with increased wheat bran and short (37.90%) and reduced soybeans (1.50%) and noug cake (8.00%);

Fishmeal groups had significantly higher ($p<0.001$) intakes of DM than the control. The significantly lowest intake was recorded for the control, whereas those with the highest fishmeal inclusion (T_6) had the highest intake due to the combined effect of the simultaneous reduction in soybean and noug cake and the increase of wheat flour byproducts and fishmeal. When the level of fishmeal increased from 3.32 % (T_2) to 13.28 % (T_5), the DM intake didn't vary significantly. The DM intake in this experiment seems to be affected not by energy but protein intake. The depressed appetite in the control diet is probably due to an amino acid imbalance in soybean and noug cakes (Agdebe and Aletor, 1997).

T_4 had the lowest CP intake among the fishmeal groups and with no significant difference ($p\geq0.05$) compared to the control diet. The rest of the diets had significantly superior % CP intakes ($p<0.001$) than the control diet. Crude protein intake did not show linear increase with fishmeal inclusion; T_3 and T_6 had significantly higher intakes than the rest of the fishmeal groups. T_4 had low DM, CP, ME and OM intakes compared to T_6 but was, at least, numerically higher in the production parameters. According to Isika *et al.,* (2006), high mineral intake impairs nutrient digestibility and, thus, the higher P contents and intakes observed in T_4 and T_5 might have reduced nutrient intakes compared to other diets. On the other hand, the relatively higher CF intakes in T_2 did not impair nutrient intake as well as utilization, thereby contradicting the expectation.

Ca intake significantly ($p<0.001$) and linearly increased with increasing levels of fishmeal inclusion. Chicks fed diets with the highest level of fishmeal consumed 3.5 times more Ca than the control group. On the other hand, P intake was significantly influenced by fishmeal inclusion. Fishmeal groups consumed higher P from the fishmeal source. Those groups maintained on T_5 had the highest P intake (approximately two fold) compared to others. The P in fishmeal, as it is of animal source, is assumed to be highly available for chicks and could thus minimize the problem of environmental pollution through the excretion of P.

The result of this trial is in agreement with the work of Ponce and Gernat (2002) who found a significant increase in feed intake when tilapia byproduct meal was to added up 6% in broiler's ration; but it disagrees with the author's report that feed intake was depressed at higher levels of fishmeal inclusion because, in this trial, DM and other nutrients' intake were stimulated with fishmeal levels. Similar results in feed intake were also reported by Karimi (2006) where chicks fed with 1.25 or 2.5% fishmeal had a higher average feed intake compared with chicks fed diets without fishmeal. In disagreement with these findings, Maigualema and Gernat (2003) found no significant differences in feed intake as protein from tilapia byproduct meal substituted soybean meal protein.

3. 3. Daily Body Weight Gains (DBWG)

Data on mean DBWG and feed conversion ratio are presented in Table 4. The mean DBWG of groups fed with rations containing fishmeal is significantly higher ($p\leq0.05$) than the control. Differences between T_1, T_3 and T_6 were not statistically significant. The highest DBWG was achieved in T_4 (9.96% fishmeal inclusion). However, it was not significantly different from the rest of the groups fed with diets containing fishmeal and, in fact, further increased the level of fishmeal depressed growth rate. Thus, 9.96% fishmeal inclusion rate in the present experiment could be taken as an optimum when only maximizing DBWG is considered. In line with this finding, Maigualema and Gernat (2003) also observed growth depression effects caused by a high (beyond 50%) level of fishmeal substitution of soybean meal in broilers. The optimum gain in T_4 might be due to the nutrient synergism of the animal and plant protein sources, which balances the limiting amino acids. Neggusie and Alemu (2005) suggested that rations containing fishmeal have the best assortment of high levels of amino acids and so should be used to balance rations of plant protein sources that are severely limited in critical amino acids. This work is also in agreement with earlier reports by Karimi (2006) who indicated that the average daily gains of broiler chicks were significantly improved by fishmeal supplementation to the diets. The factors responsible for

lower gains observed beyond 9.96% fishmeal inclusion level in contrast to their respective higher nutrients and DM intake is difficult to explain. However, in the earlier works of Ponce and Gernat (2002), significant differences were found at 3 and 6% fishmeal inclusion rates for broilers. In close agreement with these findings, Donald and William (2002) suggested that fishmeal levels up to 8% usually induce performance in broilers. Maigulema and Gernat (2003) and Karimi (2006) reported that the inclusion of fishmeal should be limited due to poor growth performance at higher levels.

Table 4. Mean daily nutrient (g/chick/d) and ME (kcal/chick/d) intakes, mean daily body weight gain (g/head), feed conversion ratio (g feed/g gain) and protein efficiency ratio (g gain /g CP intake) of RIR chicks fed diets with different levels of fishmeal.

	Treatment diets						
Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM
Dry matter intake	68.5^{a}	75.7^{bc}	74.9^{b}	74.4^{b}	75.4^{b}	77.0^{c}	0.325
Crude protein intake	13.3^{a}	14.3^{b}	14.8^{c}	13.6^{a}	14.3^{b}	14.8^{c}	0.069
Organic matter intake	63.9^{a}	69.1^{b}	68.3^{b}	68.0^{b}	68.0^{b}	68.4^{b}	0.292
Calcium intake	0.54^{a}	0.99^{b}	1.07^{c}	1.07^{c}	1.41^{d}	1.81^{e}	0.01
Phosphorus intake	0.35^{a}	0.48^{c}	0.46^{b}	0.62^{e}	0.64^{f}	0.58^{d}	0.003
Crude fiber intake	6.9^{a}	10.4^{e}	7.9^{c}	8.9^{d}	7.6^{b}	6.9^{a}	0.044
Metabolizable energy intake	231^{c}	213^{a}	230^{c}	220^{b}	234^{c}	243^{d}	0.983
Body weight gain	10.65^{a}	13.14^{b}	12.82^{ab}	13.46^{b}	13.2^{b}	12.78^{ab}	0.52
Feed conversion ratio	6.79^{a}	6.03^{b}	6.12^{ab}	5.83^{b}	6.03^{b}	6.35^{ab}	0.17
Protein efficiency ratio	0.901^{a}	1.025^{d}	0.973^{c}	1.098^{e}	1.013^{d}	0.957^{b}	0.004

Means within a raw with different superscript letters are significantly different ($p < 0.05$)

T_1= Maize (34.10%) + Wheat bran and short (21.00%) + Soyabeans, roasted (27.00%) + Noug cake (16.00%) + Limestone (1.20%) + Salt (0.50%) + Vitamin-mineral premix (0.10%) + Lysine (0.05%) + Methionine (0.05%); T_2= Fishmeal (3.32%) + T_1 but with increased wheat bran and short (24.00%) and reduced soybeans (20.88%) and noug cake (15.80%); T_3= Fishmeal (6.64%) + T_1 but with increased wheat bran and short (27.16%) and reduced soybeans (15.20%) and noug cake (15.00%); T_4= Fishmeal (9.96%) + T_1 but with increased wheat bran and short (29.74%) and reduced soybeans (10.30%) and noug cake (14.00%); T_5= Fishmeal (13.28%) + T_1 but with increased wheat bran and short (33.72%) and reduced soybeans (5.00%) and noug cake (12.00%); T_6= Fishmeal (16.60%) + T_1 but with increased wheat bran and short (37.90%) and reduced soybeans (1.50%) and noug cake (8.00%);

It was expected that chicks in T_3 should have had a higher mean DBWG than T_2. Other intrinsic metabolic differences such as the efficiency of nutrient digestion, absorption, metabolism of absorbed nutrients and natural biological variations of chicks might have been responsible for this difference (Donald and William, 2002) and conclusions could not be drawn from this trial alone.

The growth for these chicks peaks at about a daily body weight gain of 15.44 g/head/day and then it shows a tendency to drop after maximizing the corresponding CP intake (Figure 1). Extrapolated from the quadratic equation, the amount of CP required to support the optimal gain of RIR chicks, probably with optimum N-utilization, was 22.27g/head/day, beyond which DBWG starts to decline. The determination of CP level in the diet for optimum efficiency of N utilization during growth is important because of the various nutrients involved in the feeding of livestock, protein being the most expensive (Tegene *et al.*, 2001).

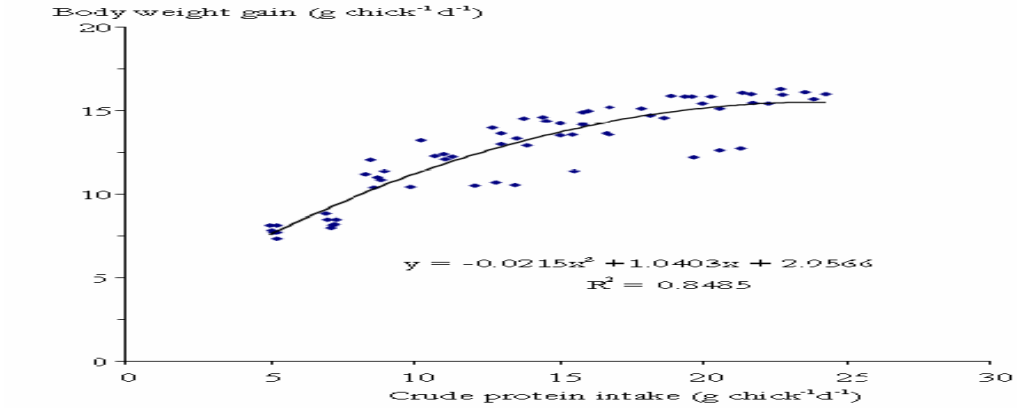

Figure 1. The relationship between crude protein intake and body weight gain (BWG, g/chick/d) of Rhode Island Red chicken fed diets with different levels of fishmeal.

The ME intake, extrapolated from the quadratic equation in Figure 2, that supports the maximum DBWG was found to be about 350 kcal/day, which is higher than the average ME recommended (228.3 kcal/d). This

discrepancy might have perhaps arisen from the low ambient temperature of Wolaita Soddo that could have increased the maintenance requirement of the chicks.

The interaction of age with diet is presented in Figure 3. The mean DBWG of T_4 varied significantly all the way up to 5 weeks of feeding compared to the rest of the diets. From then onwards, T_5 took the leading position up to 8 weeks of feeding and, between 9 and 11 weeks of the feeding period, T_2, T_4 and T_5 performed similarly. The performance on the control diet became significantly low, starting at the 3rd week when the fishmeal groups began to grow faster (commencement of active growth), indicating that better weight gain was supported by protein from fishmeal than the plant protein in the control diet.

The significant ($p<0.001$) improvement in the mean DBWG of fishmeal inclusion was consistent with age. Mean DBWG was significantly different ($p<0.05$) among groups until the chickens were 70 days old (8 weeks after the onset of the trial). Between 77 and 91 days of age (between 9 and 11 weeks after the onset of the trial), the chicken achieved a significantly ($p<0.05$) higher gain than the earlier periods. However, the differences in gain during the last three weeks of the experimental period were not significant ($p>0.05$). This indicates that the chicks' growth rate started to slow down or level off when they were 10 to 12 weeks of age after attaining maximum rate of growth. However, with fast growing broilers, the peak of growth is attained at around 6 or 7 weeks of age (Donald and William, 2002). Similarly, Maigulema and Gernat (2003) reported higher body weights up to 6 weeks of age in broilers. As a dual purpose breed, RIR than broilers chicks manifest their growth potential at later ages, presumably at about 10 weeks.

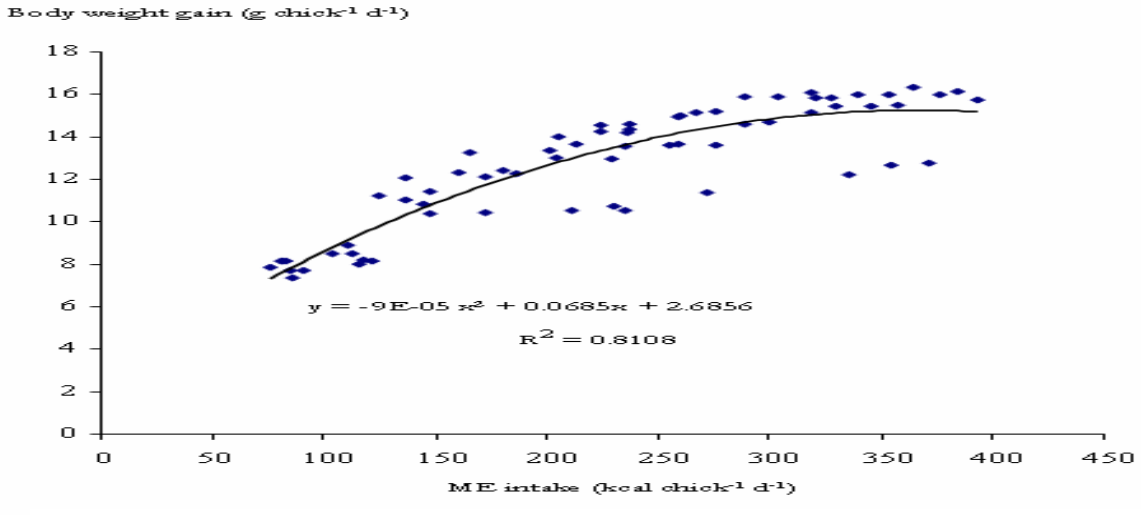

Figure 2. The relationship between ME intake and daily body weight gain of RIR chicks supplemented with different levels of fishmeal.

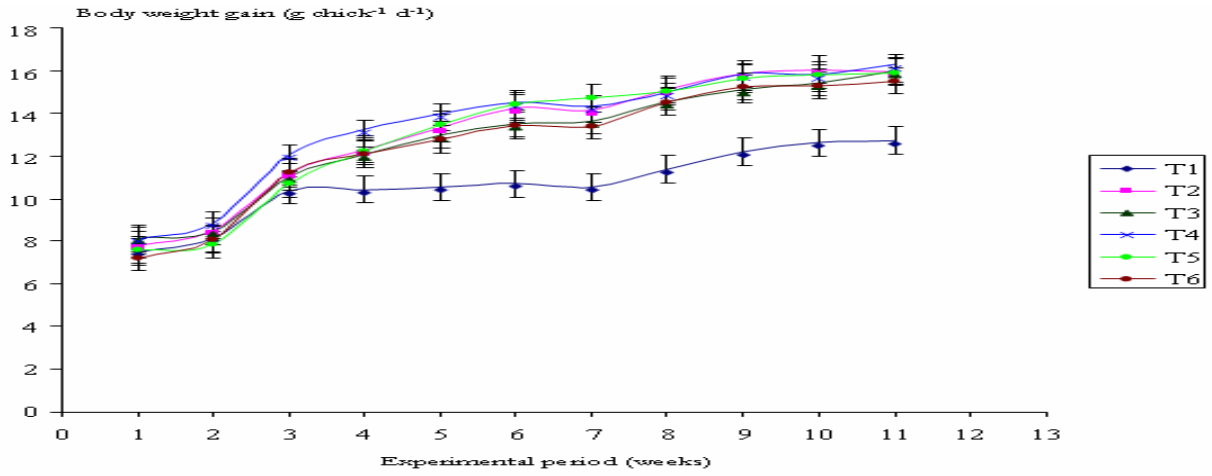

Figure 3. Mean daily weight gain (MDWG, g/chick) of RIR chicken fed diets with different levels of fishmeal. Error bars are at 95 % confidence intervals and means with overlapping error bars are not significantly different.

3. 4. Feed Conversion Ratio

The ANOVA declared that feed conversion ratio (FCR) was significantly ($p<0.01$) improved by fishmeal inclusion (Table 5). The Tukey test indicated the groups fed with rations containing fishmeal (T_2, T_4 and T_5) required a significantly ($p\leq0.05$) lower amount of feed per unit of body weight gain than chicks fed on the control diet (T_1), but T_3 and T_6 were not statistically different in FCR than the control diet. The highest level of fishmeal inclusion (16.6 %, T_6) has shown a depressing effect on the growth of the chicks where a relatively higher amount of feed was required per unit of gain.

Chicks fed T_4 (9.96% fishmeal inclusion level) had higher DWG and were efficient in converting feed to body weight. Faster growth rate is also correlated with feed utilization (Scanes *et al.,* 2004). At inclusion levels of fishmeal beyond 9.96% chicks became less efficient although the mean values were higher than the control diet. Consistently, Karimi (2006), in his work with broilers, observed higher feed intakes rather than an improvement in the feed conversion ratio at higher inclusion levels of fishmeal. It is also in line with the findings of Ponce and Gernat (2002) who reported a significant improvement in the feed conversion ratio due to fishmeal supplementation.

The effect of age on the overall mean feed conversion ratios (MFCR) is shown in Figure 4. The overall MFCR of treatment diets were significantly different ($p<0.05$). The weekly MFCR were significantly different among treatments ($p<0.05$) throughout the experimental period. A drop in the feed conversion ratio observed in the 3rd week may be inconsequential. It indicates that the FCR of the chicken was higher when they were younger. Young animals are expected to be more efficient in converting feed to body mass than older animals that have a higher maintenance requirement (Donald and William, 2002).

3. 5. Protein Efficiency Ratio

The protein efficiency ratio (PER) of the treatment diets, calculated as the ratio of weight gained over protein intake, is indicated in Table 4. Chicks fed T_4 were the most efficient ($p<0.001$) of all the treatment groups. The protein intakes of T_5 and T_6 were relatively higher than T_4, but they were not as efficient as T_4. The control group had the poorest protein efficiency, presumably because the protein sources were of plant origin. Surprisingly, the protein intake of the T_4 was comparable to that of the control diet but the protein efficiency ratio was much higher, partly because fishmeal inclusion was not high and, probably, also due to the balanced amino acids profile from plant and animal protein sources. Limiting inclusion of fishmeal has been recommended by Ponce and Gernat (2002), Maigulema and Gernat (2003) and Karimi (2006) as it reduces growth performance, which agrees with the results of this study.

3. 6. Mortality

In the present study mortality was not observed by fishmeal inclusion throughout the experimental period which disagrees with the finding of Ponce and Gernat (2002), and Maigualema and Gernat (2003) who observed 1.74 to 2.43% and 5.35% mortality, respectively, in broilers when soybean meal was replaced with tilapia byproduct meal. In line with the present work, Babu *et al.* (2005) observed no impact on the health of hens fed fishmeal at a 6% inclusion rate.

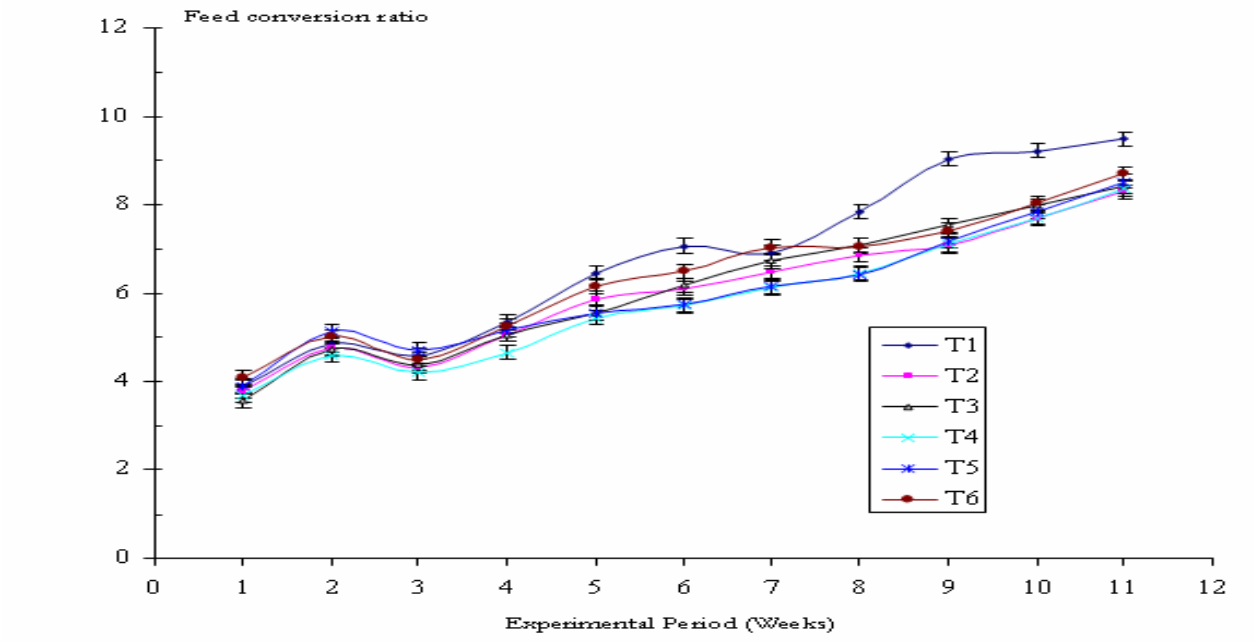

Figure 4. Weekly mean feed conversion ratio of RIR chicks fed diets with different levels of fish meal. Error bar refers to 95% confidence interval and means with overlapping error bars are not significantly different from each other.

4. Conclusion

The study pointed out that cooked and sun dried fish offal, fishmeal, can be incorporated into up to 16.6% of the diets of growing RIR chicken without affecting feed intake, growth and health. However, best results were obtained at a 9.96% inclusion level. Thus, fishmeal is a satisfactory and cheap animal protein that can partly replace expensive plant protein sources such as soybean and oil seed cakes.

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The Potential of Cowpea, Pigeonpea and Greengram to Supply Mineral Nitrogen to Maize under Intercropping on Ferralsol in Muheza-Tanga Tanzania

Marandu A.E.T [1]*, J.P. Mrema[2], E. Semu[2] and A.S. Nyaki[1]

[1]Mlingano Agricultural Research Institute, P O Bx 5088, Tanga -Tanzania
[2]Sokoine University of Agriculture P O Box 3008, Morogoro-Tanzania

Abstract: An incubation experiment was carried out at Mlingano Agricultural Research Institute, Tanga-Tanzania, to study the nitrogen mineralization in the Ferralsol under maize- cowpea, pigeonpea and greengram intercropping systems. Top soil (0 – 20 cm) samples were collected from previously maize intercropped and monocropped plots before the onset of rains in subsequent seasons. The fresh soil samples were sieved through a 6 mm screen, 250 g portions placed in 500 ml capacity volumetric flasks, then incubated at 60% field capacity for 42 days in a glasshouse. Destructive samplings were done at 14 day intervals and analysed for mineral N. The N mineralization increased with incubation time, with cowpea and pigeonpea having significantly higher quantities at the 42nd day sampling compared to the maize monocropped plots. The proportions of the mineral N at 14th day where maize stover was removed were 77%, 55%, 92% and 90% of that at the 42nd day for soil from the cowpea, pigeonpea, greengram and monocropped maize treatments, respectively. The respective proportions where the stover was incorporated were 81%, 53%, 96% and 77%. Such high proportions coupled with low maize N demand indicated possibilities of the N being leached, leading to maize plants' N deficiency symptoms. It was concluded that the N mineralization in this cropping system is not in synchrony with the maize N demand, necessitating top dressing of N fertilizers.

Keywords: Ferralsol; Incubation; Intercropping; Mineral N

1. Introduction

Nitrogen is one of the major elements required by plants for growth and production. The nutrient is depleted in most soils in Tanzania with a negative balance of 27 kg ha^{-1} $year^{-1}$ (Stoorvogel *et al.*, 1993). Sustainable land productivity efforts, therefore, need to be in place to address the trend of decreasing soil N levels. The growing of leguminous crops which fix atmospheric N_2, either solely or in association with cereal crops, contributes to the efforts to alleviate this problem of low N levels in soils. The legumes in such cropping systems contribute to the soil N through the decomposition of above and below ground residues, part of the N being derived from N_2 fixation. Maize legume intercropping is one of such associations widely practised by small-scale farmers in different parts of the world. Improvements in maize grain yields resulting from enrichment of soil N by the legumes have been reported in different places (Adetunji, 1996; Bloem and Barnard, 2000; Jeranyama *et al.*, 2000).

The enrichment of the soil N by the legumes residues is realized after the decomposition process, whereby the organically bound N undergoes an ammonification process leading to production of ammonium (NH_4^+) which is a relatively immobile form of N (Roy and Singh, 1995; Brady and Weil, 2000). The NH_4^+, under aerobic soil conditions, is subject to nitrification leading to formation of nitrate (NO_3^-), which is the relatively mobile form (Wong and Nortcliff, 1995; Mekonnen *et al.*, 1997; Purnomo et al., 2000). The NO_3^- has great potential for loss from the soil through denitrification under anaerobic soil condition and leaching (Hagedorn *et al.*, 1997). The concentration of NO_3^- has been reported to be high in the upper 0-15 cm soil layer soon after the start of rainfall and lost through denitrification within a week when soil water content was more than 10% (Weber *et al.*, 1995; space Warren et al., 1997). Purnomo *et al.* (2000), in a 16 weeks' incubation of soil sampled from 0 - 20 cm, observed that mineral N increased with incubation time, and was highly correlated with concentrations of organic C and total N. The availability of soil N to crop plants has also been reported to be related or not related to the pre-season mineral N. Barrios *et al.* (1998) for example, observed that pre-season inorganic N (NO_3^- and NH_4^+) correlated more with maize grain yield than pre-season NO_3^- alone whereas Stephens (1967) and Weber et al. (1995) found that soil NO_3^- was highly correlated with maize yields one month after planting.

Most farmers in Muheza district grow maize intercropped with cowpea, pigeonpea or greengram. The legume residues are usually left to decompose in the fields after grain harvest whereas the maize stover is sometimes harvested for livestock feeding. Although the legumes fix atmospheric N_2 with the native rhizobia of the soils (Marandu, 2005), thereby improving the soil N levels for the companion maize crop, maize grain yields are often as low as 1.3 t ha^{-1} (Ministry of Agriculture and Cooperatives, 1998), and, in some fields, N deficiency symptoms are common. Such situations indicate indicate an inadequate supply of N to maize in this cropping system. To get an insight into the contribution of the legumes to the N nutrition of the maize, nitrogen mineralization patterns of incubated soil sampled before the onset of rains in subsequent season from the field previously under maize legumes intercropping was carried out. The preseason mineral N was also determined from the soil sampled in the field at the time of maize planting.

2. Materials and Methods

This research was carried out at Mlingano Agricultural Research Institute in Muheza district, Tanga, Tanzania located at 39° 52'E and 5° 10'S and at an altitude of 183 m.a.s.l.. The site is characterized by a bimodal rainfall pattern with long and short rainy seasons. The long rains are more reliable in distribution and onset, and fall between March and June, and the short rains fall between October and December.

A field experiment of maize - cowpea, maize - pigeonpea and maize - greengram intercropping was initiated in 2003 on a Rhodic Ferralsol during the long rains. The experiment comprised eight treatments

*Corresponding author. E-mail: atanasiom@yahoo.co.uk

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replicated four times. The treatments included maize intercropped with cowpea, pigeonpea, greengram and monocropped maize with maize stover removed or left on the plots. The cowpea and greengram varieties were determinate types which take 65 days to mature whereas the pigeonpea was determinate and of short duration which matures at 120 days. The maize variety was TMV-1 which takes 120 days to mature. Nitrogen fertilizer was not applied to either maize or legumes, but P fertilizer, in the form of TSP, was applied to maize and legumes at the rate of 30 kg P ha^{-1}.

In 2004, season soils for the incubation study were sampled from the treatment plots from 0 - 20 cm before the onset of rains. The samples were randomly collected at four positions in a plot and mixed to constitute one sample per treatment. The fresh soil samples were passed through a 6 mm sieve, and an amount equivalent to 250g oven dry basis (in triplicates) was placed in wide mouth 500 ml volumetric flasks, then incubated for 42 days at 60% field capacity in a glasshouse. The incubation was at room temperature which ranged between 30.3 to 20.0 ^{o}C. The moisture content was maintained at this level by the addition of distilled water as necessary. Destructive sampling was done at 14 day intervals and analyzed for mineral N (NH_4^+- N and NO_3^-- N). After the onset of the rains (i.e at maize planting time in the field), soil was also sampled randomly from the treatment plots from 0-20 cm using an auger for pre-season mineral N determination. The determination was by steam distillation as outlined by Okalebo *et al*., (1993).

Before the layout of the field experiment, site characterization was done on a composite soil sample collected at 0 -20 cm, air dried and sieved through a 2 mm screen before being analysed for physical and chemical characteristics using internationally accepted procedures.

3. Results and Discussion

The chemical and physical characteristics of the field experimental soil are presented in Table 1. The texture of the soil at the study area is clay. According to Landon (1991), the experimental soils' reaction was medium acid, which is suitable for most annual crops. The total N was low indicating a need for external N input for high maize yields. The organic carbon was very low, whereas the C: N ratio indicated the presence of a good quality soil organic matter. The site had low available P and exchangeable Ca. The exchangeable K and Mg were low and high, respectively. The CEC was low, whereas the base saturation was high.

Table 1. Physical and chemical characteristics of the soil from experimental field.

Texture (%)			pH H_20	O. C (%)	Total (%)	C:N	Bray 1 $mgkg^{-1}$	Exchangeable bases (cmol (+) kg^{-1})					
sand	silt	clay			N		P	Ca	Mg	K	Na	CEC	BS (%)
42	6	52	6.0	1.59	0.12	13	7	4.2	2.4	0.33	0.09	10.34	68

As far as N mineralization is concerned, the soil reaction of the study site is favourable for microorganisms involved in the decomposition process of organic material to release NH_4^+, and also for the nitrification of the ammonium to NO_3^-. A great loss of this form of N through ammonia volatilization is not likely in a such clay-textured soil with the pH below 7 (Harris,1988; Brady and Weil, 2000). The availability of the mineralized N to crops at the site, therefore, will depend on the moisture status of the soil. Continuous heavy rains for example, may cause leaching of the NO_3^- beyond the plant root zone or cause anaerobic soil condition which favours the denitrification process, leading to the loss of N in gaseous forms.

3.1. Incubation Experiment

The cumulative quantities of total mineral N (NH_4^+ + NO_3^-) during incubation of soils sampled before maize planting are presented in Table 2.

The quantities of mineral N at the start of incubation (0 day) ranged from 7.7 mg kg^{-1} (maize - pigeonpea with stover removed) to 23.9 mg kg^{-1} (maize - greengram with stover left on the plots). The cumulative quantities of mineral N obtained at 14, 28 and 42 day intervals increased with incubation time. The highest increase was obtained between 0 and 14 days of incubation. In treatments where the stover was removed, out of the total N mineralized during the entire period of incubation, 77, 55, 92 and 90% had been mineralized on the 14th day for soils from cowpea, pigeonpea or greengram intercrop and that from monocropped maize plots, respectively. For the soil from treatment where the maize stover was left on the plots, the respective proportions were 81, 53, 96 and 77%. At all sampling intervals, the quantities of cumulative mineral N for the soil from treatments where the stover was removed were numerically lower than where the stover was left on the plots, indicating the N contribution of the stover.

At 14 days, in the treatments where the maize stover was removed, the quantities of cumulative mineral N for soils from the cowpea, pigeonpea or greengram intercrop and that from the monocropped maize plots were not statistically different. In treatments where the maize stover was left on the plots, the quantity of cumulative mineral N for the soil from monocropped maize plots was statistically similar to those from cowpea and pigeonpea plots, but significantly lower ($P < 0.01$) than that from the greengam intercropped plots. At 28th day sampling, the quantities of cumulative mineral N from the plots in the treatments where the maize stover was removed or those under the stover left on the plots were not statistically different ($P < 0.05$).

At the end of incubation, in the maize stover removed treatment, the soil from the pigeonpea intercropped with maize had significantly higher mineral N ($P < 0.001$) than that of soil from the cowpea or greengram intercrop, or monocropped maize. The quantity of cumulative mineral N under the cowpea intercrop was significantly higher ($P < 0.001$) than that under the maize monocrop. There was no significant difference ($P < 0.05$) in the quantities of cumulative mineral N between the soil from the greengram intercrop and maize monocropped plots.

In the treatment under maize stover left on the plots, the soil from maize pigeonpea intercrop had significantly higher mineral N ($P < 0.001$) than that of the soil from the cowpea or greengram intercrop and that from monocropped maize. The quantities of mineral N under the cowpea or greengram intercrop were statistically similar to that of monocropped maize.

The higher mineral N determined during incubation than at the time of soil sampling in the field (0 day) could be attributed to an increased microbial decomposition of crop residues and/or native soil organic matter as a result of increased soil moisture content during incubation. Such effects of soil moisture on decomposition were also reported by Andren *et al.* (1993) and Schomberg *et al.* (1994), who observed that more N immobilization in buried rather than surface applied residues attributed to increased microbial activity due to more soil moisture for the buried residues.

The observed increased cumulative mineral N with incubation time shows the potential of the soils to supply mineral N to the crop grown in the field. The trend of higher mineral N for the soils from intercropped legumes compared to that from monocropped maize for both maize stover removed and left on the plots (although not statistically different), indicates that the legumes had a positive effect in increasing mineral N in the soil. During legumes' harvesting in 2003, the average quantities of above ground residues left on the plots were 1.2, 1.0 and 0.9 t ha^{-1} for the cowpea, pigeonpea and greengram, respectively (Table 3). The above and below ground residues could explain the observed higher mineral N above monocropped maize. On the other hand, the contribution of the maize stover to the soil N is indicated by the higher mineral N for the soils from plots in the treatments where the stover was left on the plots than those with the maize stover removed. In the 2003 season, the quantities of N removed through the above ground stover ranged from 13.5 to 18.8 kg N ha^{-1}, whereas those added to the soil by leaving the above ground stover ranged from 14.7 to 23 kg N ha^{-1} (Table 3). The highest proportions of mineral N, as determined at 14th day sampling, imply that a maize crop grown in the field would get maximum N at the early stages of crop growth. The equivalent quantities of mineral N per hectare at this time of sampling were 82, 75, 85 and 68 kg N in the soils from the cowpea, pigeonpea or greengram maize intercrop and those from the monocropped maize plots with the stover removed, respectively. In treatments with the maize stover left on the plots, the quantities were 89, 79, 97 and 74 kg N for soils from the cowpea, pigeonpea or greengram intercrop and monocropped maize plots, respectively.

Table 2. Cumulative mineral N (mg kg^{-1}) of incubated soils from different intercropping systems.

Treatment	Sampling interval (days)			
	0	14	28	42
Maize + cowpea – stover	13.1	37.2 abc	41.7 abc	48.2 b
Maize + pigeonpea – stover	7.7	34.2 bc	38.6 bc	61.8 a
Maize + greengram – stover	16.1	38.8 abc	39.1 bc	42.2 bc
Maize monocrop – stover	18.0	30.7 c	34.3 c	34.1c
Maize + cowpea + stover	13.9	40.3 ab	47.0 a	50.0 b
Maize + pigeonpea + stover	19.2	35.8 abc	43.8 ab	68.1 a
Maize + greengram + stover	23.9	44.3 a	45.7 ab	46.0 b
Maize monocrop + stover	15.1	33.7 bc	39.8 abc	43.7bc
F- test	Ns	**	**	***
C V%		8.7	6.8	8.0

Ns = not statistically different

***, *** = Statistically significant at P= 0.01 and 0.001, respectively*

Means followed by the same letter within the column are not significantly different according to the Duncan's New Multiple Range Test

Table 3. Quantities of legume residues and the N added or removed by maize stover in 2003 season.

Treatment	Legume Residue (kg ha^{-1})	N added (kg ha^{-1})	Maize Stover (kg ha^{-1})	N removed/added (kg ha^{-1})
Maize Cowpea / minus stover	1421	24.7	1898	- 13.5
Maize / P pigeonpea minus stover	998	20.6	2649	-18.8
Maize /Greengram/ minus stover	958	11.7	1927	-13.7
Maize minus stover	na	na	2312	-16.4
Maize / Cowpea plus sover	1033	18.0	2553	+18.1
Maize / Pigeonpea plus stover	1048	21.6	2601	+ 18.5
Maize /Greengam plus stover	890	10.9	2071	+ 14.7
Maize plus stover	na	na	3237	+ 23

na : Not applicable

Considering such high levels of N two weeks after maize planting, maize plants will not be able to utilize much of the N at a growth stage of low N demand. Therefore, there is a high possibility that the heavy rains at this stage of maize establishment would subject the mineral N to leaching and/or erosion losses.

This incubation study shows that intercropping of maize with the legumes contributed to the potential of the soils to supply N to a subsequent maize crop. Corresponding amounts per hectare of the cumulative mineral N obtained at the end of the 42nd day of incubation are presented in Table 4. Based on the data in Table 4, the quantities of mineral N per hectare that

could be attributed to the effects of the three legumes under an intercropping system were of the order greengram < cowpea < pigeonpea in the treatments where maize stover was removed or where it was left on the plots.

Except for the maize pigeonpea intercrop, the potential of the legumes under intercropping to supply N to the subsequent maize crop was lower than the 50 kg N ha^{-1}, which is the recommended N level for maize production under a monocropping system at the site. Even with such low potential, much of the N (including that under pigeonpea intercrop) would be released within the first two weeks of planting when the maize plant requirement for N is low, with possibilities of some of the N getting lost through leaching. This could account for the N deficiency symptoms encountered in maize intercropped with the legumes in farmers' fields around the study area.

3.2. Preseason Mineral N

The quantities of preseason mineral N ($NH_4^+ + NO_3^-$) are presented in Table 5.

Generally, NO_3^- was the main form of mineral N in all treatments. The moist condition of the soils at sampling time seemed to have favored the biological processes in the soil including nitrification, leading to the conversion of NH_4^+ to NO_3^-. The mineral N could be attributed to mineralization of native soil organic matter, legumes residues from the 2003 season and/or maize stover in the treatments where stover was left on the plots (Table 3). The increased soil moisture, which resulted from the rains received at the beginning of the season, increased the soil microbial activities resulting in N- flush caused by a rapid decomposition of the residues and mineralization of N from the native soil organic matter (Birch, 1958; Wong and Nortcliff, 1995; Hagedorn *et al.*, 1997).

The mineral N trends, as in the case of incubation, were also higher in plots where the maize stover was left on the plots rather than where the stover was removed, indicating the contribution of the maize stover to the soil N. Although the maize stover was of low quality, implying the possibility of initially immobilizing mineral N, the eight months between maize harvesting and the time the soil was sampled was long enough for the immobilization to be overcome.

The total mineral N in all treatments at this time of sampling, when converted on a hectare basis, is above the 50 kg N ha^{-1} recommended for maize production at the site. Such high concentration of mineral N at maize planting has agronomic implications. The NO_3^-, which was the main form of the mineral N, is easily leached to the subsoil layers by heavy rains at this stage of maize crop development. For this cropping system, to take maximum advantage of the potential to supply mineral N, early planting is necessary.

Table 4. Cumulative mineral N at 42nd day of incubation, net mineral N and corresponding quantities per hectare.

Treatment	Mineral N (mg kg^{-1})	Net N[a] (mg kg^{-1})	N equivalent [b] (kg ha^{-1})
Maize + cowpea minus stover	48.2	14.1	31.0
Maize + pigeonpea minus stover	61.8	27.7	60.9
Maize + greengram minus stover	42.2	8.1	17.8
Maize monocrop minus stover (control)	34.1	Rd	na
Maize + cowpea plus stover	50.0	15.9	35.0
Maize + pigeonpea plus stover	68.1	34.0	74.8
Maize + greengram plus stover	46.0	11.9	26.2
Maize monocrop plus stover	43.7	9.6	21.1

a : Net N is the difference between mineral N of intercropped treatments and monocropped maize with that of monocropped maize with stover removed (control)
b : Equivalent N ha^{-1} was obtained by converting the net N on hectare basis (i.e. 2.2 x 10^6 kg of furrow slice soil)
Rd = Data for mineral N was a reference
na = not applicable

Table 5. Quantities of mineral N, NH_4^+ and NO_3^- at the time of maize planting.

Treatment	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	$NH_4^+ + NO_3^-$ (mg kg^{-1})	N equivalent[a] (kg ha^{-1})
Maize/cowpea minus stover	7.4	18.5	25.9c	57
Maize/cowpea plus stover	11.5	24.2	35.3b	78
Maize/ pigeonpea minus stover	12.4	23.4	35.8ab	79
Maize /pigeonpea plus stover	13.6	25.8	39.4a	87
Maize / greengram minus stover	10.2	18.4	28.5c	63
Maize/ greengram plus stover	13.2	20.0	33.2b	73
Maize minus stover	8.6	16.7	25.3c	56
Maize plus stover	11.3	22.7	34.0b	75
F- test			***	
CV%			6.7	

a: Equivalent N ha^{-1} was obtained by converting the net N on hectare using the 2.2 x 10^6 kg of furrow slice soil
**** : Statistically significant at P = 0.001*
Means followed by the same letter within the column are not significantly different according to the Duncan's New Multiple Range Test

4. Conclusion

Low maize yields experienced by farmers in the study area are more heavily influenced by a lack of synchronization between the mineralization of N from crop residues and /or soil organic matter and maize N demand. Most of the mineral N is released when the maize crop is at a stage of low N demand, leading to the possibility of losses through leaching. It is suggested that, to improve the

levels of maize production in this cropping system, possibilities of supplementing the maize a with top dressing of N fertilizer should be considered.

5. Acknowledgements

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The Effect of Removal of Buds and Younger Leaves on Growth, Tuber Yield and Quality of Potato (*Solanum tuberosum* L.) Grown Under Hot Tropical Lowland

Bizuayehu Desta[1] and Tekalign Tsegaw[2]*

[1]Haramaya University, School of Graduate Studies, Department of Plant Sciences, P O Box 138, Dire Dawa, Ethiopia
[2]Haramaya University, Department of Plant Sciences, P O Box 150, Ethiopia

Abstract: A study was conducted to determine the effects of the removal of buds and younger leaves on the growth, yield and quality of potato grown in the hot tropical lowlands of Humbo, southern Ethiopia using two potato cultivars in 2006. Five pruning treatments viz. normal growing, removal of only terminal buds, removal of terminal buds along with associated younger leaves, removal of terminal buds and axillary buds, and removal of terminal buds and axillary buds along with associated younger leaves were applied to the two potato cultivars, Wochecha and Tolcha. A split plot design with three replications was employed and cultivars were assigned to the main plots while the pruning treatments were applied to the subplots. Although Wochecha cultivar developed tuber initials 1.28 days earlier than Tolcha, non-significant differences were observed between them with regard to plant height, total leaf area, above- and underground biomass yield, physiological maturity, tuber number and yield, mean tuber mass, tuber specific gravity and dry matter content. The removal of terminal and axillary buds as well as nipping off terminal buds, axillary buds and associated younger leaves decreased total leaf area, above and under-ground dry mass, delayed days to physiological maturity by about 8 days, increased tuber number by about 29%, tuber yield by 63.5%, average tuber mass by 26.5%, specific gravity by 1.56%, and dry matter content by about 17.5% the compared to the check. The results clearly indicated that removal of buds and younger leaves can improve tuber yield and the quality of potato grown under high temperatures. The results are of paramount importance to increase the productivity of potato in the hot lowland tropics where high temperatures are limiting factors in its successful production.

Keywords: Axillary Bud; Debudding; Lateral Bud; Potato; Tropical Lowland

1. Introduction

Potato prefers cool weather and temperatures between 16-25^{o}C favor foliage growths, net photosynthesis, and tuberization. Although potato is a remarkably adaptable crop, its expansion has been restricted by high temperatures in some regions of the world (Levy, 1986). For instance, in Ethiopia, about 35% of the available agricultural land is situated in the semi-arid regions of the country, where potato cultivation is not being practised due to unfavorably high temperatures throughout the year. High temperatures cause yield and quality reduction and are considered the major constraints for potato production in the tropics.

Tuberization in potato is influenced by environmental factors, primarily temperature and photoperiod (Gregory, 1956; Salter, 1968). Low mean temperatures (15-19^{o}C) under a short photoperiod (12 hr) are most suitable for early tuber growth (Vandam *et al*., 1996). Under such conditions, the onset of growth and bulking are early, and absolute tuber growth rates and dry matter partitioning rates are high. High temperatures promote foliage growth, decrease assimilate partitioning to the tubers, delay the onset of tuber initiation and bulking, decrease absolute growth rate and net photosynthesis while increasing dark respiration (Menzel, 1980; Hammes and De Jager, 1990; Vandam *et al*., 1996). Some cultivars are more sensitive to high temperatures than others (Manrique, 1989; Reynolds and Ewing, 1989). Nevertheless, it seems safe to say that, for all genotypes, high temperatures, such as long photoperiods, decrease the partitioning of assimilate to tubers and increase partitioning to other parts of the plant (Wolf *et al*., 1990).

The inhibition of tuberization by high-temperature is believed to be mediated through the production of high levels of endogenous gibberellins (Menzel, 1983) that are known to delay or inhibit tuberization (Vreugdenhil and Sergeeva, 1999). The hormonal balance controlling potato tuberization can by altered by using anti gibberellin chemicals such as chloroethyl trimethyl ammonium chloride (CCC) (Menzel, 1980) and paclobutrazol (Simko, 1994; Tekalign and Hammes, 2005a). An alternative approach to the control of tuberization involves the manipulation of endogenous phytohormone levels by pruning.

Vegetative buds and younger leaves are important sites of gibberellin synthesis (Jones and Phillips, 1966), and their removal can substantially modify the phytohormone levels in the plant. This was observed in potato in which tuberization was promoted under non-inductive long days by the removal of buds (Hammes and Beyers, 1973). Menzel (1981) studied the effect of pruning on potato grown under high temperatures and found an increment in tuber weight per plant a decrease in haulm weight per plant due to a substantial diversion of dry matter to the tubers. Das Gupta (1972) showed that decapitation resulted in an increase in the growth rate of the storage root in *Beta vulgaris* and significantly increased the root/shoot ratio.

Many researchers have studied the effect of high temperature on growth, tuber yield, and quality of potato

*Corresponding author. E-mail: tekaligntsegaw@yahoo.com

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(Menzel, 1980; Steward *et al*., 1981; Reynolds and Ewing, 1989). However, very little work has been done regarding the effect of disbudding on the growth and productivity of potato under hot tropical conditions where unfavorably high temperatures limit its successful production.

It has been suggested that the removal of buds and associated younger leaves could increase the tuber yield and quality of potato grown under high temperatures. Therefore, this study was initiated to investigate the effect of the removal of terminal buds, axillary buds and younger leaves on the growth, tuber yield and quality of potato grown in the hot tropical conditions of Humbo, Southern Ethiopia. The ultimate objective of the study was to generate information to improve potato production in marginal areas where high temperatures are limiting factors.

2. Materials and Methods

2.1. Description of the Study Site

The experiment was conducted at Humbo, Wolaita Zone of Southern Ethiopia in 2006. The experimental site is located at 6° 34' N, 37° 43' E and at an elevation of 1320 m.a.s.l. It is situated about 35 km away from Wolayta town very close to the main road to Arbaminch.

The area is a typical tropical lowland with mean minimum and maximum day air temperatures of 28.4°C and 34.4°C, respectively. It is characterized by a long dry season from October to February. The area has a bimodal rainfall distribution with low rainfall from February to April and the main rainfall from July to September. It receives a mean annual rainfall of 948 mm. During the study period, the mean monthly minimum and maximum day/night temperatures were 28.1/21.7and 32.4/26.7°C, respectively.

2.2. Experimental Materials and Treatments

Wochecha and Tolcha, both medium maturing cultivars, were used for the study. The following pruning treatments were applied.

Treatment 1: Normal growing plants (Control).
Treatment 2: Removal of terminal buds.
Treatment 3: Removal of terminal buds and associated younger leaves (<4 cm long).
Treatment 4: Removal of terminal and axillary buds.
Treatment 5: Removal of terminal and axillary buds with associated younger leaves (<4 cm long)

2.3. Experimental Design

The experimental plots were arranged in a split-plot design with three replications. The two cultivars were assigned to the main plots and the pruning treatments to the sub-plots (size=11.025 m^2). Sub-plots within the main plots were arranged continuously without bordered rows, and the end plots were bordered by two rows of potato plants.

Well-sprouted medium sized tubers from each cultivar were planted on February 02, 2006 at a spacing of 75 cm between rows and 30 cm between plants. Phosphorus was applied as DAP at a rate of 90 kg P_2O_5/ha and nitrogen was side dressed at a rate of 76 kg N/ha in the form of urea. The whole rate of phosphorus was applied during planting. Half rate of nitrogen fertilizer was applied after full emergence and the remaining half was applied as a two side dressing at 50% flowering stage. Plots were irrigated regularly to maintain adequate moisture in the soil. Other cultural practices were carried out as per the recommendation. No major disease or pest incidences were encountered.

2.4. Data Collected

Days to tuber initiation were recorded when the stolon tip attained a size at least twice the diameter of the stolon (Ewing and Struik, 1992). Plant height was measured from the base of the stem to shoot apex at maturity. A month after the last treatment application, the total leaf area was estimated from the individual leaf length using the formula developed by Firman and Allen (1989). Six weeks after flowering, while the vines were green but had practically ceased growth (CIP,1983), five randomly selected plants were harvested and dried in an oven at 72°C to a constant mass to determine aboveground (stem, branch, and leaves) and underground (root, stolon, and parts of the stem remaining under-ground) dry mass. Days to physiological maturity were recorded when the haulms of 50% of the plants in each plot turned yellowish. Tuber fresh mass and tuber numbers represent the average of 15 plants per treatment. Average tuber mass was determined by dividing the total fresh tuber yield to the respective total number of tubers. Tuber specific gravity was determined using weight-in-air and weight-in-water method (Murphy and Goven, 1959). For tuber dry matter content, tubers were oven dried at a temperature of 72 ºC to a constant mass. Tuber dry matter content was determined as the ratio between dry and fresh mass expressed as a percentage.

2.5. Statistical Analysis

All data were subjected to analyses of variance, using MSTAT-C statistical software (MSTAT-C 1991). Means were compared using least significant differences (LSD) test at the 5% probability level. Correlations between parameters were computed when applicable.

3. Result and Discussion

3.1. Days to Tuber Initiation

Significant variation in days to tuber initiation was exhibited between cultivars and among treatments (Table 1). Wochecha cultivar developed tuber initials 1.3 days earlier than Tolcha which could be due to genotypic difference with respect to the rate of net photosynthesis and the efficiency of assimilate partitioning to the tubers. Nagarajan and Bansal (1990) reported genotypic differences regarding the onset of tuber growth at high temperatures. Similarly, Prange *et al.* (1990) reported that leaf net photosynthesis and dry matter partitioning are influenced by the growing temperature, harvest date and cultivars.

Compared to the control, the removal of terminal and axillary buds, and terminal and axillary buds along with younger associated leaves shortened days to tuber initiation by about a week. Similarly, the removal of terminal buds along with younger leaves reduced the days required for tuber initiation by about two days. Pruning

significantly reduced days to tuber initiation and this could be due to reduced GA synthesis which, in turn, reduced top growth and promoted early tuberization. High levels of endogenous GA promote shoot growth (Menzel, 1980) and delay or inhibit tuberization (Abdella *et al*., 1995; Vandam *et al*., 1996). The delaying or inhibiting effect of GA on tuberization may be partly attributed to its effect on carbohydrate metabolism especially sucrose utilization (Jackson, 1999). The involvement of GA in regulating the pattern of assimilate partitioning was suggested by Yim *et al*. (1997) who noted that high GA activity leads to higher carbohydrate allocation to the shoots, while low GA level results in more dry matter allocation to the roots. Application of Paclobutrazol, GA-biosynthesis inhibitors, significantly reduced top growth and promoted early tuberization in potato grown under hot tropical condition (Tekalign and Hammes, 2005a).

3.2. Plant Height

Non-significant difference was observed between the cultivars with regard to plant height (Table 1). However, the pruning treatments significantly reduced plant height compared to the normal growing plants. This is attributed to the direct effect of removal of the growing tip and reduction in stem elongation and increased partitioning of assimilates to the tubers due to reduced GA activity in response to the treatments. Menzel (1980) and Mares *et al*. (1981) reported that the exogenous application of GA_3 inhibited tuber formation, decreased tuber sink strength and encouraged stem extension. Menzel (1980) and Tekalign and Hammes (2005a) reported that the application of GA biosynthesis inhibitors favored the partitioning of more assimilates to tubers by reducing stem extension. Similarly, Benoit *et al*. (1983) reported that treated plants appeared to be short because of the reduction in stem elongation and increased partitioning of assimilates to the tubers.

Table 1. Days to tuber initiation, plant height, total leaf area, biomass yield, and days to physiological maturity of potato as influenced by cultivars and pruning treatments.

Main effect	Days to tuber initiation	Plant height (cm)	Total leaf area (cm^2)	Biomass (g)		Days to physiological maturity
				Above ground	Under ground	
Cultivar						
Wochecha	53.24 b	42.50 a	2985.60a	65.92 a	9.45 a	96.97 a
Tolcha	54.52 a	40.46 a	2940.10a	58.91 a	8.05 a	99.70 a
Treatment						
Normal growing plants (control)	57.41 a	58.11 a	3742.10b	65.67 a	9.28 a	95.83 b
Terminal buds removed	56.04 ab	38.68 b	4463.10a	67.60 a	9.51 a	96.16 b
Terminal buds and younger leaves removed	55.96 b	37.54 bc	4243.10a	67.01 a	9.41 a	97.50 b
Terminal and axillary buds removed	50.32 c	36.75 bc	3114.70c	56.22 b	7.81 b	103.83 a
Terminal buds, axillary buds and younger leaves removed	49.64 c	36.39 c	2962.90c	55.51 b	7.72 b	104.33 a
CV (%)	2.16	4.05	4.68	11.31	11.74	1.52

Means of the same main effect in the same column followed by the same letters are not significantly different at 5% probability level.

3.3. Total Leaf Area

The two cultivars produced comparable total leaf area and significant variation in total leaf area per plant was exhibited among the bud and leaf removal treatments (Table 1). Removal of terminal buds and terminal buds along with associated younger leaves encouraged the production of higher total leaf area than the control as well as the other two treatments. This could attributed to the observed high number of lateral branches and expanded leaves in response to pinching of the terminal buds. Shoot apex meristem maintains its role as the primary site of growth by inhibiting the growth of axillary meristem through the phenomenon called apical dominance and its effect is mediated by auxin levels (Chatfield *et al*., 2000). Removing terminal buds stimulated the growth of lateral branches along with the expanded leaves in potato (Tsegaw and Zelleke, 2002; Tekalign and Hammes, 2005b).

The removal of terminal and axillary buds, and terminal and axillary buds along with younger leaves gave the lowest total leaf area, resulting in short and compact plants. This response is attributed to the reduction in total leaf area and restriction in stem elongation. Menzel (1981) reported that the most noticeable potato growth response to pruning treatment was the reduction in shoot growth. Higher leaf area is essential for higher biomass and tuber yield; however, in this study, it has been observed that the treated plants exhibited a higher tuber yield despite the reduced leaf area. This compensation could be due partly to an enhanced net assimilation rate in response to the treatment.

3.4. Biomass Yield

The two cultivars produced comparable above and underground biomass yield (Table 1). However, a significant variation in above and under ground dry mass was observed among the treatments. The removal of terminal buds, and terminal buds and associated younger leaves significantly increased above ground biomass yield, compared to pruning of terminal and axillary buds, and terminal and axillary buds along with associated younger leaves. This could be due to the production of more lateral branches and expanded leaves in response to the removal of terminal buds. Salisbury and Ross (1992) indicated the existence of apical dominance in the stems of most plant species and the removal of the terminal buds favors the growth of lateral buds and thereby increases branching.

Since the plants were grown under high temperatures that encourage vegetative growth, the untreated plants exhibited higher haulm dry mass than the treated groups which may be due to the higher GA activity. Elevated temperatures and/or long days stimulate GA biosynthesis and thereby encourage top growth (Menzel, 1981; Vreugdenhil and Sergeeva, 1999). Exogenous application of GA_3 inhibited tuber formation, decreased tuber sink strength and encouraged shoot and stolon growth (Menzel, 1980; Mares *et al*., 1981). Similar reports have been published indicating that high temperatures decreased tuber growth rate, reduced the partitioning of assimilates to the tubers and increased the amount allocated to other parts of the plant (Menzel, 1980; Struik *et al*., 1989; Vandam *et al*., 1996).

Removal of terminal buds and terminal buds along with associated younger leaves did not significantly reduce underground biomass yield compared to the control. This could be due to high GA activity that encouraged stolon branching. This is in agreement with the finding of Menzel (1981) who reported that the removal of terminal bud and terminal bud along with younger leaves alone had a negligible effect on the distribution of dry matter to tuber but had a positive effect on the distribution of dry matter to stolons and stimulated their branching. He also noted that high temperatures increased GA activity and thereby inhibited tuberization and favoured stolon elongation rather than swelling. Similarly, Struik *et al.* (1989) reported that stolon branching is stimulated by high temperatures due to increased GA activity.

3.5. Days to Physiological Maturity

Although not statistically significant, Wochecha matured three days earlier than Tolcha which required almost 100 days to attain physiological maturity (Table 1). Pruning treatment significantly prolonged days to physiological maturity. Removal of terminal buds, axillary buds and associated younger leaves delayed days to physiological maturity by about 8 days which could be due to reduced GA activity in response to the disbudding treatments. High temperatures accelerate the onset of senescence in potato due to high concentration of GA (Menzel, 1983) and favor rapid growth and shorten the growing period (Vander Zaag *et al*., 1990). The observed yield advantage, in response to the removal of buds and younger leaves, may be attributed to the prolonged canopy life of the potato plant which enables the plant to produce photoassimilates for an extended period. A highly significant and positive correlation (r=0.99**) between total yield and days to physiological maturity substantiates this hypothesis.

3.6. Tuber Number

The mean total tuber number per hill for cultivars was such that Wochecha (10.53 per hill) produced a comparable number of tubers with Tolcha (10.24 per hill) (Table 2). The removal of terminal and axillary buds, as well as nipping off terminal buds, axillary buds and associated younger leaves increased total tuber number by about 29% compared to the control. The existence of buds and younger leaves decreased the number of tubers that could be due to high GA activity which favoured stolon elongation rather than swelling. In agreement with the present finding, Menzel (1981) reported tuber number increment in response to the removal of the terminal bud alone and more so by the removal of all buds. The use of different types of growth regulators known to inhibit GA-biosynthesis had a positive effect on the number of tubers formed *in vitro and in vivo* (Simko, 1993; Harvey *et al*., 1991; Tekalign and Hammes, 2005a).

Although there was no significant difference in the total tuber yield between the two cultivars, Wochecha produced a higher total tuber yield (565.3 g $hill^{-1}$) than Tolcha (503.4 g $hill^{-1}$). Compared to the control, the removal of terminal and axillary buds as well as nipping off terminal buds, axillary buds and younger leaves increased total tuber yield by about 63.5%. This appears to indicate that buds and younger leaves development has a depressing effect on tuber development which may be due to active synthesis of GA at high temperature that limits assimilate partitioning to the tubers. Buds are major sites of gibberellins synthesis in the potato and high temperatures stimulate the synthesis of gibberellins and their export to the stolons, where they inhibit tuber formation (Menzel, 1981). Potato grown under high temperatures is characterized by high levels of endogenous GA (Vreugdenhil and Sergeeva, 1999) that promote shoot growth (Menzel, 1980) and delay or inhibit tuberization (Abdella *et al*., 1995; Vandam *et al*., 1996). In addition, the accumulation of gibberellin in tuber tissue can specifically impede starch accumulation (Paiva *et al*., 1983; Vreugdenhil and Sergeeva, 1999) and inhibits the accumulation of patatin and other tuber specific proteins (Vreugdenhil and Sergeeva, 1999).

3.7. Average Tuber Mass

The mean tuber mass of cultivars indicated that Wochecha (53.7g) gave heavier tubers than Tolcha (49.2g) though it was not statistically significant (Table 2). The removal of terminal and axillary buds and the removal of terminal buds, axillary buds along with associated younger leaves increased average tuber mass by about 26.5% compared to the control. The increase in average tuber, as a consequence of the removal of buds and younger leaves, may be explained on the basis of the absence of competition for assimilates between shoots and tubers. It is speculated that, in the absence of buds and younger leaves, presumably since developing tubers are the predominant sinks, a large amount of dry matter is diverted to the tubers which would otherwise be utilized for shoot growth. As a result, most of the initiated tubers increased in size (Tekalign and Hammes, 2005a). Similarly, Menzel (1981) reported that the removal of buds and younger leaves caused a substantial diversion of assimilates to tubers and thereby increased tuber size (mass) and the number of tubers. Tuber dry mass per plant was increased in response to the application of Paclobutrazol (inhibitor of GA biosynthesis) which may be linked to early tuberization, more dry matter partitioning to the tubers, and delaying the onset of senescence (Balamani and Poovaiah, 1985; Simko, 1994; Tekalign and Hammes, 2005a). Positive and significant correlation was observed between total yield and average tuber mass (r=0.77**) indicating that the yield advantage

obtained in response to pruning treatments could partly be attributed to individual tuber mass increment.

Table 2. Total number and yield, average tuber mass, tuber specific gravity and dry matter content as influenced by cultivars and pruning treatments.

Main effect	Total tuber number	Tuber yield (g hill^{-1})	Average tuber mass (g tuber^{-1})	Specific gravity (gcm^{-3})	Dry matter content (%)
Cultivar					
Wochecha	10.53 a	565.3 a	53.73 a	1.0788 a	19.60 a
Tolcha	10.24 a	503.4 a	49.16 a	1.0786 a	19.27 a
Treatment					
Normal growing plants (control)	9.14 b	407.4b	44.57 b	1.0710 b	18.03 b
Terminal buds removed	9.48 b	457.8b	48.26 b	1.0734 b	18.42 b
Terminal buds and younger leaves removed	9.62 b	473.8b	49.09 ab	1.0736 b	18.49 b
Terminal and axillary buds removed	11.67a	657.6a	56.32 a	1.0873 a	21.06 a
Terminal buds, axillary buds, and younger leaves removed	11.95a	675.3a	56.48 a	1.0881 a	21.33 a
CV (%)	11.27	11.66	13.79	0.19	2.42

Means of the same main effect in the same column followed by the same letters are not significantly different at 5% probability level.

3.8. Specific Gravity and Dry Matter Content

There was no significant difference in tuber specific gravity and dry matter content between the two cultivars (Table 2). Compared to the control, the removal of terminal buds alone and terminal buds along with associated younger leaves did not significantly influence specific gravity and dry matter content. However, the removal of terminal and axillary buds, as well as nipping off terminal buds, axillary buds and associated younger leaves increased specific gravity by about 1.56%, and dry matter content by about 17.5% compared to the control. This could be due to a high concentration of GA which reduces the diversion of assimilates to the tubers and decreases starch accumulation. This study is in line with Booth and Lovell (1972) who explained that the application of GA_3 to potato shoots reduced the export of photosynthates to the tubers, decreased starch accumulation, increased sugar levels and resulted in the cessation of tuber growth. Improvement in tuber specific gravity as well as dry matter content in response to the pruning treatments may be due to the diversion of the largest proportion of assimilates to the tubers due to reduced GA activities that would be used for excessive top growth. This finding is supported by Menzel (1981) who reported that the largest proportion of assimilates were diverted to the tubers and enhanced starch synthesis after removing buds and younger leaves. Similarly, Tekalign and Hammes (2005a) observed an increased tuber specific gravity and dry matter content in response to paclobutrazol (GA biosynthesis inhibitor) which may be due to an increased sink strength to attract more assimilates and enhance starch synthesis. Highly significant positive correlation (r= 0.94**) was observed between specific gravity and the percentage of dry matter, indicating that specific gravity is a true indicator of the amount of dry matter (total solid) of tubers. In agreement with the present study, Tsegaw and Zelleke (2002) reported highly significant positive correlation between specific gravity and the percentage of dry matter.

4. Conclusions

There was no significant difference between the two cultivars for all of the parameters considered, except days to tuber imitation. Generally, it was observed that the removal of terminal and axillary buds, along with younger leaves, significantly shortened days to tuber initiation, delayed the onset of senescence, increased tubers' number and yield, average tuber mass, specific gravity and dry matter content while reducing plant height and the total leaf area of potato grown in the hot tropical lowlands where potato production is not being prasticed due to the existence of high temperatures throughout the year. This indicates that buds and younger leaves have a depressing effect on tuber development, perhaps due to competition for assimilate between them. These results revealed the possibility of increasing tuber yield and improving the quality of potato through pruning treatments in hot tropical and sub-topical areas where high temperature is a limiting factor for introduce potato culture.

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Genetic Variability Studies in Ethiopian Shallot (*Allium cepa* L. var. *ascalonicum* Backer) Genotypes

Fasika Sendek [1], Hailu Tefera [2] and Kebede W/Tsadik [3*]

[1]Sirinka Agricultural Research Center, P O Box 74, Weldia, Welo, Ethiopia
[2]Debre Zeit Agricultural Research Center, P O Box, 32, Debre Zeit, Ethiopia
[3] Haramaya University, P O Box 138, Dire Dawa, Ethiopia

Abstract: Field experiments were conducted on forty-nine shallot genotypes to estimate the nature and magnitude of variability for bulb yield and other related characteristics at Sirinka and Girana trial sites in north-eastern Ethiopia. The experimental design was simple lattice with two replications. Observations were made on ten plant samples. A wide range of genetic variability was obtained for all the traits, except for leaf diameter at Girana and the number of lateral branches and maturity date at Sirinka. Highly significant genetic differences were recorded for plant height, leaves per plant, bulb splits, bulb diameter, total, marketable and biological yields, harvest index, total soluble solids, bulb dry weight and pungency. The PCV values ranged from 5.91% for days to maturity at Sirinka to 41.57% for total yield per plant at Girana. At both locations, wide PCV and GCV as well as phenotypic (δ^2p) and genotypic (δ^2g) variances were observed for biological, total and marketable yields, bulb dry weight and leaf number per plant. The lowest PCV and GCV levels were observed in days to maturity and harvest index. Similarly, lower phenotypic and genotypic variances were observed for lateral branches per plant and bulb splits, bulb diameter, total soluble solids and the pungency of the bulbs. At both locations, moderate to high heritability estimates coupled with moderate to high genetic advances as a percentage of the mean were recorded for bulb yield and related traits considered. This study demonstrates the existence of adequate genetic variability in Ethiopian shallot genotypes for further exploitation through breeding. Selection for biological, total and marketable yields, bulb dry weight, bulb splits and pungency characteristics is likely to be effective for improvement of the crop as high heritability values have been associated with high genetic advance.

Keywords: Shallot; Bulb Yield; Genetic Variability; Heritability; Genetic Advance

1. Introduction

Ethiopia, with its diverse agroecologies, has the potential to produce diverse types of vegetable crops ranging from temperate types in the highlands to the tropical and sub tropical types in the lowlands. According to Seifu (1981), Ethiopia is considered as a center of diversity for shallot. Shallot is widely produced in Fedis in Hararghe; Huruta, Sire, Sirka, Bekoji and Arsi Negelle in Arsi; Ambo, Wolliso, Godino, Kessem and Majete in Shewa; Bure and the vicinities of Debre Markos in Gojam; Waraillu and Wara Babo in Wello (Getachew and Asfaw, 2000).

Shallot is the most vital vegetable crop used as a condiment in most Ethiopian cuisine (Seifu, 1981). It is widely cultivated as a cash crop, mainly by subsistence farmers in the mid-and high-altitude areas of the country. According to the CSA (2005), Allium spp. were grown on 17,980 hectares of land and a total of 229,678 tons of bulbs were produced in 2004/05.

Understanding the extent of variability in a crop species is of utmost importance in breeding to develop better varieties. Genetic variability is of immense importance to the breeder because it could be transmitted to the progeny and proper management of this diversity could produce gain in the performance of the plant (Welsh, 1981). Wide phenotypic and genotypic variations in important traits such as plant height, number of leaves per plant bulb diameter, and bulb dry weight can provide a great opportunity for improving these traits through effective selection (Kallo *et al.*, 1982; Barta *et al.*, 1983). Genetic coefficient of variation, together with heritability estimates, the proportion of genetic variance to the total phenotypic variance, is considered to give the best picture of the amount of advance to be expected from selection (Johnson and Hernandez, 1980). In Alliums spp., high estimates of heritability and genetic advance with respect to bulb weight, leaf length, leaf number, bulb diameter have been reported (Kallo *et al.*, 1982; Dowker, 1990; Abayneh, 2001).

Ethiopian shallot germplasm collections have been found to vary in shape, colour, pungency, storability and other characters (Getachew and Asfaw, 2000). According to the same authors, Ethiopian farmers grew different lines and named them after major production belts like 'Fedis', 'Huruta', 'Bure', etc. Furthermore, different types of plants, including bolters and non-bolters, spreading and compact types, and bulbs with various shape size and colour are commonly observed within a farm. However, as shallot is propagated mainly by an asexual method, it is unclear whether these variabilities reflect a diverse genetic background or not. Currently, shallot improvement activities in the country focus on traits such as high bulb yield, bulb size and colour through simple selection from national germplasm collections (Getachew and Asfaw, 2000). Information on the nature and extent of variability and the degree of transmission of traits in local shallot germplasm is believed to be of paramount importance in enhancing the efficiency of selection. Therefore, this study was initiated to estimate the nature and magnitude of variability for bulb yield and other related characteristics in a collection of local shallot genotypes.

2. Materials and Methods

This study was conducted at Sirinka and Girana trial sites of Sirinka Agricultural Research Center (SARC), north Wello, during the main growing season (July to November) in 2003. Sirinka station is located at latitude of 11°83' N and longitude 39°68' E with an altitude of 1850 m.a.s.l. The mean annual rainfall is 950 mm with a

*Corresponding author. E-mail: tsadike@haramaya.edu.et

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mean maximum and mean minimum temperature of 26ºC and 13ºC, respectively (SARC, 2000). The soil type is eutric vertisol (Samuel, 2000). Girana research site is located south of Sirinka at about 50 kilometer from SARC. It is situated at an altitude of 1450 m.a.s.l. and the soil type is clay with a pH of 7.6 (SARC, 2000). Forty-nine shallot genotypes (Table 1) including one local and one standard check were evaluated in a simple lattice design with two replications. Plot sizes were 2m x 2m, consisting of 50 plants in five rows. Uniform size bulbs of the lines were planted at a spacing of 40 cm x 20 cm between rows and plants within rows, respectively, and the plots received cultural practices uniformly. Observations were made on fourteen yield and related traits by randomly taking 10 plant samples from the three middle rows of the plots. The parameters recorded included plant height, leaf number, leaf diameter, number of lateral branches and bulb splits, bulb yield (total, marketable and biological), harvest index, bulb dry weight total soluble solid and pungency of bulbs. The data were subjected to analysis of variance using MSTATC computer software (MSTATC, 1989).

The variability present in the population was estimated by calculating genotypic and phenotypic coefficients of variation as suggested by Burton and DeVane (1953).

Table 1. Accession number and origin of shallot genotypes used in the study.

		Origin				Origin	
No	Accessions	Province	Locality	No	Accessions	Province	Locality
1	004-4A	Shewa	Kimbibit	26	042-1	Shewa	Mafud
2	012-1	Shewa	Moret & Jihur	27	043-2	Shewa	Mafud
3	068-2	Shewa	Efrata	28	DZ-Sht-75	Shewa	D. Zeit
4	023-3	Shewa	Ankober	29	AFS-3	Hararghe	chiro
5	024-1	Shewa	Ankober	30	061-1	Shewa	Kewet
6	034-1	Shewa	Dulecha	31	028-2	Shewa	Dulecha
7	040-2	Shewa	Dulecha	32	284-1	Shewa	Gerar Jarso
8	074-1	Shewa	Efrata	33	176-1	Gojam	Bahir Dar
9	051-4	Shewa	Mafud	34	207-1	Gojam	Gozamin
10	DZ-Sht-70	Shewa	D. Ziet	35	135-1	Wello	Ambasel
11	140-1	Wello	Goba Lafto	36	044-1	Shewa	Mafud
12	123-2A	Wello	Desie Zuria	37	274-1	Shewa	Were Jarso
13	305-2A	Shewa	Kuyu	38	054-3	Shewa	Kewet
14	306-3	Shewa	Kuyu	39	DZ-Sht-50	Shewa	D. Ziet
15	205-3	Gojam	Gozamn	40	066-4	Shewa	Efrata & Jile
16	272-1	Shewa	Were Jarso	41	066-3	Shewa	Efrata & Jile
17	097-2	Wello	Kalu	42	278-2	Shewa	Dejen
18	DZ-Sht-68	Shewa	D. Ziet	43	065-5	Shewa	Efrata& Jile
19	096-1	Shewa	Antsokya	44	176-1A	Gojam	Bahir Dar
20	064-2	Shewa	Efrata	45	AFS-2	Hararghe	Fediss
21	DZ-Sht-23	Shewa	D. Ziet	46	102-5	Wello	Desie Zuriya
22	070-2	Shewa	Efrata	47	Huruta*	Shewa	D. Ziet
23	DZ-Sht-Op-5s	Shewa	D. Ziet	48	278-2A	Shewa	Degem
24	088-3	Gojam	Gemza	49	Local **	Wello	Gubalfto
25	DTKG-4	Hararghe	Chiro				

** Standard check ** Local check*

Source of data: Debre Zeit Agricultural Research Centre

Genotypic variance

$$(\sigma^2 g) = \frac{MSg - MSe}{r}$$

Environmental variance ($\sigma^2 e$) = Error mean square (MSe)

Where, MSg and MSe are the mean squares for the genotype and environmental factors, respectively and r is the number of replications.

Phenotypic variance

$$(\sigma^2 p) = (\sigma^2 g) + (\sigma^2 e)/r$$

The genotypic and phenotypic coefficients of variation were then estimated as:

Genotypic coefficient of variation

$$(GCV) = \sqrt{\sigma 2g} \;/\; \text{grand mean} \times 100$$

Phenotypic coefficient of variation

$$(PCV) = \sqrt{\sigma 2p} \;/\; \text{grand mean} \times 100.$$

Heritability in broad sense

$(h^2) = (\sigma^2 g / \sigma^2 p) \times 100$, Where, $\sigma^2 p = \sigma^2 g + \frac{\sigma 2e}{r}$

Genetic advance (GA) was estimated using K value of 2.06 at 5% selection intensity as described by Allard (1960).

GA = (K) (σ p) (h^2), Where, K= selection differential (2.06) at 5% selection intensity, h^2 = heritability, and σ p = phenotypic standard deviation

The genetic advance as a percentage of the mean was estimated as: $\frac{GA}{grandmean}$ x100, where grand mean is population mean

A homogeneity test of data was carried out for the two locations for each character and the test showed heterogeneity of the two locations for almost all characteristics considered. And so combined analysis of the two locations was not considered. The experiment was conducted using lattice design with two replications.

However, randomized complete block design was used instead of lattice design for the analysis, because randomized complete block design was more efficient than lattice for estimating the variance components of the different tested characteristics.

3. Results and Discussions

3.1. Analysis of Variance

The results of the analysis of variance using randomised complete block design at Sirinka and Girana are presented in Tables 2. At Girana, mean squares due to genotypes were highly significant for all the traits studied except for leaf diameter, indicating the existence of high genetic variability within different collections of shallot genotypes from different regions. The same trend was observed at Sirinka, where the genotypes showed highly significant difference for all traits studied, except for days to maturity and lateral branches per plant. These results indicate the existence of wide genetic variability among the genotypes at both locations.

Table 2. Analysis of variance for 14 parameters in shallot (*Allium ascalonicum* var *ascal*onicum) genotypes at Sirinka and Girana, north Wello, Ethiopia.

	Mean squares							
	Sirinka				Girana			
Character	Replication (df=1)	Genotype (df=48)	Error (df=48)	CV (%)	Replication (df=1)	Genotype (df=48)	Error (df=48)	CV (%)
Plant height	197.47	19.85**	8.47	9.82	98.60	22.50**	9.65	12.73
Leaves per plant	34.16	325.60**	68.73	22.04	908.23	93.43	58.60	24.00
Leaf diameter	0.000	0.028**	0.007	12.38	0.002	0.007	0.006	16.29
Lateral branches per plant	3.27	3.26	2.13	22.89	62.16	2.18	1.44	25.18
Bulb splits per plant	4.33	7.34**	1.74	15.56	1.16	274.45**	96.05	18.90
Bulb diameter	102.04	24.59	16.33	5.28	80.83	32.36*	16.10	6.18
Total yield per plant	9.68	18.43**	6.34	9.35	2.06	8.67	5.48	10.73
Marketable yield per plant	2223.97	1054.96**	240.45	20.11	25.66	248.40**	104.39	31.98
Biological yield per plant	2273.02	1261.03**	276.14	24.20	162.26	155.27**	54.20	28.94
Harvest index per plant	2776.19	1336.1**	304.18	20.06	61.62	338.69**	130.35	29.35
Bulb dry weight	0.44	41.38**	20.21	5.06	18.13	122.24*	63.45	9.78
Days to maturity	9.68	50.06**	13.86	19.57	94.24	10.48**	4.41	25.96
Total soluble solid	0.004	2.93**	1.33	8.65	4.29	4.08**	1.66	11.57
Pungency	1.60	13.69**	0.84	7.21	2.48	6.17**	0.52	5.25

***, * Indicate significant difference at 1% and 5% probability levels, respectively.*

3.2. Estimates of Phenotypic and Genotypic Variations

The Phenotypic Coefficients of Variation (PCV) values ranged from 7.58% for days to maturity to 41.57% for total yield per plant at Girana and from 5.91% for days to maturity to 40.38% for marketable yield per plant at Sirinka. The Genotypic Coefficients of Variation (GCV) ranged between 4.39% for days to maturity to 27.95% for marketable yield per plant at Girana whereas it ranged from 2.65% for days to maturity to 32.32% for marketable yield per plant at Sirinka.

Both at Girana and at Sirinka experiment sites, wide differences between PCV and GCV for total bulb yield, marketable yield, bulb diameter, biological yield, bulb dry weight, lateral branches, leaf diameter, harvest index, bulb splits, total soluble solids, plant height and leaves per plant were observed (Tables 3 and 4). At both locations, high GCV was recorded for marketable yield, leaves per plant, total yield, biological yield, bulb dry weight, pungency and bulb splits per plant. The variations in these traits could be attributed to the geographical origin of these genotypes (Table 1), which offers relatively wide scope for selection among these traits. On the other hand, a small difference (less than 5%) between the PCV and GCV values was observed in days to maturity and pungency, indicating the lower influence of the environment in determining these traits.

Both at Girana and Sirinka, high phenotypic (δ^2p) and genotypic (δ^2g) variances were recorded for biological yield, total bulb yield, marketable bulb yield and leaves per plant. From this, it appears that there is wide scope for selection among genotypes for these characteristics based on phenotypic appearance. On the other hand, lower phenotypic and genotypic variances were observed for the number of lateral branches and bulb splits per plant, bulb diameter, total soluble solids and pungency of the bulbs, indicating the difficulty in improving these traits through selection.

These observations are in general agreement with those of Singh (1981) and Abayneh (2001) who reported moderate to high phenotypic and genotypic coefficients of variation as well as phenotypic and genotypic variances in onion for these characteristics. Similarly, Mohanty (2001) reported a moderate to high genotypic coefficient of variation in bulb weight and leaf number per plant among twelve Indian onion varieties. The presence of considerable genetic variation for such traits offers a great opportunity for the improvement of shallot through simple selection.

3.3. Estimates of Heritability in Broad Sense and Genetic Advance

Heritability estimates in a broad sense (h^2) for the 14 characteristics, ranged from 20.4% to 84.40% at Girana and 20.2% to 88.4 % at Sirinka (Tables 3 and 4). High heritability values were recorded for pungency, marketable yield, total bulb yield per plant, bulb diameter, total soluble solids, bulb splits, biological yield, bulb dry weight and plant height, indicating the possibility of improving these traits through selection.

Table 3. Estimates of phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability in broad sense (h^2), genetic advance (GA) and GA as a percentage of mean for different characters of shallot genotypes at Girana, north Wello, Ethiopia.

Characters	Range	PCV (%)	GCV (%)	δ^2p	δ^2g	h^2 (%)	GA	GA (%mean)
Plant height	18.6-31.05	16.42	10.38	16.07	6.42	40.0	3.30	13.52
Leaves per plant	14.35-48.90	27.33	13.08	76.00	17.41	22.9	4.11	12.88
Leaf diameter	0.36-0.68	19.80	6.08	8.66	8.17	9.40	0.02	4.26
Lateral branches per plant	3.10-7.45	28.23	12.75	1.81	0.37	20.4	0.57	11.97
Bulb splits per plant	4.65-12.55	26.25	18.22	3.86	1.86	48.2	1.95	26.07
Bulb diameter	16.65-26.20	12.20	5.80	7.07	1.60	22.6	1.24	5.69
Total yield per plant	13.04-69.71	41.57	26.56	176.40	72.01	40.8	11.17	34.96
Marketable yield per plant	12.41-46.32	40.24	27.95	104.71	50.52	48.3	10.17	39.99
Biological yield per plant	19.92-80.80	39.37	26.24	234.55	104.19	44.4	14.01	36.02
Harvest index per plant	59.04-95.41	11.83	6.66	92.78	29.40	31.7	6.29	7.73
Bulb dry weight	3.90-13.85	33.73	21.54	7.45	3.04	40.8	2.29	28.31
Days to maturity	52.50-72.00	7.58	4.39	24.23	8.13	33.6	3.40	5.23
Total soluble solid	7.00-14.50	15.23	9.90	2.87	1.21	42.3	1.48	13.30
Pungency	8.15-16.55	13.23	12.15	3.34	2.82	84.4	3.18	23.01

Table 4. Estimates of phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability in broad sense (h^2), genetic advance (GA) and GA as a percentage of mean for different characters of shallot genotypes at Sirinka, north Wello, Ethiopia.

Characters	Range	PCV (%)	GCV (%)	δ^2p	δ^2g	h^2 (%)	GA	GA (%mean)
Plant height	24.05-35.80	12.69	8.05	14.16	5.70	40.2	3.12	10.52
Leaves per plant	25.00-64.80	37.34	30.13	197.22	128.41	65.1	18.84	50.09
Leaf diameter	0.42-0.98	19.81	15.46	0.02	0.01	60.9	0.17	25.37
Lateral branches per plant	4.25.9.00	25.75	11.81	2.69	0.57	21.0	0.71	11.15
Bulb splits per plant	5.10-12.95	25.12	19.72	4.54	2.80	61.6	2.71	31.96
Bulb diameter	21.40-33.90	13.07	9.13	12.39	6.05	48.8	3.54	13.14
Total yield per plant	34.97-149.81	33.00	26.17	647.68	407.33	62.9	32.96	42.25
Marketable yield per plant	24.70-138.86	40.38	32.32	768.67	492.44	64.10	36.59	53.29
Biological yield per plant	40.18-166.49	32.93	26.12	820.02	515.92	62.9	37.12	42.69
Harvest index per plant	79.02-97.30	6.25	3.66	30.80	10.56	34.4	3.93	4.43
Bulb dry weight	11.20-33.70	29.72	22.36	31.95	18.09	56.6	6.59	34.65
Days to maturity	71.00-84.50	5.91	2.65	20.47	4.12	20.2	1.88	2.46
Total soluble solid	9.90-16.30	10.94	6.70	2.13	0.80	37.5	1.13	8.47
Pungency	6.74-17.30	21.21	19.94	7.27	6.42	88.4	4.91	38.63

At both locations, moderate heritability estimates were recorded for days to maturity, lateral branches per plant, harvest index per plant and total soluble solids. In addition, the number of leaves and bulb diameter were observed to have moderate heritability at Girana. Such moderate heritability values indicate the limited scope in improving these characteristics. Generally, heritability values were higher at Sirinka than at Girana for most characteristics considered. There seem to be clear differences between the two environments and Sirinka appeared to be a better place for the genotypes to express their genetic potential.

According to Singh (1990), very high heritability characteristics feature is an indication of a fairly easy selection for that characteristics as there would be a close correspondence between the genotype and phenotype due to a relatively small contribution of the environment to the phenotype. In conformity with this finding, Pike (1986), Abayneh (2001) and Mohanty (2001) observed moderate to high heritability estimates for bulb yield per plant in onion.

The expected genetic advance (GA) as a percentage of the mean by selecting the top 5% of the genotypes, varied from 4.3% for leaf diameter to 40% for marketable yield at Girana (Table 3). At Sirinka, it ranged from 2.5% for days to maturity to 53.3% for marketable yield (Table 4).

Expected high genetic advances were observed for biological yield, total yield and marketable yield, bulb dry weight, bulb splits and pungency at both locations, while leaf number and leaf diameter also showed high genetic advance at Sirinka. Hence, selection according to these characteristics is likely to be effective for the improvement of the crop as high heritability values has been associated with high genetic advances in this observation. In support of this finding, Dowker (1990), Abayneh (2001) and Mohanty (2001) reported expected high genetic advances for pungency, leaf number and bulb yield per plant in onion.

4. Conclusion

Information on the nature and magnitude of variability for yield and related characteristics are essential for designing breeding strategies in crop improvement. To generate and understand such information, 49 shallot genotypes were grown in triple lattice design at Sirinka Agricultural Research Centre and Girana trial site, north Wello. The study has indicated the presence of adequate genetic variability, high heritability in a broad sense and high genetic advance for most of the characteristics studied that can be exploited for shallot improvement.

A Girana the estimate of PCV ranged from 8% to 42% while for GCV it ranged from 4% to 28%. At Sirinka, PCV ranged from 6% to 40% and from 3% to 32%. High

heritability, coupled with high genetic advances, were recorded for marketable yield per plant, biological yield per plant, total yield per plant, bulb dry weight, bulb splits and pungency at both locations. This study has proved that phenotypic differences observed for various traits reported by different authors show the existence of genetic variability in the Ethiopian shallot genotypes. Therefore, further study on shallot genotypes is essential to exploit the rich germplasm resource of the country.

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The Combining Ability of Maize Inbred Lines for Grain Yield and Reaction to Grey Leaf Spot Disease

Dagne Wegary[1], Habtamu Zelleke[2]*, Demissew Abakemal[3], Temam Hussien[2] and Harjit Singh[2]

[1]Bako National Maize Research Project, P O Box 3, West Shewa, Bako, Ethiopia
[2]Haramaya University, P O Box 147, Haramaya, Ethiopia
[3]Ambo Plant Protection Research Center, P O Box 37, West Shewa, Ambo, Ethiopia

Abstract: Considering the potential threat of grey leaf spot (GLS) to maize production, the national maize research program of Ethiopia has been screening local and exotic maize genotypes against the disease as the commercially available and advanced inbred lines susceptible to GLS. This experiment was, therefore, conducted to investigate the general (GCA) and specific (SCA) combining ability of selected maize inbred lines for grain yield and GLS disease resistance. Eight maize inbred lines with contrasting reactions to GLS were crossed in diallel mating to generate 28 hybrids. The parents and hybrids were evaluated in randomised complete block design with three replications at Bako, Ethiopia in 2001 and 2002. Significant differences were observed among entries for disease parameters, plant height, days to maturity and grain yield. GCA and SCA effects were significant for all traits. Mean squares due to GCA were higher than that of SCA for all traits, except for grain yield, indicating the predominance of non-additive gene effect for grain yield and additive gene effect for other traits. Some inbred lines with resistance reaction have been identified. Parental lines 143-5-i and CML-387 showed a better *per se* performance and GCA effects while A-7016 was the worst parent for GLS disease reaction. Inbred parent 143-5-i was the best general combiner for grain yield; but a poor general combiner for plant height and days to maturity. The information from this study will be useful for the development of high-yielding and GLS disease-resistant maize varieties.

Keywords: Combining Ability; Disease Resistance; Grain Yield; Grey Leaf Spot; Maize

1. Introduction

Maize is a major food crop in Ethiopia and is cultivated on about 1.4 million hectares, which accounts for 20% of the seven million hectares of land allocated for all cereals. It ranks second after teff (*Eragrotis teff*) in area coverage, first in total national production and yield per hectare (CSA, 2004). In the major producing regions, maize is the major source of protein and calories (Mosisa *et al*., 2002). Considering its importance in terms of wide adaptation, total production and productivity, maize has been selected as one of the high priority crops to feed the increasing population of Ethiopia. Past research efforts in Ethiopia resulted in the development and release of open pollinated and hybrid varieties for different agro ecologies of the country. However the national average yield (1.9 t ha^{-1}) (CSA, 2004) is still very low compared to the global average of 4.2 t ha^{-1} (Paliwal *et al*., 2000). This low productivity is attributed to maize production constraints such as drought stress (Mandefro and Habtamu, 2001), low fertility (Mosisa *et al*., 2004) and poor management practices (Tolessa *et al*., 2002). Pests and diseases also cause significant losses to maize production in different regions (Tewabech *et al*., 2002).

Earlier surveys indicated that foliar diseases such as *Turcicum* leaf blight caused by *Exserohilum turcicum* and common leaf rust incited by *Puccinia sorghi* have been recognized as the most important maize production problems that occur widely on maize (Assefa, 1999). Currently, grey leaf spot, caused by *Cercospora zeae-maydis* Tehon and E. Y. Daniels, is the principal maize disease in the country. The disease first became serious in 1997. Since then, there have been widely distributed severe epidemics every year, especially in the warm and humid areas of the country (Tewabech *et al*., 2002). GLS has been recognized as one of the most significant yield-limiting diseases of maize in Africa since the 1980s (Ward *et al*., 1999). GLS was first reported as a cause of economic losses in South Africa during the 1990/1991 growing season (Ward *et al.,* 1999). The GLS disease was first observed in Uganda in 1994 and in Zimbabwe during the 1995/96 cropping season. The same year, GLS was observed in Kenya and Cameroon, and during 1996 in Zaire (Ward *et al*., 1999; Pixley, 1996). Since then, GLS has become important in the southern and eastern African regions, where the incidence and severity of its epidemics have been increasing (Tembo and Pixley, 1999).

The disease is most severe and damaging when extended periods of high relative humidity occur, caused by slow-drying dews and prolonged long season fogs (Beckman and Payne, 1983). In addition, the widespread adoption of reduced tillage practices, maize monocropping and planting of susceptible varieties have resulted in a build-up of inoculum reservoirs and GLS incidence (Gevers *et al*., 1994; Pratt *et al*., 1997). A study conducted in Ethiopia by Dagne *et al.* (2004) indicated that GLS caused a yield loss of 37%, with estimated higher losses in years of severe epidemics. Losses associated with GLS are greatest when photosynthetic tissues are blighted and prematurely killed prior to grain fill (Donahue *et al.,* 1991).

Host resistance is the most effective and cost-efficient means of managing GLS and preventing leaf blighting (Graham *et al*., 1993; Coates and White, 1994). However,

*Corresponding author. E-mail: habtaamu@yahoo.com

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no commercial hybrids with adequate resistance are currently available in Ethiopia, as they have not been improved for resistance to this specific disease. Several published reports, mostly from diallel studies, have concluded that additive effects are far more significant than non-additive effects in determining resistance to GLS (Huff *et al*., 1988; Elwinger *et al*., 1990; Ulrich *et al.,* 1990; Bubeck *et al*., 1993). Host resistance to *C. zea-maydis* is regulated by a small number of quantitative loci, with five or more genes involved, which are inherited additively (Saghai-Maroof *et al*., 1996). Such disease progress rate reducing polygenic resistance increases the level and stability of resistance. GLS resistance was not very complex and could be evaluated effectively using inbreds *per se* (Thompson *et al*., 1987).

Recognizing the seriousness and potential destructiveness of GLS disease, the Ethiopian national maize research program has been conducting a series of experiments on evaluation and selection of maize genotypes from local and exotic sources. In these tests, some of the entries exhibited fleck type lesions (resistance reaction) when artificially inoculated with *C. zea-maydis*. Conversely, some of the advanced and commercial inbreds with good agronomic characters show susceptible reaction to the disease. The combining ability effects of the identified GLS resistant lines for low disease reaction and yield need to be worked out for Ethiopian conditions. The combining ability of inbred lines is the ultimate factor determining their usefulness in developing the hybrids as well as the composites and the synthetics (Caraballosso *et al.,* 2000). The most suitable way to achieve this goal is the use of a diallel mating system; a method whereby the progeny performance can be statistically separated into components related to general (GCA) and specific (SCA) combining ability effects. Therefore, this study was undertaken with the objective of investigating the combining ability of selected maize inbred lines for yield and grey leaf spot resistance.

2. Materials and Methods

2.1. Experimental Environment and Germplasm

The study was conducted at Bako Agricultural Research Center, western Ethiopia during the main seasons (May to November) of 2001 and 2002. Bako is situated at an altitude of 1650 meters above sea level, 9°06' north latitude and 37°09' east longitude. Average annual rainfall at this location is 1246 mm. The rainy season lasts from April to October, with maximum rainfall in July and August. The soil type of the center is reddish brown Nitosols, according to FAO/UNESCO soil classification, which were developed from basalt parent materials. The soil is deep-weathered, well-drained, slightly acidic in reaction, clay to sandy clay loam at the surface, low in available P, total N, organic matter and available water holding capacity (Wakene, 2001).

Eight maize inbred lines with contrasting reaction to GLS obtained from the Ethiopian national maize research co-ordination center, Bako and International Maize and Wheat Improvement Centre (CIMMYT) were used for the study. The selection of the lines was based on *per se* performance evaluation for reaction to GLS disease and general agronomic performance. Inbred lines 143-5-i, Gotto LMS_5, A-7016 and SC-22 were obtained from the national maize program of Ethiopia while CML-197, CML-202, CML-387 and CML-395 were received from CIMMYT. The Ethiopian national maize program inbred lines were locally developed from the materials introduced during 1960s and 1970s from East Africa and CIMMYT-Mexico. Based on their reaction to GLS disease, the lines were categorized as resistant (143-5-i and CML-387), moderately resistant (Gotto LMS_5, SC-22 and CML-395), moderately susceptible (CML-202) and susceptible (A-7016 and CML-197). The categorization was mode on the basis of disease severity ratings using a 1-5 scale (Roane *et al.,* 1974) in the previous years' screening nursery, where 1.0–2.0=resistant; 2.1-2.5=moderately resistant; 2.6–3.0=moderately susceptible; and >3.0 susceptible. The lines were crossed in an 8 x 8 diallel mating system in 2000 and 2001 to develop 28 possible crosses, excluding the reciprocals.

2.2. Field Procedures

The 28 F_1 crosses and eight inbred parents were evaluated in a randomised complete block design with three replications. Each plot consisted of four rows of 5.1 m length, spaced at 0.75 m apart. Plots were hand sown with high density and later thinned to a final plant density of 44,444 plants per hectare. Fertilization (P_2O_5 and nitrogen at the rate of 100 kg ha^{-1} each) and standard agronomic management practices recommended for the area were applied. The pathogen, *C. zeae-maydis*, was artificially inoculated using infected leaves collected in previous years from infected maize fields showing distinct GLS symptoms. The infected dried leaves were ground into powder and stored in paper bags at a temperature of 4°C. The pulverized leaf was dusted into the whorls of the leaves of the plants where it remained long enough to permit spore germination. The inoculation was done twice under dew conditions with a ten-day interval starting from the 6-leaf stage of the plant to ensure adequate infection.

2.3. Field Measurements

Measurements were taken from the two middle rows. Latent period was measured as the number of days from disease inoculation to the date on which a clear grey leaf spot lesion was observed on 50% of the plants in a plot. Since the first inoculation alone may not ensure enough infection, the second inoculation was considered for calculating the latent period. To accommodate any variations and peculiarities of disease progression attributed to the stage of plant infection and prevailing weather conditions, disease severity was recorded four times at intervals of ten days using a widely-used 1-5 scale. Rating was started when obvious genotypic differences for GLS reaction became apparent and continued until the leaves started to senesce. Disease incidence was recorded at the third week after silking as the ratio of infected leaves to the total number of leaves on a particular plant and expressed as a percentage.

Lesion type was also scored at the third week after silking on a scale of 1-4, where 1=flecks to chlorotic lesion, 2=chlorotic lesion with some necrosis, 3=chlorotic lesion with considerable necrosis and 4=susceptible or wilted lesion (Pratt *et al.*, 1997). Plant height was measured in centimetres two weeks after pollen shed had ceased, as the distance from the soil surface to the base of lowest tassel branch. Days to maturity were recorded as the number of days from emergence to when 50% of the plants in a plot formed a black layer at the tip of each kernel on the ears. All ears harvested from each plot were weighed, and representative samples of ears were shelled to determine the percentage moisture. Grain yield, adjusted to 12.5% moisture was computed from ear weight and grain moisture, assuming a shelling percentage of 80%.

2.4. Statistical Analysis

Area under disease progress curve (AUDPC) values were calculated for each experimental unit with the formula suggested by Tooley and Grau (1984) to integrate overall assessments for disease severity. AUDPC is a quantitative summary of the disease epidemic and is based on trapezoidal integration program (Berger, 1981). Percent disease incidence data covered a wide range of values; hence, it was transformed using arcsine transformation to satisfy the assumption of analysis of variance (Steel and Torrie, 1980). Analyses of variances were performed using AGROBASE™ software (Agronomix Software Inc., 2000) for each year separately (data not shown) and then combined over years after testing homogeneity of error variances using Bartlett's test. Entries were considered fixed effects, and years and replications random effects. Pearson correlation coefficients were calculated between pairs of traits using over years means to determine their relationships. To identify the best representative disease assessment methodology, all disease parameters means averaged over years were subjected to principal component analysis. The first two principal component axis (PC1 and PC2) scores for all the genotypes were correlated with disease parameters. The diallel cross analysis was carried out using Griffing's (1956) model I (fixed), method II (involving parents and one set of F_1 hybrids) to estimate components of variance due to general combining ability (GCA) and specific combining ability (SCA). Smaller values of SCA and GCA effects were considered desirable for plant height, days to maturity and all disease parameters except for latent period. Higher values were considered desirable for grain yield. GCA effects were correlated with *per se* performances of lines for disease incidence and grain yield. Predicted disease incidence and grain yield of crosses were calculated as the sum of GCA effects of the two parents and correlated with the observed mean performance of crosses for these traits.

For F-tests, main effects such as entries and its partitions, GCA and SCA mean squares were tested against their respective interactions with the environment, and all the interactions with the environment were tested against the pooled error. Significances of GCA and SCA effects were determined by t-test, using standard errors of GCA and SCA effects.

3. Results

3.1. GLS Reaction and Performance of Genotypes

Combined analysis of variance over years showed significant year effects for most traits, except for latent period and plant height (Table 1). The effects of entries and crosses, and their interaction with year were significant for all the traits considered. The effect of parents was significant for all disease parameters and grain yield. Parent x year interaction was significant for disease incidence, AUDPC, plant height and days to maturity. The effect of parents versus crosses was not significant for all traits, but its interaction with year was significant for most traits with the exception of latent period, lesion type and grain yield.

Among all entries, the means of latent period averaged over two years varied from 30.0 to 58.0 days (Table 2). The parent CML-387 had the longest latent period of 56.8 days, followed by 143-5-i with a latent period of 38.5 days. Most crosses involving one of these inbred lines as their parents had a longer latent period than other crosses. Inbred lines A-7016 and CML-197 exhibited lower latent periods of 34.2 and 33.8 days, respectively. Crosses with short latent period; vis-à-vis, Gutto LMS_5 x A-7016 (30.0 days), A-7016 x CML-197 (30.8 days) and A-7016 x CML-202 (30.8 days) involved A-7016 as one of their parents. Disease incidence ranged from 8.4 % for CML-387 to 90.0 % for A-7016 and CML-197, respectively for the inbred parents. For crosses, it ranged from 32.6% for Gutto LMS_5 x CML-387 to 83.2% for A-7016 x CML-197. CML-387 showed lower AUDPC of 38.8 while A-7016 exhibited the highest (102.9) AUDPC among the parents. With respect to lesion type, parents 143-5-i (1.50) and CML-387 (1.00), and crosses 143-5-i x CML-395 (1.08) and CML-387 x CML-395 (1.00) showed lower values.

In terms of plant height, 143-5-i (266.5 cm) was the tallest parent and tall crosses also involved this inbred as one of their parents. Parents 143-5-i and CML-387 were the latest (146.2 days) and the earliest (137.0 days) in maturity, respectively. Crosses involving 143-5-i generally matured late while those involving A-7016 were early. The mean grain yield combined over two years was 7.91 t ha^{-1}, with a range of 3.05 to 6.76 t ha^{-1} for inbred parents and 6.80 to 10.86 t ha^{-1} for crosses. Among the parents, 143-5-i was the highest yielding (6.76 t ha^{-1}) parent and its progenies 143-5-i x A-7016 and 143-5-i x SC-22 were also the highest yielding crosses, 10.78 and 10.86 t ha^{-1}, respectively.

Table 1. Combined analysis of variance for GLS disease parameters, agronomic traits and grain yield in diallel cross among eight maize inbred lines evaluated at Bako, Ethiopia in 2001 and 2002.

Source	Degrees of freedom	Latent period (d)	Disease incidence (%)	AUDPC	Lesion type (1-4)	Plant height (cm)	Days to maturity (d)	Grain yield (t ha^{-1})
Year (Y)	1	686.23	1463.1**	722.34*	13.01**	70.04	4134.38**	208.00**
Replication/Y	4	128.27	60.15	70.43	0.28	564.32	14.09	2.72
Entry (E)	35	334.94**	2646.86**	1389.01**	3.26**	5323.03**	87.84**	24.83**
Parents (P)	7	347.00**	4519.08**	2155.28**	0.76*	4051.81	79.32	10.81**
Crosses (C)	27	344.20**	2237.84**	1173.28**	3.54**	2062.37**	69.71*	6.88**
P vs. C	1	0.05	584.86	1849.83	13.20	102259.40	636.99	607.62
GCA	7	927.27**	9861.12**	5421.84**	12.06**	8346.06*	243.24*	21.54**
SCA	28	189.87**	843.3**	380.79*	1.06**	4567.44**	48.99*	27.03**
E X Y	35	101.96**	406.22**	213.77**	0.38**	743.43**	32.24**	3.16**
P x Y	7	47.55	344.2**	180.28**	0.15	1293.71**	25.33**	1.53
C x Y	27	118.52**	409.12**	214.31**	0.45**	571.95**	29.79**	2.76**
P vs. C x Y	1	35.71	762.06*	433.62*	0.10	1521.43**	146.76**	25.37
GCA x Y	7	165.36**	647.28**	382.08**	0.44**	1377.24**	71.58**	5.65**
SCA x Y	28	86.10*	345.96**	171.69**	0.37**	584.76**	22.41**	2.54*
Error	140	52.39	129.05	35.19	0.14	179.04	4.04	1.52

, Significant at 0.05 and 0.01 level of probability, respectively; GCA=General combining ability; SCA=Specific combining ability; AUDPC=Area under disease progress curve calculated using disease severity score of 1–5 scale*

Table 2. Mean performance of eight maize inbred lines and their crosses over two years (2001 and 2002) of evaluation at Bako, Ethiopia for disease parameters, agronomic traits and grain yield.

Entry	Latent period (d)	Disease incidence (%)	AUDPC	Lesion type (1-4)	Plant Height (cm)	Days to maturity (d)	Grain yield (t ha^{-1})
143-5-i	38.50	41.40	54.17	1.50	266.50	146.17	6.76
Gutto LMS_5	35.33	55.42	64.17	2.17	228.50	143.83	6.57
A-7016	34.17	90.00	102.92	3.83	216.00	136.50	4.30
SC-22	36.67	76.48	65.42	2.33	209.50	144.17	4.43
CML-197	33.83	90.00	82.08	3.17	184.50	142.67	4.19
CML-202	34.83	70.00	62.50	2.25	195.00	138.00	3.54
CML-387	56.83	8.35	38.75	1.00	192.17	137.00	3.05
CML-395	37.33	71.55	69.17	2.67	219.33	141.33	5.30
143-5-i × Gutto LMS_5	58.00	29.55	42.50	1.42	292.17	147.67	10.05
143-5-i × A-7016	34.50	75.19	68.75	2.25	299.67	146.17	10.78
143-5-i × SC-22	42.83	55.57	52.08	1.42	286.50	150.67	10.86
143-5-i × CML-197	42.33	72.94	52.92	1.67	264.50	148.50	8.77
143-5-i × CML-202	36.50	60.31	52.50	1.50	285.83	141.83	9.16
143-5-i × CML-387	37.33	59.23	55.83	1.92	296.17	147.83	9.93
143-5-i × CML-395	49.17	42.63	42.92	1.08	302.50	146.00	9.52
Gutto LMS_5 × A-7016	30.00	79.76	86.67	3.33	255.67	140.83	7.14
Gutto LMS_5 × SC-22	32.00	53.40	53.75	1.58	246.17	147.00	7.49
Gutto LMS_5 × CML-197	36.00	68.93	62.92	1.83	261.17	146.00	9.63
Gutto LMS_5 × CML-202	35.17	57.46	60.42	1.83	245.17	141.67	7.82
Gutto LMS_5 × CML-387	46.00	32.62	49.58	1.42	250.33	142.17	7.54
Gutto LMS_5 × CML-395	34.83	48.32	56.25	1.75	244.00	149.83	8.17
A-7016 × SC-22	33.00	73.90	66.67	2.58	263.83	145.83	8.60
A-7016 × CML-197	30.83	83.15	86.67	3.25	231.17	141.33	6.80
A-7016 × CML-202	30.83	82.18	85.42	3.25	267.50	139.33	8.04
A-7016 × CML-387	32.83	82.59	87.92	3.33	270.50	141.17	8.82
A-7016 × CML-395	31.83	78.86	81.25	2.92	255.17	144.17	9.24
SC-22 × CML-197	34.50	74.69	58.33	1.58	253.17	149.17	9.20
SC-22 × CML-202	34.33	69.64	63.75	2.33	259.83	145.83	8.94
SC-22 × CML-387	48.17	35.46	53.75	1.83	266.33	142.83	8.56
SC-22 × CML-395	37.50	35.63	47.50	1.42	272.50	148.17	8.71
CML-197 × CML-202	34.33	80.77	61.25	1.83	262.83	143.67	10.19
CML-197 × CML-387	50.00	49.27	48.33	1.75	246.67	151.17	8.75
CML-197 × CML-395	36.50	69.09	58.33	2.00	287.00	150.00	9.64
CML-202 × CML-387	34.83	40.18	60.42	1.50	250.50	140.50	7.05
CML-202 × CML-395	34.17	46.58	56.67	1.67	265.67	144.50	8.08
CML-387 × CML-395	54.67	12.51	36.67	1.00	273.17	145.67	9.07
Grand Mean	38.35	59.82	61.92	2.06	254.64	144.42	7.91
CV (%)	18.87	18.99	9.58	18.36	5.25	1.39	15.46
SE (m)	2.95	4.64	2.42	0.15	5.46	0.82	0.499

AUDPC=Area under disease progress curve calculated using disease severity score of 1–5 scale; CV=Coefficient of variation; SE=Standard error of the mean

3.2. Phenotypic Correlation among Traits

Assessment of relationships among traits measured using Pearson correlation coefficients (Table 3) indicated highly significant positive correlation coefficients between each pair of disease parameters. The correlation coefficients between AUDPC and lesion type were very high (0.97). This suggests that one of the rating methods used in this study may be suitable for characterizing the reaction of maize genotypes to GLS. Plant height showed positive and highly significant correlations with days to maturity and grain yield, indicating that tall plants were late maturing and high yielders. Days to maturity showed highly significant negative correlations with AUPDPC and lesion type, showing that high disease intensity caused early senescence of the plants. Grain yield did not show strong correlations with any of the disease parameters considered.

The principal component analysis was computed using the correlation matrix of all disease parameters to integrate these traits in to an index. The first two principal component axes (PC1 and PC2) accounted for 84.3 and 10.7% of the total variation in the data set (Table 4). The scores of PC1 that showed a large proportion of variation were associated with a short latent period, and high disease incidence, AUDPC and lesion type. AUDPC is the most important trait that contributed significantly to PC1 followed by disease incidence and lesion type. Principal component scores for the two axes showed highly significant correlations ($P< 0.01$) with all disease parameters. Among all these parameters, disease incidence and AUDPC showed higher correlation coefficient values with both PC1 and PC2.

3.3. Combining Ability Estimates

The effects of GCA, SCA and their interactions with the environment were significant for all traits (Table 1). Mean squares due to GCA were higher than those of SCA for all traits, except for grain yield. Though mean squares due to the interaction of GCA and SCA with the environment were significant for all traits, the values were much smaller than the main effects of GCA and SCA. Inbred parents 143-5-i and CML-387 showed highly significant positive GCA effects for the latent period and highly significant negative GCA effects for disease incidence, AUDPC and lesion type (Table 5). Conversely, inbred parent A-7016 exhibited highly significant negative GCA effects for the latent period and highly significant positive GCA effects for other disease parameters. Gutto LMS_5 and CML-395 depicted significant negative GCA effects for disease incidence, AUDPC and lesion type but CML-197 showed highly significant positive GCA effects for these traits. The parent 143-5-i showed highly significant GCA effects for plant height, days to maturity and grain yield. A-7016 was best combiner for days to maturity but poor for grain yield. CML-202 and CML-387 had highly significant negative GCA effects for plant height, days to maturity and grain yield. CML-395 was a poor combiner for both plant height and days to maturity.

As is evident in Table 6, crosses 143-5-i x Gutto LMS_5, 143-5-i x CML-395, SC-22 x CML-395 and CML-387 x CML-395 were good combinations for most disease parameters while 143-5-i x CML-387 and A-7016 x CML-387 were poor combinations. Most hybrids showed positive and highly significant plant height and days to maturity. Though many hybrid combinations showed highly significant positive SCA effects for grain yield, 143-5-i x A-7016 and CML-197 x CML-202 were the best combinations. Correlations between the *per se* performance of parental inbred lines and their GCA effects were strong and significant ($P< 0.01$) for disease incidence ($r=0.90$) and grain yield ($r=0.80$) (Figure 1). Similarly, the correlations between the sum of GCA effects of the two parents and F_1 performance (Figure 2) were positive and highly significant for disease incidence ($r=0.85$) and grain yield ($r=0.61$).

4. Discussion

Highly significant GCA and SCA mean squares (Table 1) indicated the importance of both additive and non-additive gene effects for resistance to GLS. Similar to findings of several researchers (Ulrich *et al.*, 1990; Donahue *et al.*, 1991; Gevers *et al.*, 1994; Menkir and Ayodele, 2005), this study showed that additive genetic effects played a major role in GLS resistance. However, the significant SCA mean squares observed for all disease parameters suggest that non-additive gene effects can also be exploited to develop GLS-resistant hybrid combinations. Similar conclusions have been drawn from different genetic studies conducted previously (Elwinger *et al.*, 1990; Hohls and Shanahan, 1995; Menkir and Ayodele, 2005). Significant interaction effects of entries and its partitions with years might be observed due to the differences in the magnitude of GLS scores of lines and their hybrids between years. While parental lines and hybrids had a consistent reaction to GLS across environments, Carson *et al.* (2002), and Menkir and Ayodele (2005) found significant genotype x environment interaction due to the magnitude of differences of resistance to GLS between lines and hybrids.

Based on the magnitude of mean squares, GCA effects were more important than SCA for plant height and days to maturity. In contrast, SCA effects are much more important for grain yield, indicating the need for the exploitation of heterosis in single cross hybrids for increasing maize productivity. Such a dominant role of SCA effects in the grain yield of maize was previously reported by Bhatnagar *et al.* (2004), Kemi *et al.* (1999), and San-Vicente *et al.* (1998).

For all disease parameters considered, genotypes showed a broad range of reaction to GLS disease (Table 2), indicating the inherent differences of the genotypes for resistance to GLS. Parents143-5-i and CML-387 showed consistent resistance reaction to GLS disease. Similarly, most hybrids with resistance reaction to GLS disease contain one of these lines as a parent, indicating the usefulness of these inbred lines in the development of GLS resistant hybrids. A-7016 was the most susceptible parent and all hybrids carrying this line exhibited a susceptible reaction to the disease. This suggests that A-7016 may carry a dominant GLS susceptible gene.

Table 3. Phenotypic correlation coefficients among disease parameter, agronomic traits and grain yield for eight maize inbred lines and their crosses evaluated at Bako, Ethiopia in 2001 and 2002.

Traits	Latent period (d)	Disease incidence (%)	AUDPC	Lesion type (1-4)	Plant height (cm)	Days to maturity (d)
Disease incidence (%)	-0.774**					
AUDPC	-0.720**	0.827**				
Lesion type (1-4)	-0.633**	0.807**	0.968**			
Plant height (cm)	0.137	-0.167	-0.308	-0.331*		
Days to maturity (d)	0.170	-0.149	-0.483**	-0.453**	0.539**	
Grain yield (t ha^{-1})	0.070	-0.050	-0.278	-0.305	0.909**	0.669**

,Correlation is significant at 0.05 and 0.01 level, respectively; AUDPC=Area under disease progress curve calculated using disease severity score of 1–5 scale*

Table 4. Eigenvectors of the first two principal component axes (PC1 and PC2) and the correlation of PC1 and PC2 scores with GLS disease parameters in eight maize inbred lines and their 28 F_1 crosses.

Disease parameters	Correlation coefficients		Eigenvectors	
	PC1	PC2	PC1	PC2
Latent period (d)	-0.78**	-0.72**	-0.46	0.78
Disease incidence (%)	0.97**	0.97**	0.51	0.15
AUDPC	0.95**	0.67**	0.52	0.33
Lesion type (1-4)	0.92**	0.65**	0.51	0.51
Eigenvectors			3.37	0.43
Proportion of variation (%)			84.30	10.70

*** Correlation is significant at the 0.01 probability level; AUDPC=Area under disease progress curve calculated using disease severity score of 1–5 scale*

Table 5. Estimates of General combining ability effects of GLS disease parameters, agronomic traits and grain yield of eight maize inbred lines used in diallel study at Bako, Ethiopia during 2001 and 2002 cropping seasons.

Lines	Latent period (d)	Disease incidence (%)	AUDPC	Lesion type (1-4)	Plant height (cm)	Days to maturity (d)	Grain yield (t ha^{-1})
143-5-i	3.25**	-6.02**	-8.15**	-0.43**	26.85**	2.12**	1.15**
Gutto LMS_5	-0.25	-5.75**	-1.69*	-0.10*	-4.01*	0.30	-0.02
A-7016	-5.30**	19.72**	21.19**	1.00**	-1.63	-2.80**	-0.32*
SC-22	-0.95	1.28	-3.06**	-0.11*	-2.45	1.80**	0.01
CML-197	-1.30	14.04**	3.56**	0.17**	-11.63**	1.54**	0.02
CML-202	-3.53**	3.87**	0.81	-0.01	-6.45**	-2.65**	-0.48**
CML-387	7.24**	-20.98**	-8.73**	-0.38**	-5.38**	-1.45**	-0.53**
CML-395	0.82	-6.17**	-3.94**	-0.14**	4.69**	1.12**	0.19
SE(gi)	0.874	1.372	0.716	0.046	1.616	0.243	0.149
SE(gi-gj)	1.321	2.074	1.083	0.069	2.443	0.367	0.225

, Significant at 0.05 and 0.01 level of probability, respectively; AUDPC=Area under disease progress curve calculated using disease severity score of 1–5 scale; SE (g_i)±= Standard error of general combining ability effect and SE (g_i-g_j)±= Standard error of the difference of general combining ability effects*

Inbred parent 143-5-i was the highest yielding, and high yielding crosses observed in this experiment involved this line as one of their parents. This indicates that 143-5-i has high frequency of favourable factors for grain yield. However, it showed tall plant height and long days to maturity, which is not desirable. This inbred line may be better utilized in hybrid development for GLS resistance and high grain yield if it can be improved regarding the undesirable traits observed. In addition to GLS resistance, CML-387 was an early maturing inbred. GLS resistant, early maturing and high yielding recombinant inbred lines may be developed from the cross between 143-5-i and CML-387. High yielding cross combinations were observed between Ethiopian inbred lines; vis-à-vis, 143-5-i x A-7016 and 143-5-i x SC-22, indicating the presence of different heterotic groups.

Lack of correlation between grain yield and disease parameters (Table 3) showed the absence of yield reduction due to disease pressure. However, Dagne *et al.* (2004) found 37% yield loss in susceptible varieties under artificial disease pressure. This indicated that the set of genotypes evaluated in this experiment were tolerant to GLS. Lack of correlation between disease parameters and grain yield is desirable for breeders as it is possible to simultaneously improve genotypes for disease resistance and grain yield. As the first two principal component axes (PC1 and PC2) were correlated significantly ($P< 0.01$) to disease parameters (Table 4), one of these parameters may

be used to discriminate maize genotypes into groups of resistance/susceptible to GLS. However, AUDPC showed the highest eigenvector of all others parameters; and hence, was more effective in explaining genotypic variation for GLS disease reaction.

Table 6. Estimates of specific combining ability effects of GLS disease parameters, agronomic traits and grain yield in 28 maize hybrids from diallel cross of eight maize inbred lines evaluated at Bako, Ethiopia during 2001 and 2002 cropping seasons.

Cross	Latent period (d)	Disease incidence (%)	AUDPC	Lesion type (1-4)	Plant height (cm)	Days to Maturity (d)	Grain yield (t ha^{-1})
143-5-i × Gutto LMS_5	16.64**	-18.5**	-9.59**	-0.11	14.68**	0.82	1.03*
143-5-i × A-7016	-1.81	1.67	-6.21**	-0.39**	19.80**	2.42**	2.01**
143-5-i × SC-22	2.18	0.48	1.37	-0.10	7.45	2.32**	1.83**
143-5-i × CML-197	2.03	5.09	-4.42*	-0.14	-5.37	0.42	-0.31
143-5-i × CML-202	-1.57	2.63	-2.09	-0.12	10.78*	-2.06**	0.59
143-5-i × CML-387	-11.51**	26.41**	10.79**	0.66**	20.05**	2.74**	1.45**
143-5-i × CML-395	6.74*	-5.00	-6.92**	-0.41**	16.31**	-1.66*	0.29
Gutto LMS_5 × A-7016	-2.81	5.96	5.25*	0.37**	6.66	-1.10	-0.43
Gutto LMS_5 × SC-22	-5.16	-1.95	-3.42	-0.26	-2.02	0.47	-0.41
Gutto LMS_5 × CML-197	-0.81	0.82	-0.88	-0.29*	22.16**	-0.26	1.72**
Gutto LMS_5 × CML-202	0.59	-0.49	-0.63	-0.11	0.98	-0.41	0.41
Gutto LMS_5 × CML-387	0.66	-0.47	-1.92	-0.16	5.08	-1.11	0.18
Gutto LMS_5 × CML-395	-4.09	0.42	-0.05	-0.07	-11.32*	3.99**	0.09
A-7016 × SC-22	0.89	-6.93	-13.38**	-0.37**	13.26**	2.40**	1.01*
A-7016 × CML-197	-0.92	-10.44**	0.00	0.01	-10.22*	-1.83*	-0.81
A-7016 × CML-202	1.31	-1.23	1.5	0.2	20.93**	0.35	0.93*
A-7016 × CML-387	-7.46*	24.03**	13.54**	0.65**	22.86**	0.99	1.77**
A-7016 × CML-395	-2.04	5.48	2.08	-0.01	-2.54	1.42	1.47**
SC-22 × CML-197	-1.61	-0.46	-4.09	-0.54**	12.6*	1.40	1.27**
SC-22 × CML-202	0.46	4.66	4.08	0.4**	14.08**	2.25**	1.50**
SC-22 × CML-387	3.53	-4.66	3.62	0.26	19.51**	-1.95**	1.18**
SC-22 × CML-395	-0.72	-19.31**	-7.42**	-0.39**	15.61**	0.82	0.60
CML-197 × CML-202	0.81	3.04	-5.05*	-0.39**	26.26**	0.35	2.77**
CML-197 × CML-387	5.71*	-3.61	-8.42**	-0.1	9.03	6.65**	1.35**
CML-197 × CML-395	1.37	1.39	-3.21	-0.09	39.3**	2.92**	1.54**
CML-202 × CML-387	-7.22**	-2.53	6.41**	-0.17	7.68	0.17	0.15
CML-202 × CML-395	-1.47	-10.95**	-2.13	-0.24	12.78**	1.60*	0.46
CML-387 × CML-395	8.26**	-20.16**	-12.59**	-0.54**	19.21**	1.57*	1.51**
SE(sij)	2.68	4.23	2.20	0.14	4.95	0.74	0.46
SE(sij-sik)	3.96	6.22	3.25	0.21	7.33	1.10	0.67
SE(sij–skl)	3.74	5.87	3.06	0.20	6.91	1.04	0.64

, Significant at 0.05 and 0.01 level of probability, respectively; AUDPC=Area under disease progress curve calculated using disease severity score of 1–5 scale; SE (s_{ij})±=Standard error of specific combining ability effect; SE (s_j-s_{ik})±=Standard error of the difference of specific combining ability effects of the crosses having one parent in common and SE (s_{ij}-s_{kl})±=Standard error of the difference of specific combining ability effects of the crosses having no parent in common*

The GCA effects of all disease parameters showed that 143-5-i and CML-387 were the most GLS resistant parents. These parental inbred lines had positive and highly significant GCA effects for the latent period, and negative and highly significant GCA effects for all other disease parameters. The lines may be used as sources of GLS resistance as they may combine well with other maize inbred lines to produce GLS resistant hybrids. Characters associated with resistance include lesion type and size, latent period and sporulation (Freppon *et al.*, 1996). Parents with negative GCA effects for lesion type are resistant as they exhibit flecks or chlorotic lesions, which produce few conidia (Ayers *et al.*, 1984). Hybrid combinations with positive and significant SCA effects for the latent period, and negative and highly significant SCA effects for other disease parameters had higher resistant reactions to GLS than those predicted based on their parental performances. Such genetic variations can be attributed to non-additive gene effects (dominance and epistasis) (Callaway *et al.,* 1990). Estimates of SCA effects showed that some crosses between two lines with good GCA, for example, 143-5-i x CML-387, had poor specific combinations. This indicates that the two parental lines may have the same gene for GLS resistance and cannot take advantage of additive gene effects.

Parental line 143-5-i was a poor combiner for plant height and days to maturity, but best for grain yield (Table 5). Hallauer and Miranda (1988) indicated that maturity and plant height are positively correlated to grain yield. So, 143-5-i may be used in hybrid variety development for areas receiving long rainy seasons. None of the inbred lines evaluated in this experiment had good GCA for three of the agronomic traits measured (plant height, days to maturity and grain yield). How ever inbred lines that

had good GCA effects for specific traits of interest can be used. Estimates of SCA effects for plant height and days to maturity showed that most crosses were taller and later than what was expected based on GCA effects of the parents. These are undesirable traits and very important for maize breeders because of lodging susceptibility and recurrent early termination of the rainy season in Ethiopia. The positive and significant SCA effects for grain yield in most cross combinations for grain yield indicate the manifestation of good SCA as the parents used for this study were inbred lines with a narrow genetic base. Additive gene effects are more important in determining traits in the population while non-additive gene actions are more important in single crosses (Hallauer and Miranda, 1988). Hybrids with higher SCA effects identified in this experiment may be further evaluated for possible release or used in the breeding programs to develop three-way and double cross varieties. The highly significant correlation coefficients observed between GCA effects and the *per se* performance of lines for disease incidence and grain yield (Figure 1) shows the possibility of using inbred line information to indicate hybrid performance. Thompson *et al.* (1987), and Saghai-Maroof *et al.* (1996) reported that GLS resistance could be evaluated effectively using inbreds *per se*. Disease incidence and grain yield of the progeny was well predicted by the sum of the GCA values of the parents (Figure 2). Menkir and Ayodele (2005) reported that inbred lines with high levels of GLS resistance produce hybrids with high levels of GLS resistance.

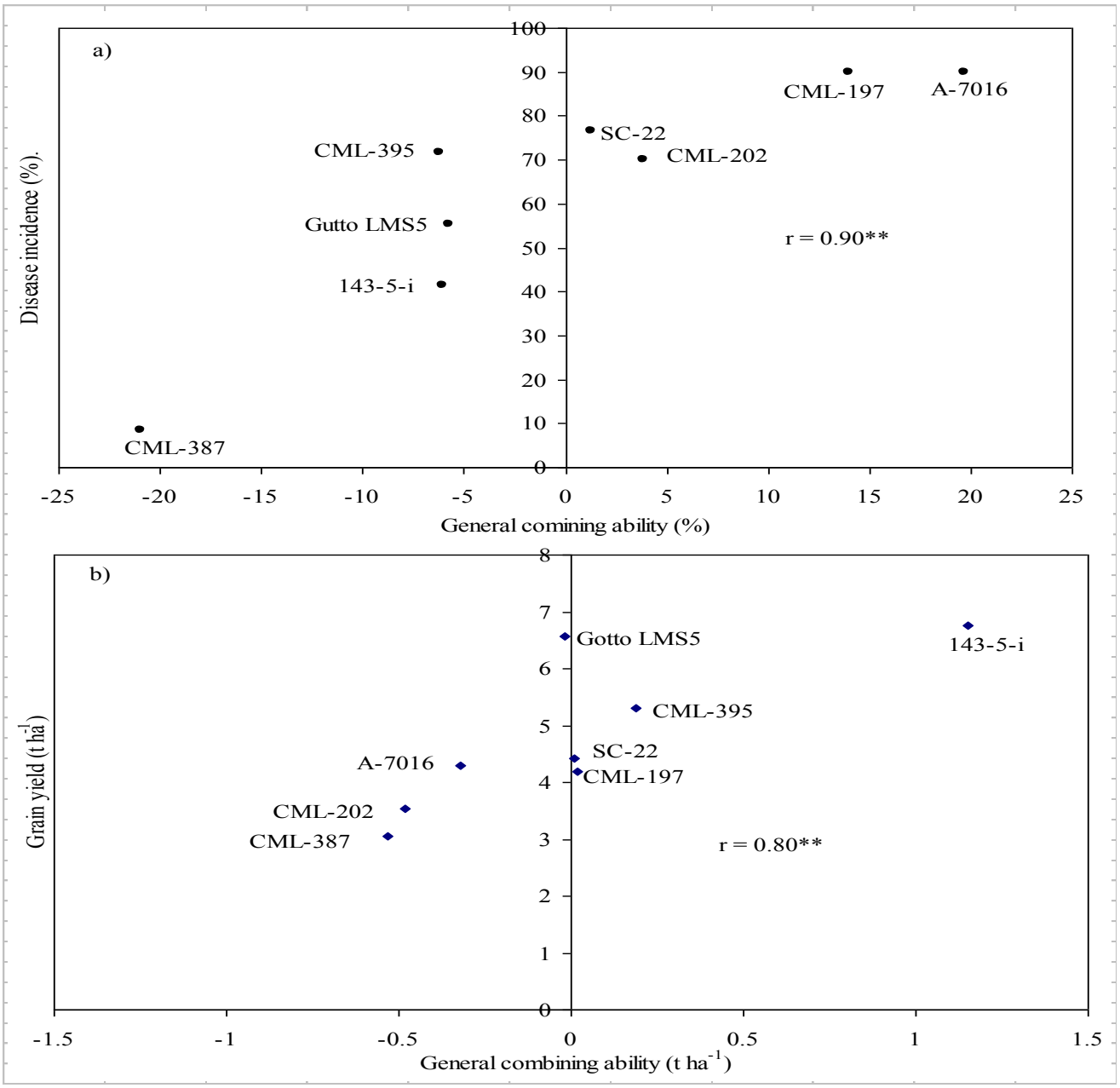

Figure 1. Relationship between GCA effects and *per se* performance for percent disease incidence (a) and grain yield (b) in eight maize inbred lines grown at Bako, Ethiopia in 2001 and 2002. ** Correlation is significant at 0.01 probability level.

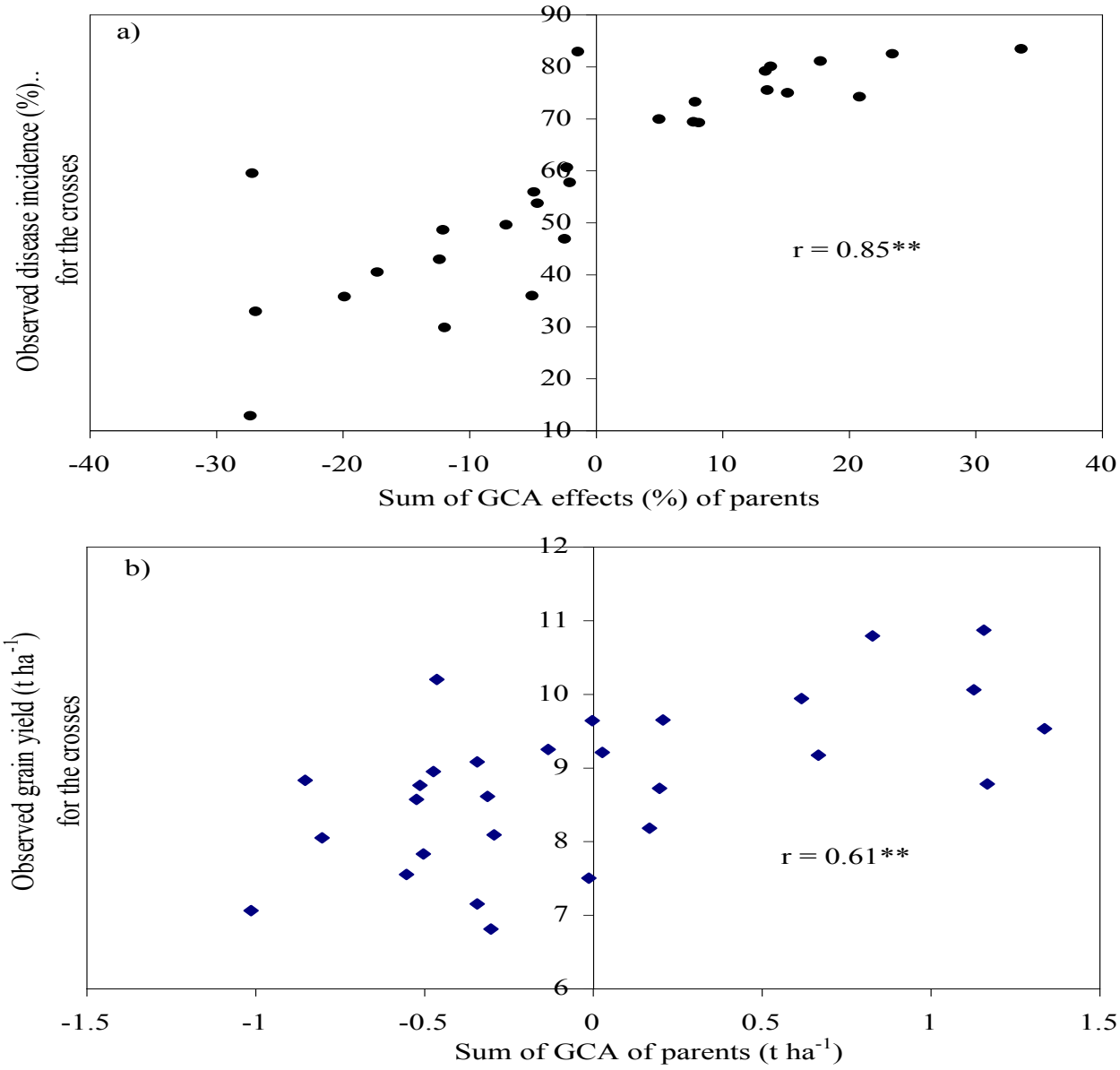

Figure 2. Relationship between GCA effects (sum of the two parents) and observed cross performances for percent GLS disease incidence (a) and grain yield (b) in 28 hybrids grown at Bako, Ethiopia in 2001 and 2002. ** Correlation is significant at 0.01 probability level.

5. Conclusion

The results of this experiment have demonstrated the importance of nonadditive gene action in the heritance of grain yield and additive gene action in the inheritance of GLS resistance. Lines with good GCA effects for grain yield and GLS reaction were identified. The information from this study will be useful for maize breeders in Ethiopia, as GLS is a relatively new disease and its mode of inheritance for resistance has not been established previously. Hence, local maize breeders can now incorporate these sources of resistance, which have shown a high level of resistance, and good combining ability, into the recurrent selection, hybridization and backcross breeding programs. In general, the findings of this study will be valuable for researchers who intend to develop high yielding as well as GLS-resistant varieties.

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Genetic Variability for Drought Adaptive Traits in A-511 Maize Population

Gezahegn Bogale[1], Van Rensburg J.B.J.[2] and Van Deventer C.S.[3]

[1] Melkassa Agricultural Research Center, P O Box 436, Nazret, Ethiopia
[2] ARC- Grain Crops Institute, Private Bag X 1251, Potchesfstroom 2520, Republic of South Africa
[3] Department of Plant Breeding, University of the Free State, P O Box 339, Bloemfontein 9300, Republic of South Africa

Abstract: Drought causes considerable yield reduction in maize (*Zea mays* L.) grown in the moisture stressed areas of Ethiopia. Increased crop production through improvement is expected if the adapted local genotypes possess variability for drought adaptive traits. Randomly taken 196 S_1 lines generated from Population A-511 were tested in 14 x 14 alpha lattice design at two plant densities in well watered and stressed conditions to estimate genetic variability (σ^2_G) and heritability for drought tolerant traits. Significant σ^2_G existed among the S_1 lines for yield, ears plant^{-1}, anthesis-silking interval, kernels ear^{-1} and kernels plant^{-1}, which are considered the most important traits for drought tolerance. Broad-sense heritability and σ^2_G for number of ears per plant (EPP) increased with increasing stress, while for yield and most other traits it increased with decreasing stress, reflecting their critical importance in selection under contrasting growing conditions. In general, there was considerable σ^2_G for important traits in Population A-511, which can be exploited for improvement in performance, both under drought and well-watered conditions.

Keywords: Drought Adaptive Traits; Drought Stress; Plant Density

1. Introduction

In sub-Saharan Africa, 40% of the maize (*Zea mays* L.) area experiences occasional drought, whereas 25% of the area is frequently affected (CIMMYT, 1990). In the moisture stress areas of Ethiopia where most farmers practice rainfed agriculture, production of this crop is seriously affected, mainly due to recurrent drought (Yitbarek, 1997). Although drought is unpredictable, maize is most susceptible to this stress at flowering (mid-season) resulting in substantial yield losses in the tropics. Drought at flowering allows no opportunity for farmers to replant or to compensate for loss yield. Thus, access to drought-tolerant genotypes that also perform well in favourable moisture conditions is considered as the only affordable option to these small-scale farmers (Bolaños and Edmeades, 1996).

Bolaños and Edmeades (1996) emphasized the effectiveness of screening simultaneously, both in drought stress and well-watered environments. This is because of the erratic nature of the rainfall during the cropping season which needs genotypes that perform well both under stress and in favourable conditions. However, screening under high plant density has been suggested for augmenting the selection for drought tolerance in areas with unpredictable rainfall patterns (Dow *et al.,* 1984). In additional, increased grain yield, ears plant^{-1}, kernels ear^{-1} and kernels plant^{-1}, and reduced anthesis-silking interval under moisture stress are considered as selection criterion and drought adaptive traits (Bolaños and Edmeades, 1996). Furthermore, for areas with high environmental variation, Ceccarelli (1996) indicated that genetic gains are possible by using locally-dapted germplasm and selecting in the target environment. Thus, the above selection strategy could increase the genetic gain in drought-prone areas if the adapted local genotypes have genetic variability for these pertinent traits.

The amount of genetic variation (σ^2_G) for various traits was determined by using unselected S_6 lines in Iowa Stiff Stalk Synthetic (Obilana and Hallauer, 1974), and S_1 to S_3 lines in six tropical maize populations (Bolaños and Edmeades, 1996). Hallauer and Miranda (1988) pointed out that, unlike any other level of inbreeding, randomly taken S_1 lines could be used to estimate σ^2_G. Evaluation of exotic genotypes for adaptation in Ethiopia is an ongoing activity. However, regarding the elite local maize populations in the drought stressed areas of Ethiopia, limited information has been reported about their available σ^2_G for drought tolerant traits (Hussein, 1999). This study was, therefore, undertaken to assess genetic variability and heritability for drought adaptive traits within the A-511 maize population.

2. Materials and Methods

In the 2001 main season, 400 S_1 lines were randomly produced from the original seeds of Population A-511 grown on 600 m^2 at Melkasa Research Centre. Out of these, 196 S_1 lines with adequate seeds were evaluated under the following four growing conditions: (i) Well-watered normal plant density (WWND), where about 44 400 plants ha^{-1} were established with a spacing of 30 cm between plants within rows, and irrigated at seven day intervals until maturity; (ii) well-watered high plant density (WWHD), where the plant density in environment 'i' was doubled with a spacing of 15 cm between plants; (iii) drought stressed normal plant density (DSND), where irrigation was suspended from 15 days prior to 50 % anthesis until 25 days after anthesis when one additional irrigation was applied, the plant population was the same as in 'i'; and (iv) drought-stressed high plant density (DSHD), drought-stressed as in 'iii' but with plant density increased as in 'ii'.

The study was conducted during the off-season (November to March 2003) on station, where a furrow irrigation system was used to apply about 40 mm of water (estimated by partial flume) every seven days. Rain did not interfere during the trial as there was drought in most parts of the country. The soil texture of the trial site was clay loam. The 196 S_1 lines in each environment were planted in a 14 x 14 lattice design with two replications. In addition, one border row of A-511 was planted at both ends of each block. Each entry was planted in a 4.2 m long row using 0.75 m inter-row spacing, and intra-row

*Corresponding author. E-mail: gezahegngb2002@yahoo.com

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spacing as determined above. The four trials were sown in adjacent blocks within the same field, while five free rows between well-watered and drought stressed conditions were left to avoid leaching to the stressed environments. Two seeds hill^{-1} were planted in all trials to ensure uniform stand and then thinned to the desired plant density (PD). As recommended by the centre, 50 kg P_2O_5 and 25 kg N ha^{-1} was applied at planting, followed by a side dressing of 25 kg N ha^{-1} 35 days later. Based on CIMMYT experience (Bolaños and Edmeades, 1996), data was collected for days to 50% anthesis (AD) and silking (SD), anthesis-silking interval (ASI) was computed by subtracting AD from SD, plant heights (PH), number of ears plant^{-1} (EPP), the uppermost ear length in cm (EL), number of kernels ear^{-1} (NKE), kernels plant^{-1} (NKP), number of primary tassel branches (TB), and grain yield plot^{-1}. The grain yield (GY) was reported in tons hectare^{-1} (t ha^{-1}) at 15% moisture content.

Data was first tested for normality, and ASI was normalized using $\log_e \sqrt{(ASI + 10)}$. Then it was analysed using the environment (plant densities and moisture levels) as fixed factors, and genotype, incomplete blocks within replicates, and replicates within environment as random factors. Analysis of variance for each trait in each environment was carried out by Alpha software. However, a randomised complete block design (RCBD) was used for covariance estimation, using AGROBASE™ 2000 software, since the relative efficiency of the alpha lattice over RCBD was below 30 % for each tested trait. In addition, combined analysis across the two plant densities in each moisture regime was conducted with PROC MIXED procedure from SAS (SAS, 1997). The genetic variances (σ^2_G) in each (Singh and Chaudhary, 1985) and across environments were estimated from the variance among S_1 lines, assuming no dominance effects (p = q = 0.5) (Hallauer and Miranda, 1988). For each growing condition, the error variance (σ^2_E) is equal to the mean square of error (MS_e), while σ^2_G was calculated on an environmental basis as: $\sigma^2_G = (MS_g - MS_e)/r$, where MS_g and MS_e were the mean squares of genotypes and error, respectively, and r number of replications. However, it has to be considered that the estimates of components of variance from one environment included a genotype x environment interaction (G x E) bias. Hallauer and Miranda (1988) reported that standard errors of estimates of σ^2_G were computed by taking the square root of $\frac{2}{r^2}\left[\frac{MS_g{}^2}{(n-1)+2} + \frac{MS_e{}^2}{(r-1)(n-1)+2}\right]$, where n was number of S_1 lines. Accordingly, broad-sense heritability (h_B^2) for a specific trait in each environment was estimated on a progeny mean basis as $h_B^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E / r)$. Since it was not possible to distinguish between the additive, dominant, and epistatic effects of the variance components, h_B^2 estimated the extent to which phenotypes were determined by the genotypes (Falconer, 1989).

From the combined analysis, with similar assumption made above, variance among genotypes (σ^2_G) provides an estimate equivalent to σ^2_A. Based on the expected mean squares as indicated in Table 1 (Hallauer and Miranda, 1988), σ^2_G was estimated as: $\sigma^2_G = [M_3 - (M_2 - M_1)/r - M_1]/re$, where M_3, M_2, and M_1 are the mean squares of S_1 lines, genotype by environment interaction (G x E), and experimental error; r and e are the number of replications and environments, respectively. The G x E variance was estimated as $\sigma^2_{GE} = (M_2 - M_1)/r$, whereas error variance (σ^2) was equal to M_1. Similarly, for S_1 lines that were evaluated in experiments repeated over environments, h_B^2 for each trait on progeny mean basis was estimated as: $h_B^2 = \frac{\sigma_G{}^2}{\sigma^2/(re) + \sigma^2_{GE}/e + \sigma^2_G}$. The standard error (SE) of σ^2_G also obtained as the square root of $2/(re)^2 \left[\frac{M_3{}^2}{n+1} + \frac{M_2{}^2}{(e-1)(n-1)+2}\right]$. As the combined analysis was done across two plant densities within each moisture regime (drought or well watered), G x E variances resulting from factors other than plant density were ignored.

Table 1. Analysis of variance of S_1 lines tested over environments.

Source	df	Mean squares	Expected mean squares
Environments, E	e-1		
Replications / E	e(r-1)		
S_1 lines (G)	n-1	M_3	$\sigma^2 + r\sigma^2_{GE} + re\sigma^2_G$
S_1 lines x E (G x E)	(e-1) (n-1)	M_2	$\sigma^2 + r\sigma^2_{GE}$
Pooled error	e(r-1)(n-1) [a]	M_1	σ^2

[a] *e, r, n refer to the number of environments, replications within environments, and number of S_1 lines, respectively.*

3. Results and Discussion

Variability in response among the S_1 progenies for the tested traits was highly significant (P ≥ 0.01) in each environment (data not shown). All traits were more sensitive to the moisture levels than to plant density. Figure 1 shows the S_1 lines' response in grain yield in each environment.

In all environments, the estimated variance components for the tested traits exceeded more than twice their standard errors (Table 2). The genetic variance (σ^2_G) and error variance (σ^2_E) for grain yield tended to be reduced with increasing relative yield reduction. Similarly, σ^2_G for NKE, NKP, EL and AD was reduced with increasing stress, especially from well-watered to drought. Consistent with the present results, Hallauer and Miranda (1988) pointed out that σ^2_G is compressed in stress environments for most of the traits. On the contrary, σ^2_G for EPP, SD, ASI and TB increased with rising density and moisture stress, with the highest magnitude under drought conditions. PH did not show a clear trend.

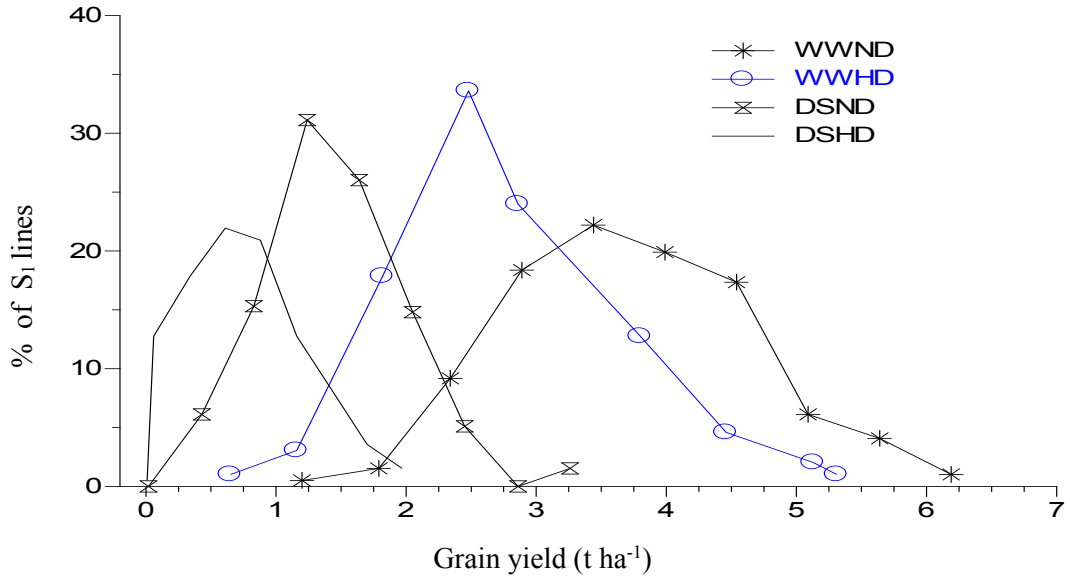

Figure 1. Grain yield performance of 196 S_1 lines across the four environments (WWND= well-watered normal plant density; WWHD= well-watered high plant density; DSND= drought-stressed normal plant density; DSHD= drought-stressed high plant density).

The estimates of the σ^2_G across PD in a well watered environment for NKE and NKP, and for TB in each moisture regime were less than their respective σ^2_{GE}. However, for all traits, the trend and magnitude of σ^2_G obtained within S_1 lines of Population A-511 was similar to those reported in six tropical lowland maize populations (Bolaños and Edmeades, 1996). In contrast to the present results, Guei and Wassom (1992) reported highest additive variance for SD and ASI within Pool 26 Sequia under non-stress, while for all the tested traits of La Posta Sequia this occurred under stress conditions. This indicates variability among populations in the expression of such traits. Except for AD and SD, the σ^2_E for most traits exceeded their respective σ^2_G, especially under drought conditions. Consistent with this result, there is higher σ^2_E than σ^2_G for various traits estimated in stress environments. Previous to these studies, Hallauer and Miranda (1988) reported higher σ^2_E for various traits estimated in stress environments compared to favourable growing conditions. The changes in magnitude of σ^2_G and σ^2_E with increasing yield were in the same direction for all traits, which also agreed with the results reported by Bolaños and Edmeades (1996). Under no-stress conditions, Obilana and Hallauer (1974) reported significant σ^2_G among the unselected S_6 lines for all traits of Iowa Stiff Stalk Synthetic. However, they indicated the difficulty of developing a group of unselected homozygous lines that adequately represent the base population. This setback was overcome in this study through randomly derived S_1 lines as suggested by Hallauer and Miranda (1988). However, since the estimation of σ^2_G through S_1 lines alone did not allow to the partitioning of additive and non-additive components of variance, further testing with involvement of mating design may be important, particularly for the hybrid program.

Broad-sense heritability for GY, NKE, NKP, EL and PH, increased with decreasing stress, while for EPP it increased with increasing stress (Table 2). Unlike the others, the broad-sense heritability for ASI was relatively higher under well-watered rather than stress conditions. Based on six tropical populations, Bolaños and Edmeades (1996) also reported the highest h_B^2 for GY, NKP and NKE under well-watered conditions, but under drought stress for EPP. These investigators indicated that the h_B^2 of ASI and EPP either increased or remained fairly constant with increasing moisture stress or declining yield level, which agreed with the current study, especially for EPP. AD showed almost similar h_B^2 across densities as well as moisture levels that agreed with Bolaños and Edmeades (1996) who noted decreased effects of environment on this trait. With Pool 26, Sequia, Guei and Wassom (1992) reported larger narrow sense heritability for GY, SD, and ASI under non-stress, and for AD and EPP under stress, but for all the traits in La Posta Sequia under stress conditions. No clear trend was exhibited for TB in h_B^2expression across environments. This may be due to the reduced effects of environmental stress as reported by Bolaños and Edmeades (1996). As expected, except for EPP and NKE, h_B^2 was reduced with increasing stress. However, the order of h_B^2 reduction due to the change in PD was smaller than the changes in moisture regimes. This was due to decreased σ^2_G rather than increased error variances. Lower σ^2_G and h_B^2 for GY under stress conditions have been reported in many other studies (Blum, 1988; Bänziger and Lafitte, 1997).

In each environment σ^2_G and h_B^2 were considered as over-estimated because σ^2_{GxE} was included in addition to σ^2_G, while relatively unbiased when estimated from combined environments. In addition to the presence of significant σ^2_G for pertinent traits within the A-511 population, their high h_B^2 obtained in environments where high σ^2_G with reduced σ^2_E indicates the possibility of its improvement for drought-stressed and favourable areas through intra-population recurrent selection.

Table 2. Estimates of components of variance and broad-sense heritability (h_B^2) for different traits of 196 randomly selected S_1 lines tested in each and across environments at Melkasa, 2002.

Envt[a]	WWND			WWHD			WWND + WWHD			
Trait	σ^2_G	σ^2_E	H_B^2	σ^2_G	σ^2_E	H_B^2	σ^2_G	σ^2_{GE}	σ^2_E	H_B^2
GY	0.60± 0.09	0.51±0.03	0.70	0.49±0.09	0.58±0.03 0.01±0.00	0.63	0.43±0.06	0.28±0.03	0.54±0.01	0.61
EPP	0.005±0.001	0.013±0.0	0.44	0.006±0.001		0.55	0.004±0.001	0.004±0.00	0.011±0.00	0.46
NKE	1438± 244	4051±204	0.42	1058±397	2536±128	0.45	990 ±216	1167 ±130	3293±83	0.41
NKP	1224± 348	4939±249	0.54	1196.5±37	3254±164	0.53	847±141	1026±66	834±69.3	0.54
EL	1.01± 0.25	2.34±0.13	0.46	0.57±0.23	2.17±0.11	0.35	0.94±0.15	0.36±0.07	2.25±0.06	0.56
AD	14.05± 1.8	7.43±0.37	0.79	13.47±2.1	11.4±0.58	0.70	11.09±1.39	2.38±0.35	9.93±0.25	0.75
SD	17.49± 2.2	8.03±0.41	0.81	18.02±2.81	15.28±0.7	0.70	15.87±1.99	4.44±0.50	12.56±0.32	0.75
ASI	3.05± 0.38	1.28±0.06	0.83	5.13±0.82		0.69	2.08±0.38	1.83±0.19	3.23±0.08	0.55
PH	167.2± 31	241.7±12	0.58	90.47±41.9	4.62±0.23	0.31	131±22.8	78.29±11.5	326±8.22	0.52
TB	2.80± 0.38	1.76±0.09	0.76	1.99±0.53	410±20.71 4.38± 0.22	0.48	0.42±0.28	3.39±0.22	3.066±0.08	0.15
Envt[a]	DSND			DSHD			DSND + DSHD			
Trait	σ^2_G	σ^2_E	H_B^2	σ^2_G	σ^2_E	H_B^2	σ^2_G	σ^2_{GE}	σ^2_E	H_B^2
GY	0.17±0.03	0.28±0.01	0.55	0.08±0.03	0.28±0.01	0.38	0.11±0.02	0.03±0.02	0.28±0.01	0.56
EPP	0.011±0.003	0.024±0.0	0.50	0.011±0.002	0.012±0.0	0.67	0.007±0.001	0.004±0.00	0.020±0.00	0.50
NKE	415±93.5	830±41.8	0.50	357.3±86		0.46	197.44±57	112.7±23.3	700.84±18	0.46
NKP	898±153	1063±53	0.48	830±159.6	700±39.8 1250±63	0.44	283±9.5	178±41.2	795±20	0.50
EL	0.65±0.22	2.42±0.12	0.35	0.47±0.28	3.50±0.18	0.21	0.40±0.15	0.22±0.1	2.96±0.07	0.32
AD	11.45±1.45	5.42±0.27	0.81	11.60±1.66	8.66±0.44	0.73	7.08±1.04	4.02±0.44	7.27±0.18	0.65
SD	26.9±3.50	14.2±0.72	0.79	24.29±3.77	22.7±1.14	0.68	18.32±2.73	9.70±0.96	18.46±0.46	0.66
ASI	4.67±0.86	6.43±0.32	0.59	6.30±1.21	9.47±0.48	0.57	3.48±0.72	1.68±0.31	8.45±0.21	0.54
PH	162±55.3	596± 30	0.35	99.16±49.7	592±29.8	0.25	133±32	3.16±14.98	594 ±15	0.47
TB	3.25±0.47	2.52±0.13	0.72	4.22±0.82	6.53±0.33	0.56	1.111±0.43	3.495±0.29	4.522±0.11	0.28

[a]*Envt = Enironment; see other abbreviations in the Materials and Methods part of the text; WWND= well-watered normal plant density; WWHD= well-watered high plant density; DSND= drought-stressed normal plant density; DSHD= drought-stressed high plant density.*

4. Conclusions

Highly significant genetic variability was observed for each tested trait within the A-511 population. The σ^2_G and h_B^2 for EPP increased with increasing stress, while for GY and most other traits it increased with decreasing stress, reflecting their critical importance in selection under contrasting growing conditions. For days up to 50% silking, ASI σ^2_G also increased with increased stress but their h_B^2 increased under well-watered conditions. This study confirmed that EPP is the best criterion for drought tolerance screening, mainly due to low σ^2_G and h_B^2 for yield in drought conditions. In general, adequate σ^2_G for drought adaptive traits as well as for other traits was detected within Population A-511, indicating its potential to be improved for better performance in both drought and non-drought conditions.

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Effects of Net Blotch (*Pyrenophora teres*) on Malt Barley Yield and Grain Quality at Holeta, Central Ethiopia

Teshome Galano[1], Chemeda Fininsa[2] and Geremew Bultosa[3]*

[1]Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, P O Box 37, Ambo, Ethiopia
[2]Department of Plant Science, Haramaya University, P O Box 138, Dire Dawa, Ethiopia
[3]Department of Food Science and Post harvest Technology, Haramaya University, P O Box 138, Dire Dawa, Ethiopia

Abstract: Barley (*Hordeum vulgare* L.) production is constrained by diseases such as net blotch caused by *Pyrenophora teres* Drechsl. The objectives of this study were to assess the effects of net blotch disease on malt barley yield and grain quality under natural infection. Four malt barley varieties (Beka, HB 120, HB 52 and Holker), three fungicide (propiconazole) spray intervals (at 7, 14 and 21 day) and no spray control were arranged in a randomized complete block design in four replications. The experiment was conducted at Holeta Agricultural Research Center, Ethiopia in the summer of 2005, on a plot of 12 m^2 for each treatment. Disease incidence and severity, yield and grain quality parameters were measured and analyzed. The results indicated a significant ($P< 0.05$) difference in disease incidence and percentage severity index at 107 days after planting and for the Area under the Disease Progress Curve among spray intervals and the varieties. Variation in yield and yield components were indicated among the varieties. Relative yield losses of 7.7–11.5% occurred in Beka and 15.3–21.2% in HB 120. The hectoliter weight (HLW) for both the varieties and the spray intervals ranged from 63.5 to 66.8 kg and were significantly different among the spray intervals and the varieties. The varieties had thousand-kernel weight (TKW) in the range of 32.5 to 46.4 g. Net blotch severity reduced yield and TKW in Beka and HB 120. However, in other grain quality factors analyzed, significant variations were observed only mong the varieties. On slotted sieve screens (mm): 2.8, 2.5, 2.8 + 2.5, 2.2 and < 2.2 the % grain mass retained ranged from 30.6–47.3, 40.6–50.6, 74.7–92.3, 6.6–18.8 and 1.0–5.7, respectively. The grain protein content (GPC) of the varieties ranged from 8.4–9.5%. The germination- capacity varied from 99.1 to 99.8% and germination energy from 98.5 to 99.4%. The study showed that the grain plumpness, GPC and germination characteristics of the varieties meet the malt and brewery industry requirements. However, HB 120 and HB 52 appeared superior to Beka and Holker.

Keywords: Grain Quality; Malt Barley; Net Blotch; Yield Loss

1. Introduction

Barley (*Hordeum vulgare L.*) is an important cereal crop in Ethiopia and covers 10% of the land under crop cultivation, with a yield of 1.2 t ha^{-1} (CACC, 2002). Many biotic factors limit the production of barley in Ethiopia (Yitbarek *et al.* 1996). Among the diseases, fungal diseases identified include: blotches, blights, mildews, rusts, smuts, and head diseases. Bacterial blight, barley yellow dwarf virus and nematodes of different genera are also known to limit the production of barley.

Barley is the most preferred cereal grain for malt (a key ingredient in brewing) production, because it has a good complement of hydrolytic enzymes, high starch to protein ratio and its hull acts as a filtering bed on the mashing (MacLeod, 2004). During malt production, grain hydrolytic enzymes are mobilized and, with the help of these enzymes, grain constituents are modified under controlled conditions (steeping, germination and kilning) (Hoseney, 1998). Malt supplies starch for hydrolysis into fermentable sugars, amylase enzymes that catalyze the hydrolysis of starch into fermentable sugars, other essential nutrients (free amino acids, vitamins and minerals) required for yeast in the fermentation and the desired flavor and color for the beer. To produce good malt, barley grain quality is determinant. The barley grain selected for malting should be sound and plumb with a high capacity for germination and should be generally free of trash, broken kernels and moulds (Hoseney, 1998).

Ethiopia faces a shortage of malt barley to meet the demand of the local breweries (Mohammed and Getachew, 2003). The yield and quality of malt barley are influenced by many factors. Net blotch disease caused by *Pyrenophora teres* Drechsl. is one of the major constraints facing barley production in Ethiopia (Yitbarek *et al.* 1996; Bekele *et al.* 2001; Asenakech, 2002; Bekele, 2005). The disease affects the foliage of barley and severely reduces its photosynthetic capacity, resulting in yield losses and excessive grain protein for malting due to reduced starch accumulation in the kernel (Horsley and Hochhalter, 2004). The net blotch infection on malt barley results in poor malt quality. The excess grain protein is undesirable for malting as it leads to a delay in germination, an excessive growth of rootlets on germination which, in turn, increases malting loss, reduces yield of extract and results in poor beer quality (Edney, 1996).

In Ethiopia, malt barley varieties under production are Beka, Holker, HB 120 and HB 52. These varieties were recognized as moderately resistant to net blotch with the exception of HB 52 (HARC, 1998/2000). Because of the inconsistent quality of malt barley and its low supply, the Ethiopian malting and brewing industries import malt barley and malt, respectively. Research on the effect of diseases such as net blotch could help to understand how yield and grain quality factors in malt barley are influenced by the disease. Such a study also could also generate information about how quality malt barley should be produced under proper net blotch disease

*Corresponding author. E-mail: Bultosageremew@yahoo.com

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management to contribute towards a reduction of dependency on imported malt barley, and thereby increase the income of local malt barley producers. Therefore, the objectives of this study were to assess the yield loss of malt barley due to net blotch, and its effect on grain quality.

2. Materials and Methods

2.1. Experimental Site

The field experiment was conducted at Holetta Agricultural Research Center (09^{O}03'N and 38^{O}30'E, 2400 m above sea level with mean annual rainfall of 1044 mm. The mean maximum and minimum temperatures were 22.0^{O}C and 6.1^{O}C, respectively with a mean relative humidity of 60.6%). The main rainy season is from June to September when it receives 70% of the annual rainfall. The experimental site has a nitisol soil with a pH of 5.24 and an average organic matter content of 1.8%. The soil contained 0.17% nitrogen, 4.55 ppm phosphorus and 1.12 potassium Meq/100 g soil (HARC, 2001).

2.2. Treatments, Experimental Design and Management

Four malt barley varieties (Beka, HB 52, HB 120, and Holker) and three fungicide (propiconazole) spray intervals (7, 14, and 21 day) and no spray control were used in a factorial randomized complete block design with four replications. The fungicide was applied at the rate of 1 L in 210 L of water ha^{-1}. To prevent drift during spraying, each treatment was sheltered with polythene sheets supported by four wooden poles. The entire experimental plot was sprayed with bayleton (active ingredient, i.e., is triadimefon) at a rate of 0.5 kg in 500 L of water ha^{-1} to protect it from barley scald and rusts only at initial stages of infection so as not to affect net blotch development.

The varieties were planted at the rate of 75 kg ha^{-1} in the experimental field with a plot size of 12 m^2, spacing of 0.2 m between rows, 1 m between plots, and 2 m between replications. Planting was carried out on 24 June 2005. Diammonium phosphate was applied at the rate of 100 kg ha^{-1}. Weeds were removed 40 days after planting (DAP), and the second weeding took place 35 days after the first.

2.3. Disease Incidence, Severity and Area under Disease Progress Curve (AUDPC)

Net blotch disease incidence and severity were assessed four times from the middle of four rows every 10 days starting from 87 DAP. Disease incidence data were obtained as a percentage of net blotch infected out of the total barley plants in the middle four rows. The severity data were taken from 10 randomly-selected and tagged barley plants from the middle four rows. A severity scoring scale of 1–10 was used, where 1= minute pinpoint or fleck-type lesions, without visible chlorosis and necrosis on the leaf blade and 10= coalescing lesions, more necrosis than chlorosis, and less than 10% green area visible on the leaf blade (Tekauz, 1985). The disease severity scores were converted to percentage severity index (PSI) as follows.

$$PSI = \frac{S_{nr}}{N_{pr} \times M_{sc}} \times 100$$

where S_{nr} is the sum of numerical ratings; N_{pr} is the number of plants rated; and M_{sc} is the maximum score on the scale.

AUDPC for each treatment was calculated from the severity assessments as described by Campbell and Madden (1990).

$$AUDPC = \sum_{i=1}^{n-1} [0.5(x_i + x_{i+1})][(t_{i+1} - t_i)]$$

where x_i is the percentage of disease severity index at ith assessment; t_i is the time of the ith assessment in days from the first assessment date; and n is the total number of days disease severity was assessed.

2.4. Grain Yield and Loss

Yield (kg) per plot and thousand kernel weight (TKW) in g in dry matter basis were collected. The relative loss (%) due to the disease was calculated for the parameters yield and TKW.

$$RYL\ (\%) = \frac{Y_{bt} - Y_{ut}}{Y_{bt}} \times 100$$

Where RYL is relative yield loss in %; Y_{bt} is the yield of base treatment (7 day spray interval); and Y_{ut} is the yield of unsprayed treatment.

2.5. Grain Quality

2.5.1. Mealiness and Glassiness

These were determined as described in the AOAC (1990) method No 935.28. Barley kernels (50) were cut cross-sectionally using a cutter (sharp knife). The cut surface of each kernel was grouped as mealy (more floury) or glassy (more vitreous) depending on the relative proportion of the two fractions and expressed as percentage.

2.5.2. Hectoliter Weight (HLW)

A measure of grain bulk density was determined on dockage-free samples using a standard laboratory hectoliter weight apparatus (EASY-WAY hectoliter weight test machine) as described in the AACC (2000) Method No 55-10. The values were adjusted to 12.5% moisture basis as described below:

$$HLW\ (12.5\%\ M\ basis) = HLW\ measured \times \frac{100-12.5}{100-\%\ moisture\ measured\ in\ the\ grain}$$

2.5.3. Sieve Test

A barley grain sample (100 g) from each plot was sieved through 2.8, 2.5, and 2.2 mm slotted sieves. The mass retained on each sieve (overs) was weighed and expressed as percentage (EBC, 1998).

2.5.4. Grain Size

Kernel size/dimension (width, length and thickness) was measured using a digital caliper (±0.01 mm) according to the modified method of Schuler *et al.* (1994). The values obtained were adjusted to 12.5% moisture basis.

2.5.5. Grain Moisture Content

Moisture content was determined by taking a 5 g barley flour sample after oven drying at 105 ^{O}C for 4 hrs, according to the AACC (2000) method No 44-15 A.

2.5.6. Grain Protein Content (GPC)

Grain protein content was estimated using the micro-Kjeldahl method of nitrogen analysis according to the AACC (2000) method No 46-11. A sample of about 1g of barley flour sample was used for the analysis. %GPC = %N x 6.25. Barley flour whose protein content was previously known was included as a standard control during the analysis.

2.5.7. Germination Energy

Barley kernels (100) were spread on wetted (4mL distilled water) filter paper lined on petri-dishes and allowed to germinate at 89.6% relative humidity set at a temperature of 16^{O}C in a relative humidity chamber (Termaks KBP 6395F, Bergen, Norway) for 3 days as described in EBC (1998) method 3.6. The germinated kernels of each treatment were counted after 3 days and expressed as a percentage.

2.5.8. Germination Capacity

This was carried out as described in the ACIB (1977). Representative 100 barley kernels were steeped in 40mL of 0.75% H_2O_2 in a clean Erlenmeyer flask of about 200mL. The steeped kernels were placed in a clean place at room temperature (23^{O}C) for 48 hrs. After 48 hrs, the chitted kernels were drained and counted; and the values were expressed as a percentage.

2.6. Statistical Analysis

Triplicate data (for quality parameters) was analyzed for descriptive statistics and ANOVA by statistical software SAS version 8.2, 1999–2000 (SAS Institute Inc., Cary, NC, USA). Means were compared by Duncan's multiple range test at 5% significance level.

3. Results and Discussion

3.1. Disease Incidence

The disease incidence differed significantly ($P < 0.05$) at 107 DAP among varieties and spray intervals (Table 1). At 97 DAP, there was a significant difference in incidence only among the varieties. At 107 DAP, the difference in both levels of factors was significant, but the interaction was not. Apart from the mean disease incidence at 14-day and 21-day spray intervals, the mean among spray intervals was significant ($P < 0.05$). The mean disease incidence among the varieties was also significant. At 117 DAP, the incidence was not significant among the varieties. Reduction in incidence after 107 DAP could be due to limited the amount of infected barley plants because of the fungicide propiconazole spray. It might also be due to the absence of conducive weather factors. Net blotch incidence is favored by humid, cool weather and susceptible early growth stage of the barley plant. Such an environmental situation did not prevail at 117 DAP. At 117 DAP, the weather was relatively drier (end of the rainy season) and the barley plant at the post-flowering stage. These factors, coupled with the controlling effect of the fungicide, could have reduced the disease incidence.

Asenaketch (2002) reported an average net blotch incidence of 94.7% on barley in central and northwest Ethiopia. Despite the conducive climatic conditions for net blotch disease at Holetta, the incidences recorded in this study were relatively low. Disease occurrence in a population of plants depends on the level of host resistance, the amount of initial inoculum and the growing environment (Campbell and Madden, 1990). Therefore, the low incidence recorded might also be due to the low amount of net blotch inoculum in the experimental field during the year. Bekele (2005) found that net blotch epidemics were influenced by seasonal weather conditions, as well as variety and effectiveness of the fungicide. In a low rainfall year, the epidemic was higher than in a high rainfall year. The effect of propiconazole application is more pronounced in most susceptible varieties.

3.2. Severity Index

The disease severity index was significantly ($P < 0.05$) different at 97, 107 and 117 DAP on all spray intervals (Table 1). However, this was not true at 87 DAP. The severity in plots sprayed at 7 day (1st spray) intervals was different to the 14, 21 day spray intervals and the control (no spray) plots. The 14 and 21 day spray intervals were also different to the control plots. However, no difference existed between the 14 and the 21 day spray intervals. Differences in percentage severity index among the varieties were recorded between HB 52 and Beka, HB 52 and Holker, HB 120 and Beka, and HB 120 and Holker at 97 and 107 DAP. But HB 52 and HB 120 were not different. Beka and Holker were found to be more susceptible to net blotch compared to HB 52 and HB 120. HB 52 was the least susceptible variety to the disease.

At 107 DAP, disease severity indexes of different spray intervals were significantly different from each other, with the lowest mean of 23.1% for the 7 day spray interval and the highest (70.2%) for the unsprayed plots (control). The differences at 107 DAP for varieties were similar to those of 97 DAP.

Among the spray intervals at 117 DAP, the lowest severity value was recorded at the 7 day spray interval and the highest for the unsprayed plots. The 14 and 21 day spray intervals were not different from each other but were different from the 7 day spray interval and the unsprayed plots. Severity reduction at 117 DAP in both treatments (varieties and spray intervals) could also be due to dry weather and the effects of successive fungicide spraying. Maximum net blotch severity of 27.4% among the three topmost leaf positions in Beka at 84 DAP, 39.5% in PGRCE 1694-5 at 76 DAP, 27.8% in Holker at 100 DAP and 15.25% in Ardu-12-60B at 76 DAP were reported by Bekele (2005) in 7–35 days spray interval of fungicide treatments in 2002 at Sinana in Bale. In the 2003 growing season, maximum disease severity indexes

of 71.3%, 85.4%, 82.1% and 19.1% were recorded at 84 to 100 DAP in Beka, PGRCE 1694-5, Holker and Ardu-12-60B, respectively. Asenaketch (2002) has reported a severity index of 28.8% in central and northwest Ethiopia. In this study, higher percentage severity indices were recorded at 97 and 107 DAP for both varieties and spray intervals. Severity index reduction after 97 DAP happened because net blotch pathogen did not have a chance to continue to infect the host under propiconazole spray.

Table 1.Incidence and severity index of net blotch (*Pyrenophora teres*) assessed at different days after planting on four malt barley varieties under three spray intervals with propiconazole fungicide, Holetta, summer season, 2005.

Treatment	Incidence (%)				Severity Index (%)			
	87 DAP	97 DAP	107 DAP	117 DAP	87 DAP	97 DAP	107 DAP	117 DAP
A. Variety								
Beka	2.9±1.7^{a*}	4.1±1.2^{a}	4.6±1.6^{a}	2.9±0.9^{a}	24.4±5.3^{ba}	52.2±12.5^{a}	53.5±24.6^{a}	22.4±8.8^{a}
HB 120	2.8±1.4^{a}	3.3±0.8^{ba}	3.7±1.6^{b}	3.2±1.3^{a}	24.6±8.3^{ba}	46.9±11.5^{b}	39.6±18.7^{b}	23.3±11.8^{a}
HB 52	3.8±3.6^{a}	3.1±1.2^{b}	3.8±1.6^{b}	3.2±1.5^{a}	21.9±6.9^{b}	45.3±11.7^{b}	34.1±19.0^{b}	24.3±15.0^{a}
Holker	2.8±1.3^{a}	4.2±1.5^{a}	4.9±1.5^{a}	3.2±0.9^{a}	28.4±10.0^{a}	53.9±11.8^{a}	56.8±24.6^{a}	23.8±9.2^{a}
B. Spray Interval (days)								
7	3.7±3.0^{a}	3.4±1.3^{a}	3.0±1.2^{c}	2.8±1.0^{b}	22.4±5.6^{a}	38.8±6.8^{c}	23.1±5.0^{d}	15.0±2.3^{c}
14	2.6±1.3^{a}	3.3±1.3^{a}	4.0±1.5^{b}	3.1±0.9^{ba}	23.6±8.3^{a}	46.4±7.7^{b}	37.3±19.1^{c}	19.1±4.3^{b}
21	3.0±1.9^{a}	3.7±1.2^{a}	4.1±1.3^{b}	2.8±1.0^{b}	25.6±9.0^{a}	48.6±9.2^{b}	53.5±21.3^{b}	18.9±3.2^{b}
No spray (control)	2.9±2.3^{a}	4.3±1.2^{a}	5.8±1.1^{a}	3.8±1.5^{a}	27.6±8.3^{a}	64.6±7.1^{a}	70.2±11.6^{a}	40.8±7.4^{a}

**Values within a column with different letters are significantly different at P < 0.05.*

3.3. AUDPC

The AUDPC was significantly different (P < 0.05) among the spray intervals and the varieties. The lowest AUDPC was obtained from the 7 day spray interval plots, followed by the 14, 21 day spray intervals, and unsprayed plots (Figure 1A). The AUDPC values between the spray intervals were different from each other. Fungicide application at the 7-days interval reduced the AUDPC compared to the rest of the spray intervals. Such significant reduction in the AUDPC has been also reported by Bekele (2005).

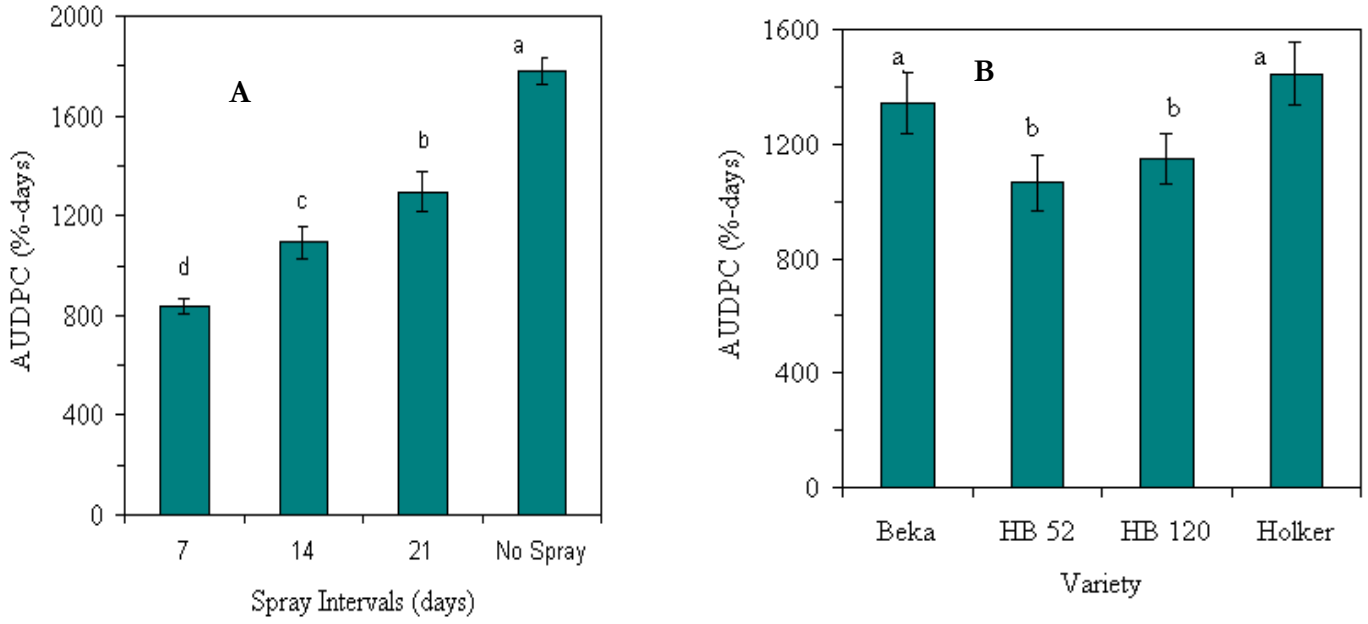

Figure 1. The AUDPC of net blotch (*Pyrenophora teres*) under three spray intervals and no spray (control) (A) and on four malt barley varieties (B). The bars are the standard errors and the different letters show significant differences at P < 0.05.

and Beka, and HB 120 and Holker (Figure 1B). However, the mean AUDPC was not significant between HB 52 and HB 120, and Beka and Holker. The AUDPC between Holker and Beka and HB 120 and HB 52 did not differ significantly, showing that the varieties have comparable resistance to net blotch. In a study reported by Bekele (2005), Holker and Beka were not significantly different in disease severity expressed as AUDPC in 2002. In terms of AUDPC reduction in Ardu-12-60B, a moderately resistant barley landrace, the range of average percentage of AUDPC reduction over Holker and Beka was similar with 13.0–41.1% (over Holker) and 14.6–61.4% (over Beka).

3.4. Yield Loss

A relative reduction in yield (kg/ha) due to net blotch occurred only in Beka and HB 120. A yield loss of 7.7–11.5% in Beka and 15.3–21.2% in HB 120 was recorded (Table 2). No yield loss occurred in HB 52 and Holker.

Various studies have shown that yield reduction due to net blotch is considerable. In Western Australia, an overall yield reduction of 21% occurred (Khan, 1987). In the semi-arid regions of Morocco, estimated yield losses due to net blotch varied between 14 and 29% with resistant varieties exceeding yielding by 39% compared to the susceptible variety without spraying and by 56% under fungicide treatment (Yousfi and Ezzahiri, 2002). Similarly, an increase in grain yield of 7.30–52.95% in the 2002 growing season and of 8.8–77.9% in the subsequent year were reported due to fungicide application (Bekele, 2005). For susceptible varieties, yield loss ranged from 30.7–40.9% in the 2002 growing season and 57.0–58.8% in the subsequent year (Bekele, 2005). In this study, a yield reduction of 7.7% in Beka and 15.3% in HB 120 appeared somewhat similar but slightly less than reported in Khan (1987), Yousfi and Ezzahiri (2002) and Bekele (2005).

3.5. TKW Loss

A reduction in TKW of 4.8% in Beka and 5.7% in HB 120 occurred in the unsprayed plots (control). On the other hand, HB 52 and Holker did not show a reduction in TKW. Holker, regardless of its highest percentage severity index at 97 DAP and 107 DAP, was not affected in both yield and TKW. The absence of TKW reduction in HB 52 might be due to the lowest severity indices at 97 DAP and 107 DAP. Low diseased leaf area allows the plant to carry out sufficient photosynthesis, thereby resulting in higher assimilate and possibly leading to higher TKW.

3.6. Grain Quality

Mealiness and glassiness

Endosperm texture, the relative proportions of mealiness (floury) and glassiness (vitreous) of the kernel showed no significant difference ($P < 0.05$) among the spray intervals but was significant among the varieties (Table 3) presumably because of genetic difference. However, no, significant difference among the varieties was observed when the means of any two varieties were compared for the percentage glassy kernels. The acceptable percentage of mealy kernels for malt is at least 95% and not greater than 2% glassy kernels according to the EBC (1998) standard adopted by Harar Brewery Share Company. The AOAC (1990) recommends mealy ≥ 90% and glassy ≤ 5%. The results obtained ranging from 40.8–80.6% for mealy and 19.4–59.2% for glassy endosperm, look fair because the data was for unmalted kernels and was estimated from visual observation of the amount of flour present when kernels were cut cross-sectionally. Glassiness indicates a reduced amount of starch carbohydrates and excessive grain proteins. The net blotch influence on the mealy and glassy fractions of the varieties appeared insignificant in this study, presumably due to its low incidence and severity.

Table 2. Effects of net blotch (*Pyrenophora teres*) under three spray intervals with propiconazole fungicide on yield and thousand kernel weight and their relative losses of four malt barley varieties, Holeta, summer season, 2005.

Variety	Spray intervals (days)	Yield (kg/ha)	Relative loss (%)	TKW (g)	Relative loss (%)
Beka	7	1625.00	0.0	33.2	0.0
	14	1332.50	18.0	31.8	4.2
	21	1437.50	11.5	33.5	+0.9
	No spray (control)	1500.00	7.7	31.6	4.8
HB 120	7	1770.00	0.0	43.5	0.0
	14	1395.00	21.2	39.7	8.7
	21	1687.50	4.7	42.8	1.6
	No spray (control)	1500.00	15.3	41.0	5.7
HB 52	7	1542.50	0.0	46.1	0.0
	14	1977.50	+28.2	45.8	0.7
	21	2060.00	+33.5	47.3	+2.6
	No spray (control)	2020.00	+31.0	46.6	+1.1
Holker	7	1270.00	0.0	39.7	0.0
	14	1480.00	+16.5	41.7	+5
	21	1730.00	+36.2	42.3	+6.5
	No spray	2062.50	+62.4	42.1	+6

The + sign shows relative yield increment.

Table 3. Effects of net blotch (*Pyrenophora teres*) under three spray intervals with propiconazole on grain mealiness/glassiness, hectoliter weight and grain size/dimension of four malt barley varieties, Holeta, summer season, 2005.

	Endosperm texture			Grain size/dimension		
Treatment	Mealy (%)	Glassy (%)	HLW (kg/hl)	GL (mm)	GW (mm)	GT (mm)
Variety						
Beka	80.6±10.0^{a*}	19.4±10.0^{c}	63.5±1.4^{c}	7.9±0.2^{c}	3.4±0.1^{c}	2.5±0.1^{c}
HB 120	40.8±13.7^{c}	59.2±13.7^{a}	66.8±1.1^{a}	8.3±0.2^{b}	3.6±0.1^{b}	2.6±0.1^{b}
HB 52	60.9±18.5^{b}	39.1±18.5^{b}	65.8±1.3^{a}	8.9±0.5^{a}	3.6±0.1^{a}	2.7±0.1^{a}
Holker	57.5±11.6^{b}	42.5±11.6^{b}	64.7±1.5^{b}	9.0±0.1^{a}	3.6±0.1^{a}	2.6±0.1^{ba}
Spray Interval (days)						
7	61.6±20.7^{a}	38.4±20.7^{a}	65.7±2.2^{a}	8.4±0.5^{b}	3.5±0.1^{b}	2.6±0.1^{b}
14	62.0±20.2^{a}	38.0±20.2^{a}	65.2±1.5^{ba}	8.5±0.5^{ba}	3.6±0.1^{ba}	2.6±0.1^{ba}
21	59.9±19.8^{a}	40.1±19.8^{a}	65.3±1.6^{ba}	8.7±0.6^{a}	3.6±0.1^{a}	2.6±0.1^{a}
No spray (control)	56.3±19.2^{a}	43.8±19.2^{a}	64.6±1.9^{b}	8.6±0.5^{ba}	3.5±0.2^{ba}	2.6±0.1^{ba}

**Values with different letters in a column are significantly different at P < 0.05. Where: HLW = hectoliter weight, GL = grain length, GW = grain width and GT = grain thickness.*

3.7. Hectoliter Weight (HLW)

This was significant (P < 0.05) among spray intervals and varieties (Table 3). According to grade specifications of malting barley in the USA, a HLW of 64.3 kg/hL for No 1 and 61.8 kg/hL for No. 2 are stipulated (Edney and Brophy, 2004). In Australia, a HLW of 65 kg/hL is specified for malt barley grade 1 and in Canada 63 kg/hL (for special select malting) and 61 kg/hL for select malting (Edney and Brophy, 2004). According to the EBC standard, a HLW of 65–75 kg/hL is required. In Ethiopia, Dashen Brewery Share Company requires a HLW of 75 kg/hL for grade 1, 70 kg/hL for grade 2 and 68 kg/hL for grade 3. The EQSA (2001) sets a HLW of 65, 65 and 60 kg/hL for 1st, 2nd, and 3rd grades malt barley, respectively. In this study, the HLW of the four malt barley varieties ranged from 63.5 kg/hL (Beka) to 66.8 kg/hL (HB 120). In terms of HLW the varieties meet most national and international requirements for malt barley production. The HLW, of the three spray intervals ranged from 65.7 kg/hL (7 day spray interval) to 64.6 kg/hL for no spray (control). The range was significantly different for unsprayed and 7 day spray interval plots indicating the negative effect of net blotch disease on the HLW of the four malt barley varieties.

3.8. Grain Size

Grain length (GL), grain thickness (GT), and grain width (GW) were significant (P < 0.05) among the varieties (Table 4). The mean GL was significant between Holker and HB 120, Holker and Beka, HB 52 and HB 120, HB 120 and Beka. The mean GT was significant between HB 52 and HB 120, HB 52 and Beka, Holker and Beka, and HB 120 and Beka. A significant difference existed between HB 52 and HB 120, HB 52 and Beka, Holker and HB 120, Holker and Beka, HB 120 and Beka for GW and GT. Grain size gives information on the soundness of the grain, because, with size, the amount of starch accumulated in the kernel may vary and also in the malting industry, grains of different sizes are not malted together.

3.9. Sieve Test (ST)

The kernels retained (overs) on the slotted sieves of 2.8 mm, 2.5 mm, 2.8 + 2.5 mm, 2.2 mm and passed through 2.2 mm (tails) indicated insignificant variation (p < 0.05) among kernels from sprayed plots, but significant among varieties (Table 4). The impact of net blotch on grain size appeared negligible. The difference between HB 52 and Beka, HB 52 and Holker, HB 120 and Beka, and HB 120 and Holker for the % kernels retained on slotted sieves (2.8 mm + 2.5 mm) was significant. Edney and Brophy (2004) classified, kernel plumpness of >85% as 1st grade and >80% as 2nd grade, when sieved on a 2.38 x 19.05 mm screen. In the Candian grading system for both grades, the throughs (tails) on a 1.98 x 19.05 mm screen should be <3%. In Australia the throughs they should be <7% when sieved over a 2.2 x 12.5 mm screen and in USA for use as < 3% when sieved over 2.18 x 19.05 mm screen 1st grade malt barley. In this study, the % retained (overs) on sieves (2.8 mm + 2.5 mm) ranged from 75–92% and the throughs (tails) of the 2.2 mm screen were <6%. Among the varieties, the kernels of Beka and Holker appeared to be less plump. Nevertheless, these two varieties meet the requirements of the malting and brewing industries.

3.10. Grain Moisture Content

There was no significant difference in the moisture content of the grain between the varieties and spray intervals (Table 4). The moisture content of the varieties ranged from 9.5–9.6%. The moisture level in the grain is influenced by field moisture drying conditions and, because of the small sample size and the dry weather conditions over the drying duration, barley grain was dried to an almost similar law moisture level. These values

are within the safe moisture range (<13%) required for malt barley storage (Edney and Brophy, 2004).

Table 4. Effects of net blotch (*Pyrenophora teres*) under three spray intervals with propiconazole on grain size, moisture and protein contents, germination capacity and germination energy of four malt barley varieties, Holetta, summer season**,** 2005.

Treatment	Sieve test (percent grain retained on various sieve sizes)								
	>2.8mm (%)	>2.5mm (%)	>2.8mm+> 2.5 mm (%)	>2.2mm (%)	<2.2mm (%)	MC (%)	PC (%)#	GC (%)	GE (%)
A. Variety									
Beka	30.6±8.9^{c*}	44.3±7.0^{b}	74.9±7.2^{b}	18.8±4.5^{a}	5.7±2.6^{a}	9.6±0.2^{a}	8.4±0.5^{b}	99.4±1.0^{a}	98.8±1.1^{ba}
HB 120	38.1±11.0^{b}	50.6±6.3^{a}	88.7±5.8^{a}	9.7±4.8^{b}	2.0±2.1^{b}	9.6±0.3^{a}	8.9±0.8^{a}	99.8±0.6^{a}	99.4±0.7^{a}
HB 52	47.3±7.9^{a}	45.0±4.5^{b}	92.3±3.9^{a}	6.6±3.4^{b}	1.0±0.6^{b}	9.5±0.5^{a}	9.5±0.9^{a}	99.3±1.2^{a}	99.4±0.7^{a}
Holker	34.1±9.3^{bc}	40.6±7.5^{b}	74.7±9.2^{b}	18.3±5.2^{a}	5.4±3.1^{a}	9.6±0.3^{a}	9.3±0.6^{a}	99.1±1.1^{a}	98.5±1.4^{b}
B. Spray Interval (days)									
7	36.2±11.1^{a}	46.6±5.4^{a}	82.8±11.2^{a}	13.5±7.9^{a}	3.6±3.5^{a}	9.6±0.3^{a}	9.0±1.0^{a}	99.6±0.9^{a}	98.6±1.3^{b}
14	36.4±11.0^{a}	46.1±5.7^{a}	82.5±8.5^{a}	14.1±6.4^{a}	3.3±2.3^{a}	9.6±0.3^{a}	8.9±0.7^{a}	99.4±1.0^{a}	99.4±0.7^{a}
21	41.0±10.7^{a}	42.9±8.6^{a}	83.9±11.8^{a}	11.4±6.6^{a}	3.5±3.3^{a}	9.6±0.5^{a}	9.1±0.7^{a}	99.4±0.9^{a}	98.9±1.1^{ba}
No spray	36.5±11.8^{a}	44.9±8.6^{a}	81.4±10.5^{a}	14.2±7.1^{a}	3.8±3.1^{a}	9.5±0.3^{a}	9.1±1.0^{a}	99.0±1.2^{a}	99.2±1.0^{ba}

**Values with different letters within the same column are significantly different at P < 0.05. # Values are on dry weight basis. Where: MC = moisture content, PC = protein content, GC = germination capacity and GE = germination energy*

3.11. Grain Protein Content

The protein content of Beka was significantly ($p < 0.05$) low compared to the rest of the varieties (Table 4). There was no variation in protein content due to spray intervals. The mean protein content of the varieties ranged from 8.4–9.5%. The desired protein content of malt barley lies in the range of 9–12% (MacLeod, 2004). High protein content in malt barley is not desirable because it leads to a reduction of malt extract caused by proportionally lower carbohydrate content (Kent and Evers, 1994 and Hoseney 1998). It also leads to a longer time of grain modification during malting, resulting in more rootlet development, greater respiratory and metabolic losses (Weston et al. 1993; MacLeod, 2004). The malt from high-protein barley can also contain relatively more soluble protein or albuminoid material compared to low-protein barley (Weston et al. 1993; Hoseney 1998). This soluble protein will pass into the extract, forming haze, possibly impairing the keeping quality of the beer (Weston et al. 1993; Hoseney 1998). Furthermore, development of bacteria is more likely to occur in liquor with a high albuminoid content (Weston et al. 1993; Hoseney 1998). On the other hand, a too low level of protein is not desirable because it limits the nutrient required for yeast growth in the course of the brewing process (Weston et al. 1993; Hoseney 1998). The EQSA (2001) specifies GPC of 9.00–11.00, 11.10–11.50 and 11.51–12.00 for 1st, 2nd and 3rd grade malt barley, respectively. This study revealed that varieties contained the desired protein content. Some varieties inclined towards the low protein content range stipulated for 1st grade malt barley by EQSA.

3.12. Germination

Germination capacity (GC %) showed no significant (< 0.05) variations due to variety and spray intervals (Table 4). Percentage GC for the varieties ranged from 99.1–99.8%. Germination energy (GE %) ranged from 98.5–99.4%. The Holker variety differed significantly in its GE from HB 120 and HB 52. The 14 days spray differed significantly in its GE from the rest of the spray intervals and the control. This study indicates that the influence of net blotch on germination is insignificant because of its low incidence. In the course of malt production, germination is required to mobilize the endogenous hydrolytic enzymes (dominantly α- and β-amylases) of the grain. These enzymes modify the structure of the grain, so that it will be readily solubilized during the brewing process to produce fermentable wort of desirable characteristic flavor and color with minimum loss of dry matter. Germination energy after three days should be >95% (Edney and Brophy, 2004). The EQSA (2001) specify germination energy of 95%, 92% and 90% for 1st, 2nd and 3rd grades of malt barley, respectively. Thus, the four malt barley varieties studied meet the germination energy requirements for the malting and brewing industries.

4. Conclusions

Varietal differences were observed in net blotch disease susceptibility and grain quality parameters. The AUDPC was significantly different among varieties and spray intervals. Yield reductions due to net blotch were observed only in Beka and HB 120. The yield of Holker and HB 52 were not significantly (< 0.05) affected. There was no significant variation in grain quality among the spray intervals. Among the varieties, Beka had the highest mealiness (%) and the lowest TKW. The % masses retained on sieve 2.8 mm + 2.5 mm for HB 120 and HB 52 were greater than that for Holker and Beka.

The HLW and the germination energy (GE) differences among the spray intervals and the varieties were significant. Unlike the HLW and the GE, grain size was significant only among the varieties. In terms of grain

length, Holker is the longest followed by HB 52. Additionally, these varieties differed from the other two in grain width and thickness.

Malt barley grain quality factors analyzed in this study were within the acceptable standards limits set by malting and brewing industries. It seems that the production of malt barley is not seriously affected in condition of low net blotch disease severity, provided that other factors are not limiting. Future net blotch or other diseases research on malt barley in Ethiopia should address hot spot areas, artificial inoculation and focus on the economic importance of the disease under consideration. A multidisciplinary research group consisting of barley breeders, plant pathologists, agronomists and cereal scientists and food technologists should collaborate to come up with high-yielding and good quality malt barley. Furthermore, malt barley producers should be provided with the complete package of information through training and demonstration. Malt factories should pay reasonable prices and even give incentives to farmers for products of premium quality. They should also communicate with barley researchers to provide feedback and convey their quality requirements.

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The Field Reaction of Long-Staple Cotton (*Gossypium barbadense* L.) Genotypes to Natural Infection of *Alternaria* Leaf Spot Disease in Ogun State, Nigeria

Popoola, A.R.[1]*, Awotide, O.G. [1], Adeoti, A.Y.A[1] and Ayodele, M.S[2]

[1]Department of Crop Protection, University of Agriculture, Abeokuta, Nigeria
[2]Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria

Abstract: Twenty-five genotypes of cotton were assessed in a randomized complete block design experiment with three replications at two locations for their reaction to natural infection by alternaria leaf spot under field conditions. Disease incidence was assessed at seedling and square formation stages. Other parameters measured were the lesion diameter, days of first symptom appearance, plant height, number of bolls, and seed cotton yield. Results showed that locations, genotypes and their interactions produced significant effect ($P \leq 0.05$) on seed cotton yield. Number of bolls and plant height were significantly ($P \leq 0.05$) affected by location, while days of first symptom appearance and lesion diameter were only significantly affected by type of cotton genotypes. GIZA 69, PIMA S_4 and BAR XL 7(79)6 had the highest average lesion size of 1.20 mm diameter in Abeokuta while PIMA S_4, BAR 14/25 (81)1 and BAR XL 7(79)6, with an average lesion size of 1.18mm diameter, had the largest lesion size in Ayetoro. BAR XL 7 (79) 33, BAR XL 7 (79) 25 and PIMA S_2 had a smaller lesion size (0.77 – 1.00 mm diameter) and a relatively higher yield, ranging between 824.89 – 976.96 kg ha^{-1} in both locations. Seed cotton yields of 871. 56 kg ha^{-1} and 976.97kg ha^{-1} for accession BAR XL 7 (79) 33 were the highest in both locations, while GIZA 68 and PIMA S_1 had the lowest seed cotton yield of 283.33 kg ha^{-1} and 347.90 kg ha^{-1} in Abeokuta and Ayetoro respectively.

Keywords: Alternaria Leaf Spot; Disease Reaction; Cotton; Nigeria

1. Introduction

Cotton is one of the most important and widely-grown cash crops in the world today. Its role as an income generator is recognized for both progressive, high input farmers and low income farmers in developing countries (Hillocks, 1992). In Nigeria, much of the cotton cultivated was restricted to the northern states in the savanna region of the country such as Sokoto, Zamfara, Kano, Borno, Adamawa, Bauchi, Gombe, Katsina, Kaduna and parts of Niger and Kwara (Nwanosike *et al.,* 2002). Diseases and pests forced farmers to abandon the crop in the early 1950s in the southern part of the country.

Alternaria Leaf Spot (ALS) is an important disease affecting cotton and has been reported from almost all the continents where cotton is grown (Munro, 1987; Hillocks, 1995). The disease is caused by a fungus *Alternaria macrospora* Zimm and is of major importance in Nigeria (Nwanosike and Adeoti, 2001). It manifests itself as small, brown, circular lesion spots on cotyledons, leaves, twigs, bracts and bolls of cotton (Bashan and Levanony, 1991). Later, the spots enlarge and coalesce with each other to form Alternaria blight (Munro, 1987). Transmission is through seeds (Rotem, 1994), wind from neighbouring fields (Munro, 1987) and infected debris from the previous year (Filajadic and Sutton, 1995).

ALS causes substantial yield loss to cotton, ranging from 25% (Cotty, 1987) to 37% in India (Padagamur *et al.,* 1989). In Nigeria, the disease is estimated to cause up to 39.7% yield loss with disease incidence of more than 80% (Nwanosike *et al.*, 2002). Earlier, Adeoti *et al.* (1995) reported an average incidence of 40-100% in the north-eastern growing zones of Nigeria. Idem (1999) observed a tremendous increase in the disease severity of a distance supporting the fact that ALS is a major disease of cotton in Nigeria. Adeoti and Popoola (2004), however, observed a lower range of 38-65% incidence in ALS in long staple cotton grown in the Northern Guinea Savanna of Nigeria.

Recently, there has been a determined effort by some state governments in the south-western part of Nigeria to re-introduce cotton cultivation. Ogun State is at the fore-front of this effort. In doing this, the onus is on researchers to offer those cultivars that have been tested and found capable of with standing the pressure of pests and diseases characteristic of high rainforest conditions in south-west Nigeria.

This paper reports the field reaction of twenty-five genotypes of long-staple cotton to Alternaria leaf spot under natural conditions in two locations in Ogun State, with a view to identifying those with low susceptibility to ALS and high seed cotton yield.

2. Materials and Methods

2.1. Crop Establishment and Management

Twenty five long staple cotton genotypes collected from the Institute of Agricultural Research, Zaria, Nigeria were assessed on the field at two locations in Ogun state, namely: University of Agriculture, Abeokuta (7^0 15'N, 3^0 25'E) and Olabisi Onabanjo University, College of Agriculture, Ayetoro campus (7^0 24'N, 3^0 06'E), for their reaction to Alternaria Leaf Spot of cotton. Cotton seeds of each of the 25 genotypes were sown on 1.5m x 3.6m plots, each having 5 ridges. This was replicated three times in a randomized complete block design. The replicates and the plots were separated by a one-meter border row. Inter-row and intra-row spacing was 90cm and 50cm respectively. Four seeds were planted per hole and the seedlings were thinned down to two seedlings per stand at three weeks after sowing. Each plot was hoe weeded four times, at intervals of three

*Corresponding author. E-mail: docakinpopoola@yahoo.com

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weeks. Compound fertilizer, N.P.K (15: 15: 15), was applied in two splits at the recommended rates of 60 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} and 40 kg K_2O ha^{-1} (Dadari *et al.,* 1994). The first application was carried out at four weeks after germination at a rate of 40 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} and 40 kg K_2O ha^{-1} (144 g/plot) while the remaining 20 kg N ha^{-1} was applied as a top dressing six weeks later as urea. The spot application method was used in applying the fertilizers.

Insecticide (Cypermethrin) was applied at the manufacturer's recommended rate of 900 ml/450 liters of water per hectare.

2.2. Disease Assessment

The plants were exposed to natural infection. Each cotton accession was assessed for disease incidence at the seedling and square formation stages by counting the number of infected plants in each plot and expressing this as a percentage of the total number of the cotton plants in the plot.

Thirty-six infected leaves were randomly harvested within the three middle rows per plot for measurement of lesion diameter, using a transparent metric rule. Diameters of ten lesions per leaf were measured, and mean values obtained.

The number of days (after planting) before the first symptom of alternaria leaf spot disease appeared were also recorded and average values calculated.

2.3. Plant Growth Parameters

Data was collected on the number of bolls per plant, plant height, and seed cotton yield. The number of bolls per plant was obtained by counting the number of bolls on each of the plants in the three middle rows assessed per plot and finding the mean. The plant height was obtained by using a meter rule to measure each of the plants in the three middle rows of each plot from the ground level to growing tip.

Hand picking of seed cotton was carried out four times. The seed data recorded.

2.4. Data Analysis

The data obtained was subjected to Analysis of Variance (ANOVA), and means separated using Least Significant Difference ($p \leq 0.05$). Data in percentage was arcsine transformed before analysis.

3. Results

3.1. Disease Incidence at Seedling and Square Formation Stages

There were significant differences in the incidence of ALS among the genotypes (Table 1) at seedling and square formation stages. At seedling stage, BAR XL7 (79) 33 had the lowest disease incidence of 40% and 37.50% at Abeokuta and Ayetoro, respectively. GIZA 45 had the highest disease incidence of 51.83% and 48.02% respectively, at Abeokuta and Ayetoro. Disease incidence was generally higher at the square formation stage than it was at the seedling stage. GIZA 45 had the highest disease incidence of 80.04% and 74.00% at the square formation stage in Abeokuta and Ayetoro, respectively. BAR XL 7 (79) 33 had the least disease incidence at the square formation in the two locations. Disease incidence at the two stages appeared to be higher at Abeokuta than at Ayetoro. This difference in disease incidence was statistically significant during the square formation stage but not so during the seedling stage ($p<0.05$) (Table 5).

Table 1. Incidence of ALS in cotton genotypes at the seedling and square formation stages at two locations (Abeokuta and Ayetoro) in Nigeria.

Cotton genotypes	Percentage Incidence of ALS			
	Seedling Stage		Square Formation	
	Abeokuta	Ayetoro	Abeokuta	Ayetoro
BAR X L 7 (79)6	47.32	44.42	74.04	67.32
BAR X L 7 (79)8	45.19	44.42	74.04	64.56
ACALA 1517C	45.11	41.76	69.60	70.44
BAR XL 7 (79) 25	41.00	37.58	72.60	66.72
BAR XL 7 (79) 33	40.00	37.50	62.64	59.28
BAR XL 7 (79) 34	44.44	43.56	64.68	63.48
BAR XL 7 (79) 35	46.2	46.01	75.96	66.00
BAR XL 7 (79) 36	45.42	45.38	76.68	64.89
BAR 14/25 (81) 1	45.28	45.28	75.48	64.68
BAR 14/25 (81) 14	45.28	43.99	73.32	64.68
BAR 14/25 (81) 16	45.69	42.62	71.04	65.28
BAR 14/25 (81) 18	45.28	41.98	69.96	64.68
BAR 14/25 (81) 23	48.05	47.38	78.96	63.63
BAR 14/25 (81) 24	48.05	48.00	72.60	68.64
BAR 14/25 (81) 39	45.70	44.21	73.68	65.28
BAR 14/25 (81) 43	46.20	44.35	73.92	66.00
PIMA S_1	44.35	47.30	67.32	63.36
PIMA S_4	46.70	43.20	72.00	66.00
BAR $14/25^A$	42.42	45.22	75.36	65.00
PIMA S_2	40.05	38.81	74.04	60.00
PIMA S_3	43.43	45.58	75.96	62.04
GIZA 45	51.83	48.02	80.04	74.00
GIZA 68	49.31	47.23	78.72	64.44
GIZA 69	47.63	44.78	74.64	68.04
BAR XL 7	44.55	43.99	73.32	68.64
LSD (5%)	2.55	3.05	2.85	3.25

3.2. Days of First Appearance and Size of Lesion in Cotton Genotypes

Table 2 shows the mean values of days after planting at which the first appearance of ALS symptoms was noticed. It also shows the lesion sizes, measured in millimeters. At both locations, it took approximately ten days from planting for symptoms to manifest themselves in seedlings of BAR XL 7 (79) 33. This was the longest latent period. Lesions were not evident in others until 7 to 9 days after planting. There were significant differences ($p \leq 0.05$) in days of first symptom appearance among the genotypes.

Lesion diameters of ALS ranged between 0.80-1.21 mm and 0.77-1.20mm in Abeokuta and Ayetoro respectively (Table 2). The smallest lesion size in Abeokuta was 0.80mm observed in BAR 14/25 (81) 1. In Ayetoro, BAR XL 7 (79) 25 had the smallest lesion size of 0.77mm. Significant differences occurred in lesion sizes of different genotypes, but locations did not confer any significant difference on the observed lesion sizes.

3.3. Plant Heights and Number of Bolls Per Plant

Plant heights of cotton genotypes ranged between 1.37-1.61m in Abeokuta (Table 3). The plants were less vegetative in Ayetoro, with plant heights ranging between 1.22 – 1.52m. Cotton plants grown at Abeokuta were taller than those grown in Ayetoro. BAR 14/25 (81) 39 was the

tallest accession, reaching a height of 1.61m in Abeokuta. The tallest cotton plant in Ayetoro with height of 1.52m belonged to cotton accession BAR 14/25 (81) 14.

The number of bolls per plant is also shown in Table 3. The number of bolls per plant was higher at Ayetoro. The average number of bolls per plant at Ayetoro ranged from 10.87 in BAR XL 7 (79) 25 to 18.27 in BAR 14/25 (81)1. At Abeokuta, the range was 8.27 – 16.47 bolls per plant.

3.4. Seed Cotton Yield

Results (Tables 4 and 5) showed that locations and genotypes significantly affected the yield of seed cotton. Table 4 shows that, BAR XL 7 (79) 33 had the highest seed cotton yield at the two locations - 871.56 kg ha^{-1} at Abeokuta and 976.96 kg ha^{-1} at Ayetoro. Table 5 shows the overall averages of seed cotton yield at Abeokuta (529.4 kg ha^{-1}) to be significantly lower ($p<0.05$) than the overall average of 618.6 kg ha^{-1} seed cotton yield at Ayetoro. A pattern of the significant effect of location was observed in the other two indices of growth – number of bolls per plant and plant height (Table 5).

Table 2: First symptom appearance and lesion diameter of alternaria leaf spot on cotton genotypes at two locations (Abeokuta and Ayetoro) in Nigeria.

Cotton genotypes	First Symptom Appearance (DAP*)		Lesion Diameter (mm)	
	Abeokuta	Ayetoro	Abeokuta	Ayetoro
BAR XL 7 (79)6	7.67	8.00	1.20	1.17
BAR XL 7 (79)8	8.33	8.00	0.93	0.97
ACALA 1517C	7.33	7.33	1.03	1.07
BAR XL 7 (79) 25	8.00	8.33	0.90	0.77
BAR XL 7 (79) 33	9.67	9.67	1.00	0.90
BAR XL 7 (79) 34	8.67	8.00	1.17	1.10
BAR XL 7 (79) 35	7.67	7.67	1.00	0.97
BAR XL 7 (79) 36	7.33	8.00	1.00	0.90
BAR 14/25 (81) 1	8.33	8.00	0.80	1.17
BAR 14/25 (81) 14	8.33	8.00	0.97	1.00
BAR 14/25 (81) 16	8.67	9.00	1.00	0.97
BAR 14/25 (81) 18	7.33	7.67	1.00	0.97
BAR 14/25 (81) 23	8.33	7.33	1.17	1.13
BAR 14/25 (81) 24	7.33	8.00	0.87	1.00
BAR 14/25 (81) 39	8.00	9.00	1.13	1.10
BAR 14/25 (81) 43	8.33	8.67	0.93	0.97
PIMA S_1	8.00	7.67	1.13	1.13
PIMA S_4	8.00	7.67	1.20	1.20
BAR $14/25^{A}$	8.33	8.67	1.00	0.97
PIMA S_2	9.00	7.67	0.87	0.90
PIMA S_3	8.67	8.67	1.00	0.87
GIZA 45	8.33	8.00	1.10	1.07
GIZA 68	7.67	9.00	1.00	0.97
GIZA 69	8.33	8.00	1.21	0.90
BAR XL 7	8.67	9.00	0.93	0.97
LSD (5%)	1.61	1.40	0.27	0.26

**DAP = Days After Planting*

4. Discussion

All the cotton genotypes were infected by *A. macrospora* to varying degrees, as reflected by the disease incidence and lesion diameter. Types of cotton genotypes contributed significantly to variations observed in such disease indices as days of first symptom appearance and lesion size. Locations, rather than genotypes, contributed significantly to plant growth indices such as plant height and number of bolls per plant. Both locations and genotypes contributed significantly to the seed cotton yield in the genotypes studied.

The susceptibility of cotton to *Alternaria macrospora* started at seedling stage and as early as seven days after planting. This pointed to the presence of high fungal inocula on the field at the two locations. In East Africa, attack by the fungus was considered by Ebbels (1976) to be the most damaging disease of cotton. Spross-Blickle *et al.* (1989) and Shtienberg *et al.* (1993) established early vulnerability of cotton to *Alternaria macrospora*.

Table 3: Growth performances of different cotton genotypes at two locations (Abeokuta and Ayetoro) in Nigeria.

Cotton genotypes	Plant height (m) Abeokuta	Ayetoro	No.of bolls per plant Abeokuta	Ayetoro
BAR X L 7 (79)6	1.59	1.36	13.80	11.67
BAR X L 7 (79)8	1.51	1.34	10.33	14.93

ACALA 1517C	1.37	1.37	10.33	12.53
BAR XL 7 (79) 25	1.56	1.22	16.47	10.87
BAR XL 7 (79) 33	1.52	1.37	15.13	16.33
BAR XL 7 (79) 34	1.52	1.23	9.60	11.47
BAR XL 7 (79) 35	1.40	1.39	8.27	12.87
BAR XL 7 (79) 36	1.51	1.19	13.00	11.93
BAR 14/25 (81) 1	1.53	1.51	13.47	18.27
BAR 14/25 (81) 14	1.43	1.52	14.27	17.67
BAR 14/25 (81) 16	1.57	1.31	9.93	15.47
BAR 14/25 (81) 18	1.53	1.35	14.87	13.53
BAR 14/25 (81) 23	1.42	1.51	11.67	16.00
BAR 14/25 (81) 24	1.46	1.24	6.80	15.07
BAR 14/25 (81) 39	1.61	1.26	9.67	15.27
BAR 14/25 (81) 43	1.43	1.48	8.87	13.00
PIMA S_1	1.57	1.35	9.33	12.00
PIMA S_4	1.39	1.35	12.40	13.10
BAR 14/25^A	1.43	1.35	14.80	15.87
PIMA S_2	1.54	1.42	14.93	15.63
PIMA S_3	1.55	1.47	5.73	15.80
GIZA 45	1.60	1.35	11.73	14.07
GIZA 68	1.51	1.28	13.73	11.13
GIZA 69	1.45	1.35	10.67	13.13
BAR XL 7	1.45	1.36	10.20	14.47
LSD (5%)	0.23	0.23	8.20	4.84

The disease appeared earlier at Abeokuta than at Ayetoro, and the lesion diameters of infected cotton leaves at Abeokuta were higher than those at Ayetoro. The differences were not significant (at $p<0.05$) at the two locations. In addition, the disease incidence was higher at Abeokuta at both the seedling and square formation stages than at Ayetoro. It was, therefore, not surprising that the number of bolls per plant was significantly higher in cotton planted at Ayetoro than that planted at Abeokuta. Bashi *et al.* (1983a) reported that free moisture and rain patterns were involved in increased Alternaria leaf spot. The higher relative humidity and rainfall at Abeokuta might have been partly responsible for the higher disease incidence and severity. Spross-Blickle *et al.* (1989) reported that the minimum temperature for the disease to occur was 10^0C, the maximum was 35^0C and the optimum was $20\text{-}25^0$C. The temperatures at both locations favored the spread of the disease.

ACALA 1517C	497.57	713.47
BAR XL 7 (79) 25	858.40	917.20
BAR XL 7 (79) 33	871.56	976.96
BAR XL 7 (79) 34	486.22	627.24
BAR XL 7 (79) 35	361.00	629.11
BAR XL 7 (79) 36	730.82	526.32
BAR 14/25 (81) 1	575.81	625.59
BAR 14/25 (81) 14	778.52	623.17
BAR 14/25 (81) 16	396.29	529.74
BAR 14/25 (81) 18	637.50	468.70
BAR 14/25 (81) 23	385.02	492.74
BAR 14/25 (81) 24	342.42	473.30
BAR 14/25 (81) 39	446.05	771.27
BAR 14/25 (81) 43	515.94	518.33
PIMA S_1	327.70	347.90
PIMA S_4	431.28	802.66
BAR 14/25^A	493.73	551.50
PIMA S_2	824.89	852.96
PIMA S_3	564.16	625.00
GIZA 45	342.07	391.21
GIZA 68	283.33	439.06
GIZA 69	460.35	539.02
BAR XL 7	408.00	688.51
LSD (5%)	169.30	177.70

Table 4. Seed cotton yield (kg ha^{-1}) of cotton genotypes in two locations (Abeokuta and Ayetoro) in Nigeria.

Cotton genotypes	Seed cotton yield (Kg/ha)	
	Abeokuta	Ayetoro
BAR XL 7 (79)6	713.01	509.97
BAR XL 7 (79)8	496.38	824.47

Table 5. Effect of locations on indices of growth and disease in cotton genotypes.

Growth/Disease Index	Mean values		Difference in mean values
	Abeokuta	Ayetoro	
Days of First Symptom	8.173	8.200	-0.027 (ns)
Lesion diameter (mm)	1.022	1.004	0.010 (ns)
Disease Incidence (%)			
Seedling stage	45.38	44.10	1.28 (ns)
Square formation stage	73.20	65.48	7.72 ($p<0.001$)
Plant height (m)	1.498	1.357	0.141 ($p<0.001$)
Number of bolls per plant	11.60	14.08	-2.48 ($p<0.001$)
Seed cotton yield (kg ha^{-1})	529.4	618.6	-89.2 ($p<0.004$)

5. Conclusion

BAR XL 7(79) 33 gave the highest yield of 976.96 kg ha^{-1} in Ayetoro and 871.56 kg ha^{-1} in Abeokuta, followed by BAR XL 7(79)25 which has 917.20kg ha^{-1} in Ayetoro and 858.40kg ha^{-1} in Abeokuta, and PIMA S_2 which gave 852.96kg ha^{-1} in Ayetoro and 824.89kg ha^{-1} in Abeokuta. These three genotypes were consistent in their low level of disease severity, low lesion diameter, high number of bolls per plant, and high seed cotton yield at both locations. BAR XL 7(79) 33, PIMA S_2 and BAR XL 7(79) 25 could be recommended to the government and farmers in Ogun State, south-west of Nigeria. They could also be recommended for other states in southern Nigeria and other areas with similar climatic conditions.

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Investigations on the Effect of Ball Burnishing Parameters on Surface Roughness and Corrosion Resistance of Hsla Dual - Phase Steels

D. Srinivasa Rao[1*], H. Suresh Hebbar[2], M. Komaraiah[3] and U.N. Kempaiah[1]

[1]Department of Mechanical Engineering, Golden Valley Institute of Technology, Kolar Gold Field -563 120, Karnataka, India
[2]Department of Mechanical Engineering, National Institute of Technology, Surathkal, Karnataka, India
[3]Department of Mechanical Engineering, Osmania University, Hyderabad, India

Abstract: Surface finish has a vital influence on most functional properties of a component, such as fatigue strength, wear resistance and corrosion resistance. This has led to processes such as lapping, honing, and burnishing. Burnishing is a fine finishing operation involving the cold working and plastic deformation of surface layers to enhance the surface integrity and the functional utility of a component. This study has been carried out to establish the effect of burnishing parameters, i.e. feed rate, speed, force, ball diameter and lubricant on surface roughness and corrosion resistance of HSLA dual – phase steel specimens. The result indicates that burnishing parameters have a significant effect on surface roughness and corrosion resistance.

Keywords: Hardness; Burnishing; Factorial Design; Inter-Critical Temperature

1. Introduction

At present, increased attention is being paid to surface integrity obtained, as surface finish is important not only on cosmetic grounds but also because it affects the functional performance of the component and is important for process control. Conventional processes have effects on surface finish, and have led to the evaluation of processes such as grinding, lapping, honing, burnishing and polishing. In recent years, however, much attention has been paid to processes which improve the surface characteristics by plastic deformation. Burnishing is such a process. It employs hard rollers and balls for the deformation. As well as improving the surface finish, burnishing secures increased hardness, wear resistance, corrosion resistance and fatigue life. The process can be automated to increase the production rate. Ball burnishing, a non-traditional finishing method that employs a hardened ball to plastically deform a surface, shows much promise. The work hardening is associated with the plastic deformation and the induction of surface compressive stresses improves the functional properties of the component.

The finishing of metals with a hardened surface layer has attracted the interest of researchers, e.g. those in the opto-mechanical industry. The functional performance of a component, such as fatigue strength, load-bearing capacity, wear resistance, and corrosion resistance depends on its surface characteristics such as hardness, surface finish, induced residual stresses and topography.

Hong and Jianying (2005) described burnishing as a cold working process which easily produces a smooth and work-hardened surface by plastic deformation of surface irregularities. In their work, the influence of the main burnishing parameters (speed, feed, force, number of tool passes and ball diameter) on surface roughness and the hardness of two different non-ferrous metals were studied. It was found that the burnishing force and the number of tool passes are the most pronounced parameters, because they had a great effect on the surface finish of the work pieces during the burnishing process.

Nemat and Lyons (2000) performed the experiment to study the effects of burnishing speed, feed, ball diameter, burnishing force and the number of passes on the quality of the work surface produced and its wearing characteristics. The wearing characteristics of the surface were measured using a specially designed experimental rig. The burnishing force and the number of passes are two of the most important parameters that govern the functional properties of final surface.

Khabeery and Axir (2001) conducted experimental work on vertical machining center to establish the effects of various burnishing parameters on the surface finish of 6061-T6 Aluminium alloys, including burnishing speed, ball material, lubricant, burnishing forces (depth of penetration) and feed. It was found that the burnishing speed and feed affect the surface finish.

Experimental work was carried out by Bonzid *et al.* (2004) to establish the effects of four ball burnishing parameters, depth of penetration, feed, ball material and lubricant on the surface roughness of AISI 1042 steel specimens. It has been noted that burnishing on AISI 1042 steel offers the best surface quality when using a small feed value. An analytical model has been defined to determine the relation between surface roughness and feed.

Hongyun *et al.* (2001) studied the effects of burnishing parameters on the surface roughness of aluminum alloy burnished with a cylindrical surfaced polycrystalline diamond tool.

Fang and Chien-hua (2003) studied the effect of ball burnishing parameters on the surface finish of a free form surface plastic injection mold on a machining center. Four burnishing parameters, namely the ball material, burnishing speed, feed and force were selected as the factors of Taguchi's experiment to determine the optimal burnishing parameters which have a dominant influence on surface roughness.

The intense interest centered on the development of ferrite-martensite dual-phase steels has led to numerous investigations. The content of such reports can be broadly classified into two groups: -

(i) Physical metallurgy aspects of dual-phase steels; which incorporate information and understanding related to the evolution of dual-phase microstructures, the effects of various alloying elements on microstructure developments, and the studies related to the kinetics of formation and nature of individual phases involved during phase transformation, and

*Corresponding author. E-mail: dsr_srinivasrao@yahoo.co.in

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(ii) Structure property relations in dual-phase steels; which include the attempts to search for correlations between the nature, volume fraction, size, and distribution of ferrite, marten site, and retained austenite, on one hand, and the strength, ductility, work hardening rate, fatigue life, corrosion resistance, toughness properties, on the other hand.

In this article, a systematic study of the effect of ball burnishing parameters on the surface roughness and corrosion resistance of HSLA dual- phase steel specimens is presented.

The overall objective of this investigation can be divided into five sections.

- To start with, achieving the optimization of the feed rate of the tool for a better surface finish;
- Optimizing the burnishing speed at an optimized feed rate to achieve a better surface finish;
- Optimizing the burnishing force at optimized feed rate and speed for a good surface finish;
- Finding out the effective lubricant at optimized feed rate, speed and burnishing force to enhance a very good surface integrity; and
- Optimizing the burnishing force at optimized feed rate and speed for good corrosion resistance.

3. Materials and Methods

3.1. Experimental Work

The experimental work was conducted on a Turn master lathe. The use of the lathe for pre-machining and burnishing operations enabled a wide range of parameter settings to be easily obtained and adjusted. A specifically designed burnishing tool shown in Figure1 is the main element in the burnishing process. It accommodates a bearing steel ball of various diameters. The ball is located in position by means of a rod and screws. The tool was held stationary and rigidly on the tool post of the lathe machine. The depth of penetration and feed terminologies are shown in Figure 2. The depth of penetration is the distance of the ball tip below the pre-machined surface; the feed is the horizontal distance between the two successive ball centers.

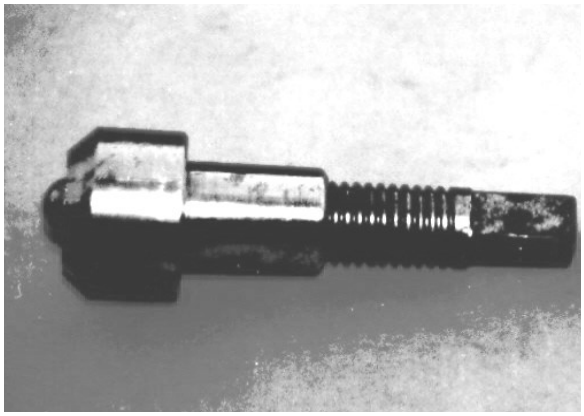

Figure 1. Ball burnishing tool.

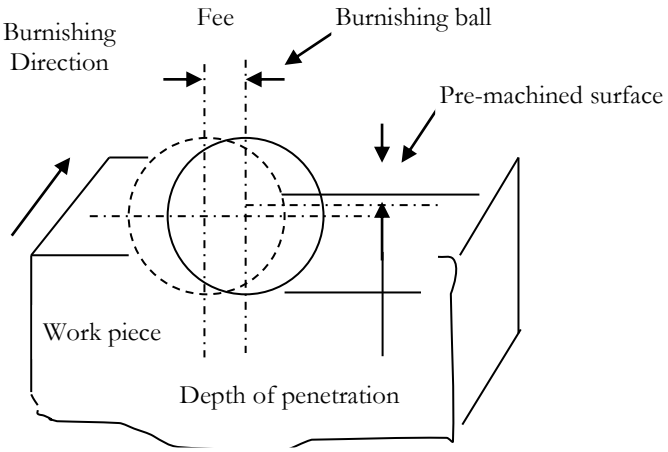

Figure 2. Schematic illustration of terminologies.

Cylindrical dual phase steel specimens were pre-machined to 18 mm diameter using a high speed steel (HSS) tool. These specimens were cut to the appropriate length of 200 mm and each was divided into 8 segments. Each segment was made 25mm in length by making grooves in between each segment with the intention of exposure to a different set of conditions during the experiment. The pre-machined surface roughness obtained was 2.644 μm to 3.0 μm .Grease was applied as a lubricant to the pre machined surface. Without removing the specimens, the surfaces were burnished by the ball burnishing tool which accommodates a constant ball diameter of 16.5 mm.

The surface roughness of the pre-machined and burnished specimens was measured using Mitutoyo SURFTEST equipment. The surface roughness traverse was taken perpendicular to the burnishing direction. The mean average roughness value was measured by taking an average of three readings. A lathe tool dynamometer was used to measure cutting force and thrust force. The thrust force was taken as burnishing force.

Dual phase steel specimens were burnished with different burnishing forces keeping optimum values of speed, feed, ball diameter and lubricant constant. The initial weight of the test specimens was measured by using an analytical balance. The test pieces were attached to copper pieces and dipped in diluted sodium chloride solution. Steel specimen and copper will form as a composite galvanic cell. The steel specimen will act as anode and the copper will act as the cathode of the galvanic cell and corrosion will occur on the steel specimens. All the test pieces, which are attached to copper, were subjected to same corrosive conditions. The test pieces were kept in corrosive media for 60 days. The test pieces were then cleaned and pickled with diluted sulphuric acid. The final weight of the test pieces was measured and the difference in weight is taken as the measure of corrosion resistance. The experimental results were shown in table 6 and figure 7.

3. 2. Material Composition

Commercial micro-alloyed steel supplied by Swedish Steel, [Oxelosund; Sweden] was selected as the starting material. The as- received steel was in the form of 20mm thick plate in the tempered condition. The chemical

composition of steel was ascertained with the help of Baird optical emission spectrometer. The analyzed composition of steel is shown in Table1.

3.3. Heat Treatment

The dual-phase microstructures were prepared by intermediate quenching (IQ). The IQ-treatment consisted of a double quench operation. The specimens were first soaked at 920 ± 2 ° C for 30 minutes and were quenched in 9% iced-brine solution (-7° C). These were then held at inter critical temperatures (ICT) of 730° to 780° C for 60 minutes and were finally quenched in oil (25 ± 2 ° C). The heat treatment process is shown in figure 3.

3.4. Micro Structural Characterization

Several stereological measurements were carried out to estimate the volume fraction of ferrite and martensite in the developed microstructures using manual point counting technique and automatic areal analysis using a LECO image analyzer. The result of volume fraction of ferrite and martensite is shown in Table 2.

Table 1. Chemical composition (wt %) of HSLA steel.

C	Si	Mn	S	P	Cr	Mo	B	Nb
0.15	0.27	1.24	0.004	0.009	0.05	0.03	0.0012	0.022

Table 2. Results of volume fractions of ferrite and martensite.

Specimen	Volume % ferrite	Volume % martensite
730° C	66.90	33.10
740° C	62.45	37.55
750° C	59.82	40.18
760° C	54.95	45.05
770° C	51.54	48.46
780° C	48.10	51.90

4. Results and Discussion

The experiments were conducted for all possible combinations of burnishing parameters and the results are shown in Tables 3 to 6.

4.1. Effect of Feed

The effect of feed on surface roughness of HSLA dual-phase steels is significant. It was observed that, with an increase of feed from 0.024mm/rev to 0.085mm/rev, the surface roughness decreases, which is shown in figure.6. The optimum feed found from the experimental results is found to be 0.085mm/rev. The work hardening effect on the burnished surface is greater at lower feed and decreases with increase in feed. The reverse phenomena could be explained by observing the relationship between the feed and the force. With excessively low feed rate, considerable surface roughness is observed. The depth and degree of work hardening are greater at low feed rate and decrease with increasing feed rate. The longer period of contact between the ball and the work metal surfaces causes excessive work hardening and, consequently, flacking of the surface layer, giving poor surface finish.

Table 3: Experimental values of surface roughness of duel phase steels after burnishing at various feed rates.

Feed in mm/rev	Surface Roughness µm					
	730^{0}C	740^{0}C	750^{0}C	760^{0}C	770^{0}C	780^{0}C
0.024	0.998	1.408	1.512	1.561	1.606	1.712
0.034	0.946	1.326	1.463	1.496	1.523	1.648
0.043	0.883	1.245	1.411	1.458	1.478	1.612
0.054	0.856	1.214	1.389	1.409	1.443	1.568
0.065	0.841	1.182	1.363	1.376	1.402	1.544
0.074	0.811	1.139	1.337	1.348	1.388	1.502
0.085	0.801	1.112	1.326	1.339	1.375	1.478
0.094	0.837	1.167	1.378	1.406	1.419	1.537
0.098	0.896	1.197	1.412	1.451	1.465	1.586

Table 4: Experimental values of surface roughness of duel phase steels after burnishing at various speed (at optimum feed of 0.085 mm/rev).

Speed in m/min	Surface Roughness µm					
	730^{0}C	740^{0}C	750^{0}C	760^{0}C	770^{0}C	780^{0}C
5.65	1.078	1.21	1.576	1.463	1.178	1.339
11.3	1.014	1.179	1.492	1.409	1.139	1.303
17	0.911	1.154	1.419	1.364	1.068	1.268
22.62	0.864	1.106	1.37	1.298	1.006	1.204
8.27	0.903	1.184	1.421	1.358	1.065	1.289
34	0.956	1.205	1.538	1.437	1.163	1.324

Table 5: Experimental values of surface roughness of duel phase steels after burnishing at various burnishing forces (at optimum speed of 22.62 m/min, Optimum feed of 0.085 mm/rev using grease as lubricant).

Force in kgf	Surface Roughness µm					
	730^{0}C	740^{0}C	750^{0}C	760^{0}C	770^{0}C	780^{0}C
5	1.364	1.483	1.558	1.572	1.403	1.342
10	1.358	1.478	1.543	1.564	1.396	1.336
15	1.351	1.47	1.537	1.558	1.388	1.329
20	1.349	1.462	1.53	1.547	1.38	1.32
25	1.336	1.458	1.522	1.532	1.372	1.314
30	1.342	1.464	1.534	1.544	1.382	1.323
35	1.35	1.472	1.54	1.552	1.389	1.336
40	1.362	1.48	1.552	1.565	1.401	1.345

Table 6. Experimental values showing reduction of weight for different volume fractions of ferrite and martensite after corrosion.

730 ^{0}C			
Burnishing Force in kgf	Initial Weight in gms	Final Weight in gms	Difference in Weight in gms
5	16.6644	16.6358	0.0286
10	16.0565	16.0296	0.0269
15	16.2632	16.2364	0.0268
20	16.0366	16.0107	0.0258
25	16.4244	16.3982	0.0261
30	16.6163	16.5892	0.027
35	16.3393	16.312	0.0273
40	16.6024	16.574	0.0283

740 ^{0}C			
Burnishing Force in kgf	Initial Weight in gms	Final Weight in gms	Difference in Weight in gms
5	16.1747	16.1175	0.0272
10	16.5194	16.4919	0.0274
15	16.2393	16.2126	0.0266
20	16.8529	16.8264	0.0265
25	16.6518	16.6256	0.0262
30	16.3503	16.3239	0.0263
35	16.3254	16.3253	0.0269
40	16.6267	16.5987	0.0279

750 ^{0}C			
Burnishing Force in kgf	Initial Weight in gms	Final Weight in gms	Difference in Weight in gms
5	16.8232	16.7954	0.0277
10	16.1175	16.0915	0.0259
15	16.4933	16.4672	0.026
20	16.3091	16.2838	0.0252
25	16.4259	16.4009	0.0249
30	16.5908	16.5647	0.026
35	16.7581	16.7309	0.0271
40	16.6699	16.642	0.0278

760 ^{0}C			
Burnishing Force in kgf	Initial Weight in gms	Final Weight in gms	Difference in Weight in gms
5	16.0344	16.0084	0.0259
10	16.4244	16.3986	0.0257
15	16.3376	16.3125	0.0251
20	16.2632	16.2389	0.0242
25	16.3148	16.2903	0.0244
30	16.2397	16.2143	0.0253
35	16.0362	16.0111	0.025
40	16.3038	16.2779	0.0259

770 ^{0}C			
Burnishing Force in kgf	Initial Weight in gms	Final Weight in gms	Difference in Weight in gms
5	16.6358	16.6095	0.0262
10	17.2654	17.2388	0.0265
15	16.3401	16.3154	0.0246
20	16.7122	16.6878	0.0243
25	16.5788	16.5554	0.0233
30	16.1477	16.1244	0.0232
35	16.0174	15.9935	0.0238
40	15.0362	15.013	0.0231

780 ^{0}C			
Burnishing Force in kgf	Initial Weight in gms	Final Weight in gms	Difference in Weight in gms
5	16.451	16.4253	0.0256
10	16.705	16.6799	0.025
15	16.0052	15.9818	0.0233
20	16.7884	16.7645	0.0238
25	16.6581	16.6349	0.0231
30	16.8264	16.8026	0.0237
35	16.3395	16.3154	0.024
40	16.6511	16.6266	0.0253

4.2. Effect of Burnishing Force

From the experimental results, it was observed that surface roughness decreases with an increase in burnishing force. After a certain burnishing force (optimum value), the surface roughness increases as shown in figure 5. In this case, the optimum burnishing force found was 25kgf. The decrease in surface roughness is due to the plastic deformation of surfaces. However, beyond the optimum value, due to the distortion of the micro profile and excessive work hardening, flaking of surface layers will occur and hence surface roughness increases.

4. 3. Effect of Speed

From the experimental values, it was observed that the surface roughness decreases with increasing speed, which is shown in figure 4. At lower burnishing speeds, due to repeated burnishing causing flaking of surfaces, roughness is high. At higher speeds there is insufficient burnishing and surface roughness is high. Hence there is an optimum burnishing speed of 22.62 m/min, which gives the highest surface finish.

4.4. Effect of Burnishing Force on Corrosion Resistance

From the experimental results, it was observed that the percentage reduction in weight of the components decreases with an increase in burnishing force that is shown in figure 6. Beyond the optimum value of burnishing force (25kgf), the percentage reduction in weight increases with an increase in burnishing force. The reason for the decrease in percentage reduction in weight with an increase in burnishing force may be due to the plastic deformation of the surface of the components by obtaining the highest surface finish. Corrosion of the metals is affected by the surface quality, rough surface usually corrodes rapidly compared to that of a smooth surface. As burnishing improves the surface finish of the metals, it also improves the corrosive resistance of the components. The corrosive resistance is improved up to a certain level of burnishing force beyond which it decreases, due to the flaking effect on severely work-hardened layers.

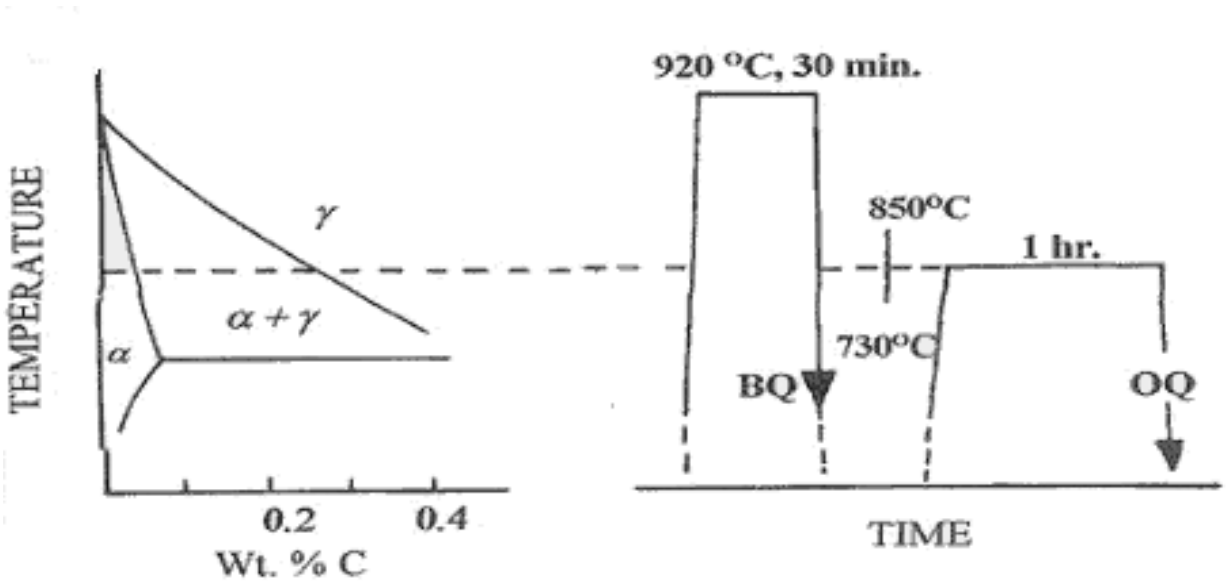

Figure 3. Intermediate quench.

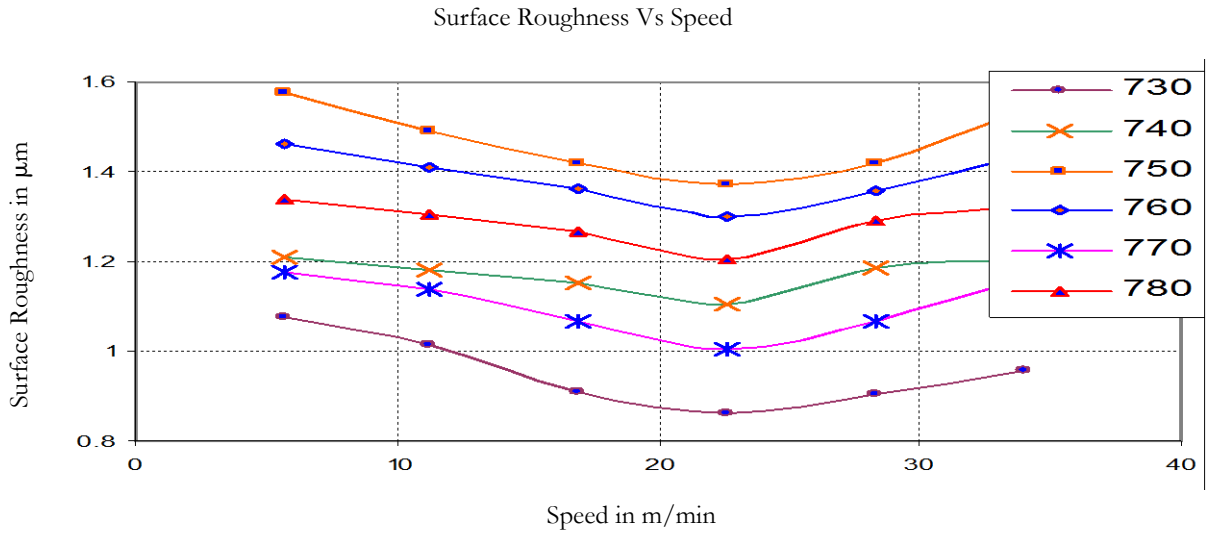

Figure 4. Variation of surface roughness with burnishing speed.

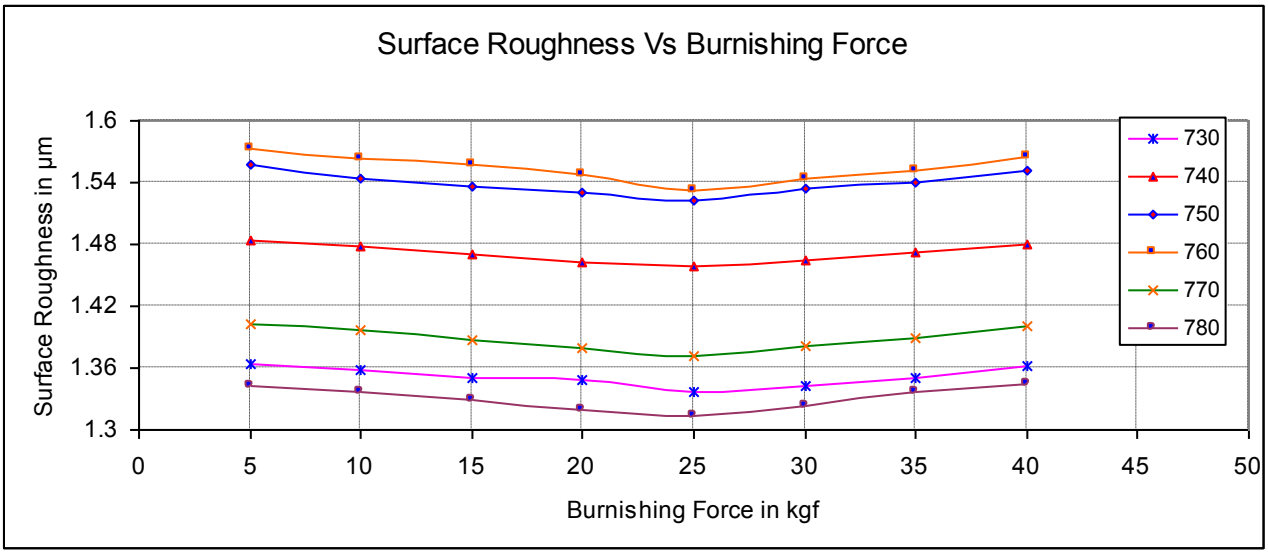

Figure 5. Variation of surface roughness with burnishing force.

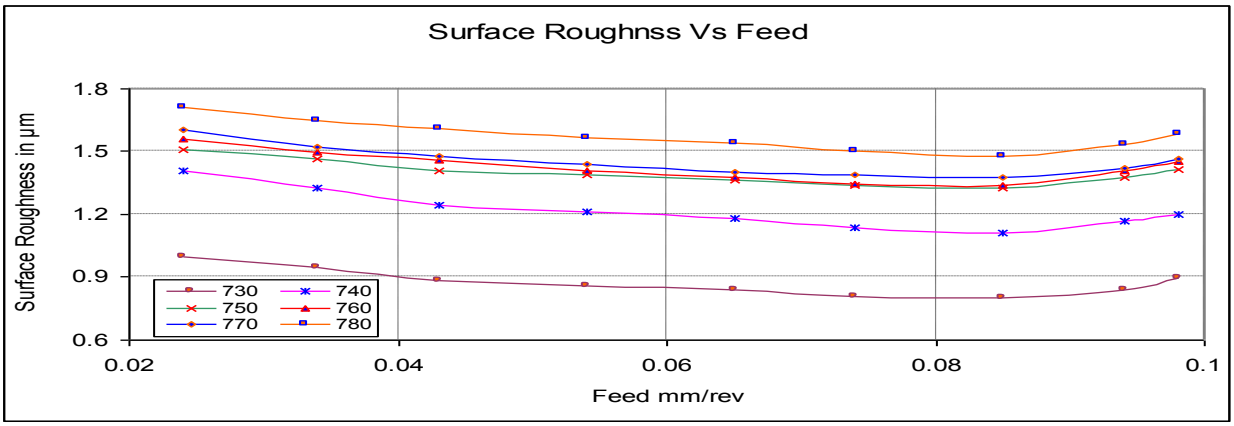

Figure 6. Variation of surface roughness with burnishing feed.

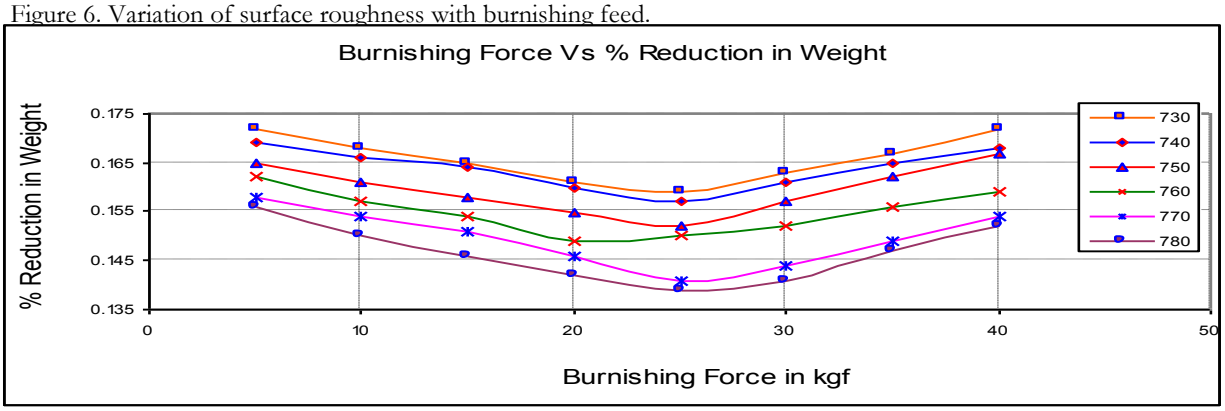

Figure 7: Burnishing force Vs % reduction in weight due to corrosion.

5. Conclusion

The effect of ball burnishing speed, feed and force on surface roughness and corrosion resistance of HSLA dual-phase Steels were studied. The main results obtained are as follows:

1. A very good surface finish is obtained in this experiment, mainly based on the maximum residual stress changes from tensile to compressive with an increase in burnishing force. The stress distribution and the depth of compressive residual stress layers are mainly controlled by the ball feed and speed.
2. Optimum burnishing parameters on dual-phase steels were established and these can be used for maximum benefit of burnishing processes.
3. It can be concluded from the experimental results that the highest surface finish and corrosion resistance can be achieved by using grease as a lubricant, a feed of 0.085 mm/rev, a speed of 22.62 m/min and a burnishing force of 25kgf.
4. Experimental work shows that an improvement of about 60% to 99% in the surface roughness of dual-phase Steels can be obtained by a ball burnishing process.

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Women and Housing in the City of Nairobi: Constraints and Opportunities

Fredrick Okoth Okaka[1*] and Dorothy N. Mutisya[2]

[1] Department of Social Sciences, University College of Education, P O Box 1933, Zanzibar
[2] Department of Geography, Kenyatta University, P O Box 43844-00100GPO, Nairobi, Kenya

Abstract: This paper examines women and housing in Kenya's urban areas. It specifically addresses the constraints and opportunities that, if explored, could enhance women's chances of accessing owner-occupied housing in the city of Nairobi. Primary data was collected through questionnaires. The questionnaires targeted two groups of women, namely renters and owner-occupiers. A total of 90 women were interviewed, comprising 45 renters and 45 owner-occupiers. Simple descriptive statistics and Chi-square analysis were performed on the data. The findings of the study showed that women faced constraints of a financial (41.2%), institutional (36.6%), cultural (18.9%) and occupational (3.3%) nature in their efforts to secure their own housing. The study demonstrates that the best opportunities for women to acquire house ownership were through self-help groups, cooperative societies, and women's finance trusts. The study recommends that the government, NGOs, and other stakeholders in the housing sector support and initiate programmes and activities aimed at increasing women's access to house ownership, especially in an urban setting such as Nairobi City where the majority of women live in a situation of insecure housing tenancy.

Keywords: Housing; Women

1. Introduction

The importance of housing to human beings, whether male or female, cannot be overemphasized. Housing carries out interrelated physical, social and economic functions. Its physical functions include the protection of the occupants from physical elements such as rain, heat from the sun, wind and cold. Housing also provides security and comfort. Socially, housing provides an environment in which members of a family are brought up and a place to socialize. From an economic point of view, a house is an investment and can be used to secure loans (Tiisetso, 1995).

Despite the crucial importance of housing, the global housing situation represents an immense challenge. Currently, over one billion people are either homeless or live in extremely inadequate conditions (ITDG, 2002). The problem of housing is more pronounced in urban areas, particularly those of developing countries due to the rapid growth in population (Thurman, 1992) where an estimated 600 million people live in life-and health-threatening homes (UN HABITAT, 1995). These are informal settlements with poor housing and inadequate or no services and where the owners usually lack secure land tenure and hence are vulnerable to eviction and violence (ITDG, 2002). In these settlements, a large proportion of the population lacks water, electricity and sewage facilities. The settlements are also characterized by a great deal of insecurity; mugging, rape, etc being prevalent. In Kenya, for example, for the estimated 700,000 people who live in Kibera, (a sprawling shantytown in the south of the capital) Nairobi, the lack of a functioning sanitation and drainage system is perhaps the greatest daily nightmare they must cope with (IRIN, 2004). Due to a lack of most basic services, each day the residents of Kibera must, among other problems, endure the sight of filthy narrow alleys, and sludge and human waste from shallow latrines flowing into nearby streams, a situation that gets worse during the rainy seasons.

In common with practically every other city in developing countries, the current housing situation in Nairobi is deplorable (Government of Kenya, 2000). Rural-urban migration has been rising steadily over the years, thus contributing to the ever-rising demand for housing. Unfortunately, most of the migrants never succeed in finding permanent employment and end up in the slums. Rural-urban migration coupled with natural population growth have contributed to the rapid growth that saw the population rise from 505,285 in 1969; 2,143,254 in 1999 (Government of Kenya, 2001) and is currently estimated at 3.5 million (UN-HABITAT and Nairobi City Council, 2006). The result has been a severe shortfall in the provision of housing with about 67% of Nairobi's population living in the slums in the squatter settlement (NACHU, 2003).

While both men and women experience the problem of housing, women are the most severely affected by this problem (UN HABITAT, 1995). This is because of their socially-ascribed gender roles and responsibilities that revolve around the household. They are, therefore, the main users of housing and related infrastructure. Any inadequacy in this area has detrimental effects on their effectiveness in executing their daily tasks (Thurman, 1993). For example, without adequate and assured housing, women are unable to perform their mandatory day-to-day chores such as the rearing of children, managing family services, domestic work, ensuring the daily and future well-being of the family among others (Lee Smith, 1997).

While general access to housing (rented or owned housing) is necessary, house ownership is particularly important because it reduces the degree of insecurity of housing tenure that largely affects women. A lack of housing renders many women unable to protect

*Corresponding author. E-mail: fredrickokaka@yahoo.com

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themselves against violence or evictions and prevents access to credit through lack of collateral (UN HABITAT, 2003). Thus, many women are today making an effort to own or rent a house; a possibility that appears to work more for women in urban areas than for those in rural areas.

Due to cultural factors, land is a crucial factor limiting women from accessing secure housing in Kenya's rural areas. In urban areas, the cultural factor is not as strong; women enjoy much freedom of choice and quite a number have succeeded in owning a house. According to Larsson (1989), urban housing is one of the few strategies available to women who have difficulties in obtaining rural land. However, most of these women end up in informal settlements in urban areas where they live in insecure housing mainly as tenants (Lee Smith, 1997). According to Matrix Development Consultants (1995), women and women-headed households are predominant in the informal settlements in Nairobi city where many are renters rather than owners. The 1999 Kenya's Population Census further revealed that only about 26% of women in Nairobi compared to 76% of men are in owner-occupier tenancy (Government of Kenya, 2002). This demonstrates that women have significantly lower house ownership rates. The purpose of this paper was, therefore, to investigate the constraints that women face in their pursuit of house ownership and opportunities that they could explore to increase their chances of owning a house in Nairobi city. The objectives of the study were to:

(a) To identify the means used by women to acquire their own housing in the city of Nairobi;
(b) To identify the constraints inhibiting women's access to house ownership in the city of Nairobi; and
(c) To find out opportunities that could increase women's access to house ownership in the city of Nairobi.

2. Methodology

The study was conducted in Nairobi City, Kenya. The city was divided into three residential categories based on data obtained from the Central Bureau of Statistics as follows; high-income, middle-income and low-income residential areas/estates. Stratified sampling was used to ensure that all the three categories of residential estates were represented in the sample. Only one residential estate was randomly selected in each category. This led to the random selection of the following estates; Kibera-Makina (low-income), Lang'ata (middle-income) and Kileleshwa (high-income). After the residential estates were sampled, the next stage involved sampling of respondents. Eligible respondents were women living in owner-occupied houses (with title as single or joint owners) and women renters who were responsible for paying the house rent. As a precursor to data collection, a preliminary door-to-door survey of the three residential areas was undertaken to establish suitable respondents who met the predetermined criteria. Subsequently, random selection was used to identify respondents for inclusion in the study.

In total, 90 women respondents were sampled. They were distributed as follows; 45 renters and 45 owner-occupiers of whom 40 were drawn from Kibera-Makina (low-income), 30 from Lang'ata (middle-income) and 20 from Kileleshwa (high-income). The sample size was restricted by time and financial constraints; however, this sample size (90) is above the minimum accepted sample size (30) in descriptive survey research (Dixon and Leach, 1978). The choice of the number of the respondents from each residential area was based on the population density. The higher the density of the population, the higher the number of respondents relative to the sample size. In this case, Kibera-Makina had the highest population density, followed by Lang'ata and lowest in Kileleshwa.

A questionnaire was used as the main data collection tool. It requested information on issues such as the socio-economic status of the respondents, means used to acquire their own house by owner occupiers, constraints that impede women from acquiring their own house and opportunities that could enhance women's access to house ownership. In addition, informal discussions were held with some respondents to seek clarification and more information to some of their responses to the questions asked. Before the final version of the questionnaires was administered, a pilot survey was conducted. This was done in order to pre-test the questionnaires to ensure that questions included were clear, understandable and would yield relevant information.

The collected data was analyzed quantitatively and qualitatively. The raw data obtained through questionnaires was first subjected to computation of simple statistics and then further subjected to significance tests using Chi-square analysis.

3. Results and Discussion

3.1 Means of Acquiring House Ownership

The findings of the study revealed four ways through which women accessed housing. These include: loans from informal groups or co-operative societies, loans from formal co-operative societies, loans from formal institutions such as banks, mortgage institutions or employers, and inheritance, as shown in Table 1.

Table 1. Mode of acquiring house ownership by women in Nairobi city.

Mode	N	%
Loan from formal co-operative society	18	40.0
Loan from informal group or co-operative society	13	28.8
Loan from formal loan institutions e.g. Banks, Mortgage institution, or employer	9	20.2
Inheritance	5	11.0
Total	45	100.0

The most frequently used means (40 %) of acquiring own housing is through loans from formal co-operative societies. The majority of those who acquired their house using this method came from the middle-income areas (50%). Over 39% were from low income area and only 11% from high income areas. The formal co-operatives included Mwalimu, Mageraza, Utumishi and Kenya Medical Association co-operative societies which draw members from the teaching fraternity, prison staff, police staff and medical practitioners respectively. These co-operatives offer very attractive flexible loans to their members for development purposes such as housing. Apart from the waiver of collateral requirements; they charge an interest rate as low as 1% on loans to their members. These co-operatives represent a good alternative to the mortgage and building societies which have strict borrowing requirements. However, these co-operatives are only open to those working specifically in the defined fields of affiliation.

About 29% of the owner occupiers acquired their house through loans from informal self-help groups or co-operatives. The majority (92%) of these were from the low income areas, while only 8% came from the middle income areas and none from high income areas. Informal groups also offer flexible loans and often do not require any collateral against such loans (which in many cases most women do not possess). Unlike formal co-operative societies, there is no strict rule on regular income, formal employment and membership to a particular profession, hence their appropriateness for the unemployed low-income women. In addition, these groups have a wide grassroots network due to their flexible operations, enabling many women to know about them and to benefit from them.

The number of women who bought houses through mortgages, banks or loans from employers was only 20%. Out of this, over 66% were in the high income group while 33.3% were in the middle income group and none were in the low income group. The study also revealed that 90% of those who had obtained their houses through banks or housing finance institutions' loans achieved this after marriage, with the assistance of their husbands (mainly joint ownership). This underscores the importance of combined earnings. It was no surprise then that the majority (67%) of house owners were married. The inability of women to access housing credit from mortgage finance institutions and banks is due to the general low-income status of women in Nairobi City, arising from low educational achievement, low job placement and unemployment, thus making their only means of acquiring property to be informal groups.

Although the legal framework in Kenya does not create barriers for women to inherit property from their relatives, spouses or parents, in many traditional Kenyan cultures women are excluded. Parents rarely let their daughters inherit their properties. This study found that only 11% (5) of the sampled women house owners had inherited their houses. Of these, over 60% (3 out of 5) were widows.

A Chi-square test showed that there is a significant difference in the ways of acquiring houses by women in the city of Nairobi. The results were significant at 0.005 confidence level. This reflects the success of some ways of acquiring own housing by women in the city of Nairobi compared to others. Co-operatives societies, for instance, is an important source of housing finance for the women.

3.2 Constraints Impeding Women's Access to House Ownership

All the respondents expressed a strong desire to own and live in their individually-owned house. This, they said, assured their security and that of their family. Most of the interviewed women lamented that, without this security, women risked being thrown out when they had a disagreement with their husband. At least 2 out of every 10 women said that they were aware of a woman who was left homeless after a quarrel with her husband. They also argued that the death of a husband could lead to a woman being thrown out of the house by the relatives of her husband. Women reported that, for them, owning a house was an uphill task faced with a myriad of problem, particularly those of gender origin.

The majority of the respondents (41.2%) identified financial constraints as the major problem impeding women's access to house ownership. This includes the escalating price of purchasing or constructing a house (Table 2) which does not favor women. This is exemplified by their lower earnings, which arise from low educational achievement and unrewarding careers. For example, according to the findings of this study, over 30% of the women renters had attained less than secondary level of education (Table 3), while over 33% were unemployed (Table 4).

Table 2. Current average cost of houses in Nairobi.

Residential Area	Average costs in Kshs	Average costs in US$ Dollar
Kileleshwa	More than 7 million	97,222
Langata	3-5 million	47,667-69,444
Kibera	500,000-2 million	6,944-27,780

Table 3. Educational levels of the respondents.

Educational Level	Tenants		House Owners	
	N	%	N	%
Primary	14	31.1	0	0.0
Secondary	14	31.1	0	0.0
Post-secondary	17	37.8	45	100.0
Total	45	100.0	45	100.0

Table 4. Occupational status of the respondents.

Occupational Status	Tenants		House Owners	
	N	%	N	%
Formal employment	18	40.0	39	86.7
Temporary employment	6	13.3	0	0.0
Informal Sector (e.g. business)	6	13.3	5	11.1
Unemployed	15	33.4	0	0.0
Retired	0	0.0	1	2.2
Total	45	100.0	45	100.0

The dominant occupations among the respondents were secretaries, clerks and primary school teachers. According to UN HABITAT (1995), even where there are no customary or legal restrictions to owning property,

financial constraints still hamper women's access to housing. A follow-up interview with one of the respondents (house-owner, Mariam) at Kibera revealed how financial constraints almost deprived her from ownership of her house through a tenant-purchase scheme. Although she was able to raise the required down payment (Kshs. 25,000 (US $347) from her share of her late husband's pension benefits, she found it extremely difficult to sustain payment of the monthly installments of Kshs. 3,000(US$ 42) due to the heavy burden of taking care of six children from her humble tailoring business. The total cost of the house at the time (1988) was Kshs. 250,000 (US$3,472). It was only through the collective support of her informal group that she was able to acquire an unsecured loan from time to time and finally complete payment for the house.

About 37% of the respondents cited institutional constraints as another factor impeding women's access to house ownership. These include unfavorable loan procedures and the bureaucratic process of obtaining a mortgage which impose unnecessary demands on women. For example, the existing housing finance institutions such as the Housing Finance Company of Kenya (HFCK) and the East Africa Building Society (EABS) require that one be introduced by someone known to the institution, a lease (the city council of Nairobi and other local councils in Kenya issues a lease of 99 or 999 years for land instead of a permanent title deed), quotations, legal documents, authenticating collateral among others. This, according to those respondents who acquired their houses through the housing finance institutions, can take up to six months. The majority of women, because of the nature of their responsibilities in the family (that of childrearing, household chores, providing for the family e.t.c.) do not have enough time to go through the long procedures to acquire a loan. Apart from time, the low literacy level of the majority of women means they are unable to comprehend and go through these procedures. Most often the majority of women cannot meet the lease requirements since they rarely own land as a result of their disadvantaged positions in society. Most of them cannot afford to pay the often high down payment because of their general low income. In this study, over 37% of the tenants had an income of less than Ksh. 10,000 (US$ 140) per month (Table 5), 90% of these were from the low income residential area where women are predominant. This means that they cannot afford to purchase a house of their own even in the low income area where the prices ranged between Ksh. 500,000 (US$6,945) to Ksh. 2 million (US$27,780) (Table 2). These results are congruent with the findings of Rembe (1995) who reports that certain requirements for loans have the effect of hindering women from accessing housing credit.

Culture-related constraints were also adduced (18.9%) as reasons why women do not purchase or construct their own housing. The paradox of the social and economic subordination of women can be clearly demonstrated by the case of some renters with financial ability but failing to acquire own housing. Four single women tenants in the high income group lamented that, though they believe they could afford to buy or construct a house of their own, they feared that they would not find suitors because most men, as a result of deep-rooted cultural beliefs, feel uncomfortable living under the roof of a woman. These single women are, therefore, forced by what can be termed as cultural circumstances to wait until they get married before jointly acquiring a house or even opting to register the house under the husband's name. This is regardless of whether they were the prime purchasers.

Table 5. Income levels of the respondents.

Income Levels (Kshs)	Tenants		House Owners	
	N	%	N	%
Below 10,000 (US$ 139)	17	37.8	5	11.1
11,000-20,000 (US$ 153-278)	9	20.0	6	13.3
21,000-30,000 (US$292-417)	7	15.6	8	17.8
31,000-40,000 (US$ 431-556)	7	15.6	8	17.8
Over 40,000 (OverUS$556)	5	11.1	18	40.0
Total	45	100.0	45	100.0

It was also reported by the majority of the respondents that they could not inherit their parents' house, even in situations where they were the only adult child or the only child in the family. In most cases the house went to younger male siblings or immediate male relatives. This was also attributed to cultural practices that discriminate against women. These findings concur with those of Keller (2000), who observes that women's access to and ownership of land and housing are constrained by customary law, traditional practices and attitudes, which reflect the subordinate position of women under customary law.

Finally, 3.3% of the respondents identified occupational constraints as factors that impede women in Nairobi from acquiring own housing. According to the results of this study, all the women who had acquired their own housing had post secondary education (Table 3) and were in formal employment (86.7%) (Table 4), a number of them (64.5%) earning a good salary of more than Kshs 21,000 (US$ 292) per month (Table 5).

However, the general situation in Nairobi is that women are represented less often in post-secondary institutions and in formal employment in comparison to men. This has compromised their financial ability to acquire property since they dominate the informal sector and low paying formal jobs. These results concur with Moser and Peak (1987), who observe that women miss out, even in low-income housing projects, because they are, prevalent in unskilled occupations or are at the bottom base of formal employment. This means that they earn lower wages and are therefore unable to buy or are not eligible for housing projects. Poor educational achievement, especially among women, has negative implications for the type of occupation they are into. They go forced to do the most menial jobs or resort to informal employment, both of which offer low and fluctuating income.

3.3 Is there Hope for the Women?

Based on the information presented above, it might appear that there is no hope for women and that they will never succeed in their endeavor to own a house. However this is apparently not the case. There is some light at the end of the tunnel. According to the results of this study, as shown in Table 6, women could explore the following:

Table 6. Opportunities that can be explored by women to access house ownership.

Opportunity	N	%
Informal self help groups	63	70.0
Formal co-operative societies	15	16.6
Women finance trust	6	6.7
Gendered approach to housing policy	6	6.7
Total	90	100.0

Informal self help groups was suggested by 70% of the respondents. These are solidarity ties that operate as crucial resources in providing access and finance opportunities without elaborate legal structures and stringent requirements. They are known to offer flexible loans. According to La Ferrara (2002), self-help groups are an important source of income for certain categories of people, especially women. Women form self-help groups to address practical gender needs in both the traditional and modern aspects of their lives. They form self-help groups to assist each other with labor on their farms, to supplement the incomes of their households and to acquire land and housing (Karega, 2002). These groups are essential because there are few individual women who have sufficient resources; consequently women see these groups as a means to achieve goods and resources. In Nairobi, among the groups identified as assisting women to acquire their own housing are; Dandora Local Women's Self-Help Group, Kabiro Women Group, Humama and Mathare Women's Self-Help Groups under the Mathare Gender Learning Resources Center (MGLRC). These groups offer flexible payment schedules on loans to members, waive collateral requirements and give long grace periods before commencement of loan payments e.g. 1 to 2 years.

Secondly, 16.6% of the respondents identified formal co-operative societies that offer flexible credit schemes as an avenue women can explore to acquire their own housing. In a number of cases, women, irrespective of their marital status, have been able to own property through formal co-operative societies. Most co-operative societies, unlike housing finance companies and banks, subsidize loans granted to members. For example, the majority of the sampled women house owners (over 40%) had acquired their houses through co-operative societies such as Mwalimu Sacco, Huruma Co-operative Society and the Magereza Sacco.

Thirdly, 6.7 % of the respondents cited Women's Finance Trust as another opening for women in Nairobi to access credit to acquire their own housing. Initiatives have been made by women to set up their own banks that take care of their special interests and, in particulars, take account of their low income position. Kenya Women Finance Trust (KWFT) is an example of such an initiative in Kenya. The trust targets women who have not generally had financial resources (low income). It has initiated a loan guarantee mechanism to finance women's income-generating activities through the existing commercial banking system as well as set up an intermediary credit institution for poor women who cannot yet qualify for commercial bank loans.

Finally, another 6.7% of the respondents suggested a gendered approach to housing policy by the government to accommodate the special needs of women. The existing official Kenya Housing Policy (contained in the Sessional Paper No. 5 of 1966/67) which has been the basis for the preparation and implementation of housing development plans, progress and projects pursued in the country has not adequately addressed women's unique problems in accessing house ownership. However, a draft housing policy, which is yet to be translated into a sessional paper, has recognized the disadvantages faced by vulnerable groups (women included) and one of its main focal issue is the housing needs of such groups (Government of Kenya, 2002). It is important that the government recognizes the unique problems of women in accessing own housing by developing an appropriate gender-sensitive housing policy. The lumping together of both men and women in low income groups, has been counter productive. For example, according to Nampuno-Parente's findings in her study of Dandora site and service low-income housing project in Nairobi, the majority of the women sold or gave away their plots because they were unable to develop them (Nampuno-Parente, 1987). Moser and Peak (1987), point out that women miss out even in low income housing projects because they are less educated and are predominantly to be found in unskilled occupation. So this calls for special programmes to address their unique needs. This finding is inline with that of Lee-Smith (1997) who suggested that a gendered approach to housing policy in Kenya had great potential. She recommends that women's property rights and their housing production capability should be the two elements of such a policy and that it should support the values and objectives of women's community-based organizations by formally incorporating these organizations into the policy-making process.

4. Conclusions

This study, using the conducted survey, brings to the surface the fact that the acquiring of owner-occupied housing for women is an uphill task. There are several hurdles that women must circumvent before they can own a house. Some of these hurdles include: financial, institutional, cultural and occupational constraints. These constraints are attributed to women's insubordinate position in society. This emanates from the entrenched gender relationships dominant in most Kenyan communities. The economic and social subordination of women is shown by the fact that, despite having legal equality with men, single women still afraid to venture into house ownership due to strong cultural beliefs. Admittedly, women, irrespective of marital status, have inheritance rights to family property and enjoy the legal protection of housing rights. For example, there are restrictions that bar husbands from disposing of houses without the knowledge or consent of wives. However, because of unbalanced power and gender relationships entrenched by patriarchy, having legal rights to property does not necessarily guarantee ownership. The non-legitimization of gender equality in some social contexts, due to prevailing cultural gender norms, prevents women from building dynamic relationships based on equality with men. However, housing rights for women are essential for their personal security and privacy, and are

meant to protect them from poverty and marginalization (Madebwe and Madebwe, 2005).

Despite these constraints, however, the study reveals that certain opportunities exist for women in Nairobi city to acquire their own housing. These include informal self -help groups that offer flexible loans, formal co-operative societies that offer flexible credit schemes, women's finance trusts and the review or development of a gender-sensitive housing policy and programmes by the government.

In conclusion, women's access to secure housing tenure in Nairobi city is still severely constrained. These findings indicate the need for the government, NGOs, and other stakeholders in the housing sector to support and initiate programmes and activities aimed at increasing women's access to house ownership, especially in an urban setting such as Nairobi City where the majority of women live in a situation of insecure housing tenancy. One such support could be to provide financial and technical assistance to grassroots self-help groups since this study reveals that these groups represent one of the main opportunities for women to access house ownership. The government should also prevail upon housing finance institutions to produce mortgage packages that cater for women and which do not jeopardize their profits. This can be done through broadening the house financing criteria to include revised collateral requirements that will accommodate more women clients. A gendered approach to housing policy that supports the values and objectives of women's community-based organization should also be adopted by formally incorporating these organizations into the policy-making process. This includes seeking their contributions before implementing a new housing policy.

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Nutrient Intake and Nutritional Status Profile of HIV-Positive Individuals Supported by Deep Griha Integrated Service for HIV/AIDS (Disha) Nutrition Center, Pune, India

Zinabu Anamo[1]* and Angelin Jeyakumar[2]

[1]Faculty of Health Sciences, Haramaya University, P O Box 1555, Harar, Ethiopia
[2]Department of Dietetics, School of Health Sciences, University of Pune, Pune-411007, India

Abstract: The intake of sufficient nutrients is important for maintaining the functional compounds of the immune system. The main aim of this study was to assess the nutrient intake and nutritional status profile of HIV positive individuals. Home dietary recall and six days' food intake from the nutrition center was used to estimate the dietary intake of the study subjects. Anthropometric measurements such as the height and weight of the subjects were also recorded. The findings revealed that the body mass indexes of 11(33.33%) were within the range of 18.5 to 20 and 14 (42.42%) were between20-25. The estimated intake of antioxidants based on home dietary recall and individual food intake from the nutrition center was 556μg, 2mg, 1mg, and 35μg for β-carotene, vitamin C, vitamin E and Selenium respectively, which are lower than the recommended levels for the specific ages and sex. The intake of □-3 fatty acid was also below the recommended level for HIV-positive subjects. Dietary habit analysis revealed that 65% of subjects were consuming rice, pulses and legumes, eggs, milk and milk products at least once a day. The intake of fruit and vegetables was less than once per day. It is therefore recommended that efforts have to be made to maintain daily requirements of nutrients.

Keywords: HIV Positive Subjects; Micronutrients; Nutrient Intake

1. Introduction

Nutritional management is integral to the care of all patients infected with human immunodeficiency virus (HIV). HIV infection results in complicated nutritional issues for patients, and there is growing evidence that nutritional interventions influence health outcomes in HIV-infected patients. The antioxidant nutrients (vitamins A, C and E, β-carotene, and selenium) have additional functions in the immune system independent of their antioxidant properties (Tracie and Sherwood, 1999).

Nutrition intervention studies from sub-Saharan Africa indicate that vitamin A supplementations reduce the risk of child mortality (Fawzi, 2003) and that multivitamins (B, C and E) given to women during pregnancy and lactation reduce the risk of child mortality (Fawzi *et al.,* 2004).

Vitamin E supplementation enhances both humoral and cellular immunity, augments phagocyte efficiency and regulates signal transduction and gene expression. In studies (Tracie and Sherwood, 1999) of the elderly supplementation of diet with 400-800 IU/day with vitamin E resulted in a significant increase in immune function, which is many times the current Recommended Daily Allowances (RDA), 8mg/day.

Deficiency in the B-vitamin complex has long been associated with impairment of the immune system. Patients in the highest quartile (1-5 times the RDAs) of intake of the B-vitamins, such as thiamin, niacin, riboflavin and B_6 had an improved survival period in an 8-year follow-up study of 281 HIV-1 patients. Serum levels of vitamin B_{12} are depressed early in the progression of HIV and normalization of serum levels results in an improvement in CD4 counts. (Tracie and Sherwood, 1999).

The immune system requires selenium to work properly. Low levels of selenium result in immune deficiency. The HIV virus causes AIDS when rapid HIV replication drains the immune system of its selenium supply. Each HIV virus uses up molecules of selenium. Hence, rapid viral replication causes rapid selenium depletion.

In a clinical trial, SAM (Selenium, Aspirin and Multivitamin) Combination therapy provided a 67% benefit in CD4 count compared with the placebo (Tracie and Sherwood, 1999). A six-month double-blinded placebo-controlled clinical trial (Howard, 2004) conducted by medical school professors in Zimbabwe used 1.2 grams aspirin, 125mcg selenium, and a vitamin tablet. After six months, the SAM arm CD4 counts rose 43% versus the placebo arm where CD4 fell 23.7%, for a total benefit in CD4 count of 67% provided by SAM Combination Therapy.

No magic yet has been identified to promote the repair of damaged tissues other than good nutrition. Thus, the dietary needs of HIV positive subjects have to be adjusted based on regular nutritional assessment with the intention that an infected person can live longer with no or minimal immune status compromised. It is, therefore, with this objective that the current study was carried out to assess the nutrient intake, nutritional status and dietary habits of HIV positive subjects.

2. Materials and Methods

2.1. Period and Site of the Study

This study was conducted from December 2005 to January 2006 as part of a nutrition program evaluation in Deep Griha Integrated Service for HIV/AIDS (DISHA) nutrition Center within the Family Welfare Center, Pune, which is about 200 km away from Bombay, capital of Maharashtra State, India. DISHA nutrition center is located near Pune railway station on the Tadiwala road beside the Ruby Hall Clinic. The Center offers services such as Childcare Programs which focus on creating a conducive environment for young children, education programs which focus on school dropout and women's Empowerment and Health Care Programs. Among the services under Health Care programs, Deep Griha Integrated Service for HIV/ AIDS is

*Corresponding author. E-mail: zinabunu@yahoo.com

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a new program that started in February 2005. Some of its services include: providing medical treatment, supply of daily nutrition (breakfast and lunch) based on a high protein diet, and counseling services. In this study all 33 HIV positive individuals supported by the nutrition center were included.

2.2. Ethical Consideration

Before administration of the study, official consent from the nutrition center and every study subjects was obtained. Moreover, the ethical committee of the University of Pune, School of Health Sciences, approved it.

2.3. Data Collection

An interview method was used to collect information from each individual. The questionnaire includes age, sex, educational status, economy and family size. Anthropometric data like height and weight; six days food intake from the center (breakfast and lunch) and home dietary recall for every person was collected.

Height: Height in centimeters was taken with the help of a measuring plastic tape. Two measuring tapes each 1.5m long were pasted to the wall of the DISHA nutrition center by making use of cello tape. The subjects were asked to take out their footwear, and stand with heels together and head positioned so that the line of vision was perpendicular to the body. A scale was used to the topmost point on the head. Height was recorded to the nearest 1 cm.

Weight: A weighing scale was used to take the weight of every individual. It was calibrated against known weights. Weight was recorded to the nearest 500 grams.

2.4. Data Processing and Analysis

The data were entered and analyzed using SPSS (Statistical Package for Social Sciences) version 11.5 and USDA SR18 nutrient analysis software packages.

2.4.1. Food and Nutrient Intake of Individuals

The average daily intake of various foods was computed according to sex and age groups. The nutrient intake was calculated using food composition tables (NIN, 2004) and (Kathleen and Sylvia, 2004). The nutrient intake was compared with the recommended nutrients requirements for HIV positive persons (Tracie and Sherwood, 1999). Based on the recommendations, 10% (approximately 40kcal/kg usual body weight) extra intake of energy (additional to RDA) was applied to adults aged more than 25. In the case of subjects whose body weight was less than 10 % ideal body weight, 50kcal/kg usual body weight was used to estimate daily needs. For children, RDA +10 % energy was considered.

2.4.2. Anthropometry

Unintentional 10% loss of body weight over six months was used to define HIV wasting syndrome. In addition, their current body weight was checked as to specific ideal body weight. BMI was computed using Nutrisurvey software 2005.

3. Results

Out of the total 33 subjects, 19 (57.6%) were females and 4(12.1%) under 10 years of age children supported by the center. All were Hindus.

The greatest proportion of the subjects were in the age group of 20-35 years followed by less than 10 years of age and their mean age was 23.2 years (Table 1).

Table 1. Selected socio-demographic variables of the study subjects.

Age categories	Respondents by Gender		
Age	Male	Female	Total
20-35	8	18	26
10-20	2	1	3
< 10	4	0	4
Total	14	19	33
Educational status	Male	Female	Total
Illiterate	4	4	8
Can read and write	1	0	1
Elementary	8	11	19
High school	1	4	5
Total	14	19	33
Family size	Frequency	Percent	
<5	18	54.54	
5-10	12	36.36	
> 10	3	9.1	
Total:	33	100.0	

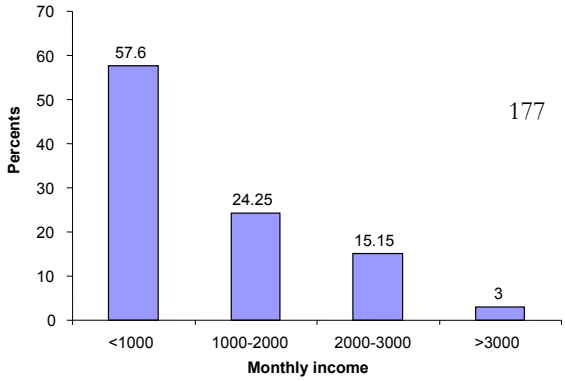
The educational status of those sampled shows that 24.2% of them were illiterate and less than 1% can read and write without formal education. More than half of the subjects had completed elementary level education.

Analysis of the findings also indicates that mean family size was 5.33 with a maximum and a minimum size of 13 and 2, respectively. As to their occupational status, 12 (63.2%) of the females were housewives followed by 21% who were working in private sectors 4 (21%). 11 (78.6%) of males who were without a job (including children) and 2 (14.3%) were working in the private sector.

Regarding their income, nearly 82% (including those in the zero income group) of the clients were earning below 2000 rupees (less than 44.5 USD at the time of study) per month (Figure 1). Those who (inclusive of children and adults) were not earning money at all were 16 (48.5%).

Almost all (97%) of them were non-vegetarian in their dietary habit. Their personal habits also signify that 97% of them were non-alcoholics, non-smokers and don't chew gutkha.

Even though 30% of the clients were diagnosed between the years 1995-2000, only a few of them (9%) had complained of associated symptoms of HIV infection (AIDS) while the remaining were asymptomatic. Common health ailments reported were fever, diarrhea, oral thrush, and nausea.



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N.B 1 US dollar = 45 rupees during the study period
Figure 1. Monthly income of study subjects in Rupees

3.1. Nutritional Status and Nutrient Intake

In this survey, no wasting syndrome was observed when their current body weight records were compared with the past six months weight records. The body Mass Indexes of 11(33.33%) were within the range of 18.5 to 20 and 14 (42.42%) were between20-25. The BMI of the others was below 18.5. Based on home dietary recall and the nutrition center individual food intake estimation, subjects' intake of energy was, on average, 2068 kcal/day from both meals (breakfast and lunch). This showed that half of them were getting what is recommended daily for their age and sex from the center. For the other half, three quarters of the daily needs were supplemented.

With regard to protein, subjects were getting an average of 114.5 gm daily from the food. Eggs and milk had supplied 60% of the daily protein intake of the study samples. More than 50% of subjects' ω-3-fatty acids daily intake in this analysis was less than half the recommended level and the rest were getting nearly half of it recommended for them. Estimated average intake of antioxidants from foods shows 0.556mg, 2mg, 1mg, and 35μg for β-carotene, vitamin C, vitamin E and Selenium respectively (Table 2).

Table 2. Summary of comparison of provisional recommendation of antioxidants and its estimated average intake.

Antioxidants	Estimated average intake per day	Daily requirement
β-Carotene	0.556 mg	30-50 mg
Vitamin E	1 mg	16-40 mg
Vitamin C	2 mg	200-500 mg
Selenium	35μg	100-200μg

NB. Requirements are based on Recommendation for HIV positive people

An analysis of the study revealed that B-vitamins estimated intake as measured by particular vitamins was Vitamin B_6 0.5μg, Thiamin 2.2 mg, Riboflavin 1.7mg, Niacin 18.7mg, Folate 169.5μg and 3.38μg Vitamin B_{12}. (Table 3)

Their Dietary habits analysis showed that more than 65 % of the study subjects were consuming rice, eggs, cereals, milk and milk products at least once in a day in their meal. But intake of vegetables and fruit for almost all of them was less than the recommended levels.

Table. 3. Comparison of supply of estimated B-complex vitamins and its requirements by adults.

Vitamins	Estimated average intake per day	Requirement (2-5 times the RDA)
Niacin, B3	18.7mg	38-95mg
Thiamin, B1	2.2mg	3.0-7.5mg
Riboflavin, B2	1.7mg	3.4-8.5mg
Pyridoxine, B6	0.5mg	4-16mg
Cobalamin, B12	3.38μg	4-16μg

4. Discussion

Demographic characteristics of the study subjects show that 24.2 % of them were illiterate and more than half were from an elementary school background. Educational status of an individual plays an important role in HIV, as educated people are more likely to be aware of how to prevent the disease progression than uneducated ones. Monthly income is an indicator which is closely associated with occupation. People who are earning an adequate income can afford to satisfy their daily basic needs. In this study nearly 82% of the subjects were earning less than 2000 rupees (during study period 1USD=45rupees) per month, which is much lower, to cover their daily expenses. However, nutritional support from the center relieved them to some extent.

Home dietary recall and nutrition center individual food intake estimation showed an average energy intake of 2068 kcal from both meals (breakfast and lunch). Half of the recommended daily allowance for their age and sex was provided by the center. Nevertheless, their food intake outside the center was less than one third of the daily requirement for almost all of them. Low food intake outside the center could be attributed to the low socio-economic status of the study samples.

Since adequate protein intake has been associated with the maintenance of the immune system, increased protein intake to 1.5-2.0 times the recommended dietary allowances (RDAs) for HIV positive/AIDS subjects is suggested (Howard, 2004). Protein average daily intake was 114.5gm from foods, which is adequate as per the requirements for specific age, weight and sex.

Research suggests that (Fanta, 2004) the chance of immune suppression by HIV infection might be reduced in individuals who have good nutritional status; the onset of the disease and death might be delayed where HIV-infected individuals are well-nourished. In particular micronutrients play an important role in delaying the progression of HIV to its advanced stages. Vitamin B complex has been reported to be associated with the immune function of individuals (Tracie and Sherwood, 1999). Its deficiency may lead to impairment of the immune system. When B-vitamins; except vitamins B_2 and B_{12}, daily intake is compared with the recommended level for HIV positive subjects, it becomes lower than half of that recommended by Tracie Miller and Sherwood (1999). But combined therapy with selenium and aspirin in the form of SAM (Selenium, Aspirin and Multivitamin) on a daily basis from the center was quite adequate to meet their daily needs.

Research on HIV-positive patients and HIV-infected cell lines point out the importance of the antioxidants (Vitamin

A, C, E, β-carotene and Selenium) in controlling Reactive Oxygen Species (ROS) intracellular and their relationship to disease status in HIV (Kupka and Msamanga, 2003). When Selenium in the body is low, fewer CD4 cells are produced and more CD4 cells die (Kupka and Msamanga, 2003). The intake of Selenium, β-carotene, Vitamins C and E from foods only were also far below the recommended levels. However subjects obtain ready-made supplements as in the form of SAM (two capsules of Antioxidants, Vitamins and Selenium, four tablets of Aspirin and one capsule of Multivitamins) and so daily requirements of these antioxidants, except for Vitamin E, were maintained. The intake of ω-3 fatty acids is also less than the daily recommended level in the case all the subjects.

5. Conclusion

This study shows that subjects were getting the amount of protein required daily and a relatively good amount of energy as per the recommendation. Even though SAM therapy was given as a supplement, intakes of most micronutrients from foods were less than the recommended levels for HIV-positive subjects. For example, vitamin E intake per day was less than the recommendation. Selenium appears to be most effective when vitamin E is also supplemented. Vitamin E supplementation should be considered to increase its utilization. The intake of essential fatty acids, n-3 was also not sufficient to meet the daily requirement. The n-3-fatty acids (α-linolenic acid, eicosapentaenoic acid (EPA) and Docosahexanoic acid (DHA)) are considered to be anti-inflammatory. Adequate intake of n-3-fatty acids from foods such as fish is very helpful for HIV positive individuals. Study subjects dietary habits also revealed that the majority of them were consuming fruits and vegetables less than once per day. Therefore, to maintain their normal health, fruit and vegetables should be eaten at least once per day, foods rich in macro and micronutrients should be included in their daily food menu.

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Short Communication

Gender Bias and Stigmatization against Women Living with HIV/AIDS in Harar, Ethiopia

Bedri Jebril

Nihon Fukushi University, Graduate School of Management, Development and Information systems, P O Box 400, Harar, Ethiopia
E-mail: albedru@yahoo.com or bedrim2003@hotmail.com

Abstract: In Ethiopia, HIV/AIDS is highly stigmatized due to the fact that sexual intercourse is the main mode of transmission. While both men and women are stigmatized and discriminated against for breaking sexual norms, the impact on women is more severe. The paper attempts to find the socio-psychological bases of stigmatization against women living with HIV/AIDS in Harar, Ethiopia and ways in which complex elements such as gender bias, socio-economic situations and traditional beliefs contribute, individually and in combination, to dual discrimination against women living with HIV/AIDS in their families, in their neighborhoods and in society as a whole. The results show that women throughout the research were subjected to stigma as women, and HIV-positive women. Stigma was reported everywhere to be more extensively directed against women than against men. It is recommended that there is a need to address issues of stigma and discrimination as part of the prevention of further spread of HIV/AIDS in Ethiopia.

Keywords: Ethiopia; Gender; HIV/AIDS; Stigma; Women

1. Introduction

HIV/AIDS is as much a social phenomenon as a biological and medical concern. Right from the beginning, the epidemic has been accompanied by an epidemic of fear, ignorance, and denial, leading to stigmatization of and discrimination against people with HIV/AIDS and their family members (ICRW, 2002a). Although this situation holds true for both men and women, women suffer from greater stigma and discrimination than men do, and it is often women who are blamed for bringing AIDS into the family. Consequently, it is believed that women are reluctant to inform their partners and families about their HIV status due to the fear of abandonment (Alubo, *et al*., 2002). In Ethiopia, which has the third largest number of people with HIV/AIDS in the world, the epidemic is spreading faster among women than men (Amhara Region HAPCO, 2006). In this context, many women may face stigmatization and discrimination in the society they live in and also in the workplace.

There is growing evidence that a large proportion of new cases of HIV infection are due to gender-based violence in homes, schools, the workplace and other social spheres. In addition, in settings of civil disorder and war, women and girls are often systematically targeted for abuse, including sexual abuse (Portnoy, 1997). In Ethiopia too, women are increasingly infected and affected by HIV/AIDS, now making up nearly more than half of the 1.5 million adults living with HIV/AIDS. As of the end of 2005, more than half of the people living with HIV/AIDS are women. (MOH/HAPCO, 2006).

In most of the developing countries, there are cases where women living with HIV have been discriminated in society. HIV-positive women are treated very differently, where men are likely to be excused for the behavior that resulted in their infection, women are not (Jenni and Annabel, 2006). HIV-related stigma is also linked to a particular group of people who are considered as specific risk groups. The segments of society in a specific risk group of infection include prostitutes and their clients, intravenous drug users, gay men, and prisoners who were already been discriminated against and marginalized even before the emergence of HIV/AIDS (Levine, 2002). According to Mann, Jen "HIV/AIDS- stigma and discrimination comes from the powerful combination of shame and fear - shame because the sex or drug injecting that transmit HIV are surrounded by taboos and moral judgments, and fear because AIDS is relatively new, and considered deadly. (Mann, 2003).

A study conducted in a number of countries found out that women living with HIV/AIDS suffer multiple layers of stigma. They are considered less valuable since they are women and HIV positive, because they are pregnant and HIV positive. In addition, in places where breast- feeding is the norm, the decision by HIV positive women not to breast-feed could draw attention to her sero –status placing her at risk of abuse and ostracism. (Panos, 2001 and ICRW, 2002b). According to Muturi, (2005) "the existing HIV/AIDS related stigma was due to ignorance of the disease and predicted that with appropriate knowledge , the level of stigma attached to the disease would decline" . Others believed that once a cure has been found, HIV/AIDS would be just like any other disease and people would no longer be stigmatized. On the other hand, few believe that if people disclose their HIV positive status openly the issue of stigma will cease. However, in spite of these views, misconceptions about transmission remain the main cause of stigma.

2. Methodology

A qualitative research approach was used for this study, as the main aim of the study was to include as much information as possible and to acquire an in-depth understanding of Ethiopian women living with

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HIV/AIDS. Harari region has the third highest HIV/AIDS prevalence rate in Ethiopia and is the most affected region (Ministry of Health, 2006). Harar city is divided into two major areas. In the oldest place, which is called "old walled city", the majority of the residents are the native people called Harari whereas in the other part there are people with different cultural and religious back grounds who come from all over the country. Given that it is difficult to easily access the people living with HIV/AIDS, the sampling strategy that was used in the study is snowball sampling. The interviewees were 35 women. This was the case because of the sensitive nature of the as topic it was difficult to get as many as population required to interview. The first interviewee introduced another person who volunteered to be interviewed. The researcher first approached the head of the organization for social services for AIDS (0SSA) in Harar. He introduced the women who could talk openly. After this first step, I visited some of the interviewees first and interviewed them at the second meeting. Some interviews were conducted in OSSA's office. In-depth semi-structured interviews were used for data collection because it allowed the respondents to express their experience of living with HIV/AIDS and could help the researcher to gain insights into their real experience of the disease (John, 1997). Secondary data was obtained from document observation. Reports, books and articles have been used in order to gather information about the stigmatization against women living with HIV/AIDS in Ethiopia.

3. Results and Discussion

3.1. Stigmatization against Women Living With HIV/AIDS

3.1.1. Women Living With HIV/AIDS Subject to Stigma

Generally, the findings of this paper show several things. The level of knowledge concerning HIV/AIDS among interviewees was good enough. However, it was observed that their understanding is low in terms of HIV-related risk taking. The answers from all respondents are not the same even though most of them seem to agree that women are subjected to HIV/related stigma more than men.

According to a respondent, though stigma surrounding HIV/AIDS is gradually getting better, women living with HIV/AIDS are still stigmatized. Associations that work for the rights of women and for the people living with HIV/AIDS have made a significant contribution to the small amount of progress achieved. This respondent is an activist in the association of people living with HIV/AIDS and hence her words might represent the outsider's way of thinking which is viewed as positive. Nevertheless, most other respondents have a more negative attitude about the situation. When asked if they are still stigmatized and discriminated against because of their known or suspected HIV status, most of them replied that they are experiencing a high level of stigma and discrimination on the basis of their HIV status. For example, 88 percent of them stated that they experienced a high level of stigma and 8 % claimed the situation is getting better than it was before.

Gender differences in the expression and experience of HIV-related stigma also come sharply into focus when one considers the experiences of married couples. Generally, it is believed that a woman should offer forgiveness and accept her husband's mistakes whereas husbands are almost expected to abandon their wives should they find that their wives are HIV positive. The participants' responses towards people living with HIV/AIDS can be explained in part as a result of their stereotypes, in particular their gender role stereotypes. The thin line between fact and image shows the persistent bias in viewing women as immoral and a source of sexual contagion. According to the vice chairman of the association, the HIV positive people who participated in a survey conducted by OSSA were blaming some one for their infection. Ethiopian men generally put the blame on their wives.

Table 1. Status of women living with HIV subjected to HIV related stigma.

	N : 35	Percent
Highly Stigmatized	22	63
Stigmatized	10	29
Showing Improvements	3	8

N= Number of samples

3.2. HIV/AIDS Remains a Taboo Issue

In most of the cases, the issue of HIV/AIDS is always related with the unacceptable behavior of a person. In responding to whether the issue of HIV/AIDS is a taboo in their society, all of them seem to agree that the issue of HIV/AIDS is still a taboo. The respondents agreed that people don't want to confront the issue of HIV/AIDS in their society. This way of considering HIV/AIDS as a taboo is clearly related with the assumption that a person who is HIV positive has had several sex partners. Most of the respondents don't feel comfortable and prefer even to avoid mentioning AIDS and prefer to refer to it as "the disease." It seems like the name AIDS has a magic force shrouded in taboo. Out of superstition, AIDS deserves to be kept at a distance, and by naming it people are afraid of catching AIDS themselves.

The very important point to solving the problem of taboo according to Bosire is early education. However, several assumptions might have hindered early education. In particular there is a belief among HIV/AIDS program facilitators that educating about HIV/AIDS may offend the community taboos or they will be accused of promiscuity and loose morals. Furthermore, there has been a reluctance to integrate any discussion of sex into discussion in some community groups (Bosire, 2005). However, the taboo nature of AIDS will aggravate the spread of the disease, if it is not checked in time.

Table 2. The issue of HIV as a taboo?

	N= 35	Percent
Shame to talk about it at any place/ with anyone.	17	48.5
Never mentioned with husband/family members.	8	23
I think many don't at all won't to mention about it.	4	11.5
A serious problem which lacks adequate attention	6	17

In most of the cases, the majority of the respondents agreed that since the main transmission of HIV is through sexual intercourse, and this itself is the issue which is taboo in society as a whole, the topic of HIV/AIDS also continues to remain a taboo to some extent.

3.3. Reluctance to Disclose HIV Status

The conceptualization of HIV/AIDS as a disease of shame, perversion and immorality has negative consequences for disclosure and help- seeking behavior. The social stigma associated with HIV/AIDS and its impact may force people to avoid getting tested and avoid seeking treatment if they test positive. Women in particular are faced with a dilemma about disclosing their status due to the fear of being thrown out of their homes and being considered as the culprit and also the loss of their job. Due to the perceived likelihood of being isolated from family, society and the workplace on discovery of their HIV status, most of the respondents experience strong pressure not to admit their HIV status to friends, colleagues and families. When asked if they have the courage to tell others about their HIV status, 88 percent of the respondents replied that they don't want to disclose their status to their community members while the rest said they don't mind letting others know about their HIV status. However, 57 percent of the participants stated that they are not unwilling to disclose their HIV status to their family members (especially to their mothers) while 43 percent don't even want to disclose their HIV status to their family members

Their fear is well –founded in most cases. In Ethiopia, women usually depend on their family or husband and communities for support and care. However, as long as HIV/AIDS is associated with stigma, disclosure can lead not only to rejection by family members, but loss of work and friends, physical and sexual assaults by male partners (Chikwampu *et al*., 2001). Given the existing potential for social stigma associated with HIV/AIDS, women living with HIV/AIDS may be extremely reluctant to disclose their HIV status to family or friends due to potentially losing the benefit of their support. Bharat *et al.* (2001) suggests that the fear people have in connection with disclosure of their HIV status have been identified as the manifestation of felt stigma. There are some people who prefer to disclose their HIV status to one or a few significant people while others yet again go fully public. Some people choose to tell no one. All these choices have their different and varying consequences. The attempt always made to avoid the impact of enacted and felt stigma may possibly create and reinforce both felt and enacted stigma. This situation exacerbates the dilemma of disclosure that may be necessary to obtain social support.

In both cases the participants' responses indicate that women living with HIV/AIDS are still reluctant to disclose their HIV status to others. As women disclose their HIV status, people around them start to express unusual sympathy towards them. Inside the home, stigma is manifested through the separation of shared objects such as bed sheets, clothes, and plates, letting those people with HIV/AIDS eat and sleep alone. The physical isolation of people with HIV is evident ranging from isolation from inside the family and community gatherings such as religious activities, market places, restaurants; to work places.

4. Conclusion

The HIV/AIDS epidemic has a clear gender bias. Women worry that they will be stigmatized; more than men suffer shame and be discriminated against if they are known to be HIV positive. The above discussion suggests that women who are HIV positive become vulnerable and risk violence, abandonment, rejection or even the loss of their children and homes because of their HIV status. Due to stigmatization and a fear of rejection from families and community members, women may be afraid to go for testing, deny their level of risk, or refuse to seek treatment more than men. As a result, information is not shared, prevention measures are less effective, and the experiences and insights of PLWHA and those most affected are not used to enhance prevention, care, and treatment programs.

Programs to reduce HIV-related stigma should be enhanced and, at the same time, should be disassociated from the sensitive and often taboo issues that are related with the transmission of HIV, such as sexual relations. This disassociation may be done without paying more attention to communication of information about prevention. Likewise, reducing stigma requires appropriate attention at the community level rather than rejecting cultural values, for instance, those who are dealing with HIV prevention activities should provide information on those voices within society that are struggling to create positive , non-stigmatizing messages or facilitate collaboration between communities that are seeking to mobilize a non-stigmatized response to the AIDS epidemic. Unreasonable rejection is always the consequences a lack of information. Hence, to combat this unreasonable rejection such as, for example, that people living with HIV/AIDS are prostitutes or promiscuous, effective ways of educating people that include everyone is a key feature to improve the situation. Finally, important relevant literature in this specific area is not readily available and so more extensive research about HIV-related stigma would be desirable, not only in Harar but also at a national level.

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Short Communication

Host Plant Resistance to Pathogen with Reference to Induction of Systemic Acquired Resistance (SAR) in Tobacco (*Nicotiana Tabacum* Var. Samsun) When Infected with *Tobacco Mosaic Virus* (TMV)

Fekadu Mariame[1]*, Anders Kvarnheden[2] and Anssi Vuorinen[3]

[1]Melkassa Agricultural Research Center, Ethiopian Institute of Agricultural Research, P O Box 2500, Adama (Nazret), Ethiopia
[2]Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences (SLU), P O Box 7080, SE-750 07 Uppsala, Sweden
[3]Department of Applied Biology, M-Building, P O Box 27 (Latokartanonkaari 7), FIN-00014, University of Helsinki, Finland

Abstract: A model study based on induction of SAR via infecting different lines of tobacco (*Nicotiana tabacum* var. Samsun) with Tobacco *mosaic* virus (TMV) was carried out at the Department of Applied Biology, University of Helsinki, Finland in December, 2006. The objective was to demonstrate the onset of defence response, spread of SAR in tobacco plants infected with TMV and detection of expression of marker gene, PR protein gene (PR1) using northern blotting and real time polymerease chain reaction (PCR). Localized necrosis was observed at 3 and 10 days post inoculation (dpi) only in TMV-inoculated NN plants in contrast to absence of similar localized symptom in the corresponding inoculated nn plants except systemic yellowing at 10 dpi. The data generated by northern blot analysis as well as real-time PCR showed existence of variation among experimental treatments with respect to PR1 gene expression. Northern blotting and real-time PCR gave similar results except a difference that was observed for sample 10 probably signifying high precision and sensitivity of real-time PCR in indicating PR1 expression compared to northern blotting. The RNA ladder was not visible in agarose gel and it was not possible to observe the actual result. This confirmed the importance of proceeding to the application of northern blotting. The result of this study indicated localized induction of necrotic lesions in infected tobacco var. Samsun (NN) as indicator for the HR programmed cell death and PRl expression as indicator for SAR in systemic leaves. All plants of nn variety are susceptible and showed only systemic mosaic symptoms in contrast to localized lesions expressed in NN plants. The result of this study indicated that the NN gene in TMV-infected tobacco var. Samsun carrying N allele recognized the corresponding Avirulence gene of the virus inducing the SAR in systemic leaves as indicated via expression of PR1 protein and hypersensitive reaction (HR) in infected leaves as visualized by localized necrosis in infected leaves. This is in agreement with the theory of gene-for-gene interaction in plant defence responses.

Keywords: Gene-for-Gene; Inoculation; Pathogenesis-Related Proteins

1. Introduction

Resistance of plants to their pathogens may be systemically enhanced by a localized induction treatment, a characteristic named systemic acquired resistance (SAR). Induction of SAR corresponds with the production of a set of proteins, pathogenesis-related (PR) proteins. PR proteins were originally characterized as acidic, relatively low molecular weight and protease-resistant proteins recovered from the plant leaf intercellular spaces induced after pathogen infection (van Loon, 1985).

Currently, 14 families of PR proteins are recognised and classified for tobacco and tomato based on their homology at the amino acid level, serological relationships and/or enzymatic or biological activity (van Loon and van Strien, 1999). Many PR proteins have been shown to have enzymatic activity, as is the case with tobacco PR-2 (glucanase) or tobacco PR-3 (chitinase). The function of tobacco PR-1 is not known, but it has been shown to have antifungal activity (van Loon and van Strien, 1999; Niderman *et al.*, 1995). On the basis of these classifications and criteria, a set of SAR marker genes have been established for some of the most widely used test plants such as tobacco, *Arabidopsis* and cucumber for monitoring the onset of SAR. The identity and the relative abundance of expression of these genes, however, vary between plant species (Ryals *et al.*, 1996).

Induction of SAR is accompanied by increased levels of salicylic acid (SA), which can also be used as an agent to induce the production of some PR proteins. Some synthetic compounds such as 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) have been shown to induce the same set of SAR marker genes as SA and also increased resistance against a wide range of pathogens in a variety of crops. Recently, the genetic dissection of the pathway leading from SA production to PR-protein induction has lead to identification of the gene *NPR1* in *Arabidopsis*, the role of which seems to be central in this signal transduction pathway among plants (Mou *et al.,* 2003).

In *N. tabacum* var. Samsun NN, the interaction with TMV is incompatible inducing HR due to the recognition of TMV replicase by the product of the tobacco *N*-gene at temperatures below 28°C. In *N. tabacum* var. Samsun nn, the interaction is compatible. The onset and spreading of SAR is monitored from the total RNA samples using specific primers for the tobacco *PR-1a* gene, which is one of the nine SAR marker genes in tobacco (Ward *et al.*, 1991). The induced levels of *PR-1a* mRNA are detected

*Corresponding author. E-mail: Fekadumarame@yahoo.com

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using real time-reverse transcription-PCR (RT-RT-PCR) and northern blotting.

In RT-RT-PCR, a cDNA is first synthesized from the *PR1a* mRNA of the sample with reverse transcriptase. Then, DNA copies of the cDNA are produced by PCR. During the PCR, the amount of DNA copies are detected real time. Primers were designed to amplify an internal region of *PR-1a* mRNA. Amplification of a region from an actin gene will be used as a control of a constitutively expressed gene to be able to compare the induction levels of *PR-1a*.

TMV belongs to the genus *Tobamovirus*. TMV infects tobacco and many other, mainly solanaceous' hosts. TMV is easily transmitted mechanically and in nature it is spread by incidental contact and wounding. The virus particle is a rigid helical rod and contains a positive-sense RNA genome, producing two replicase-associated proteins from the genomic RNA, and the movement and coat proteins from the 3' coterminal subgenomic RNA. TMV is a very stable virus and easily transmitted by contact.

PR protein(s) must be induced in the tissues that do not normally express the protein(s) and induced expression must occur in at least two different plant-pathogen combinations, or induction by one-pathogen combination must be reproducible by other laboratories. According to Nicola (1999), an isolate of Tobacco Mosaic Virus (TMV) that causes a hypersensitive local lesion (incompatible reaction) on tobacco would be avirulent, while an isolate causing systemic mosaic (compatible reaction) would be virulent regardless of the severity of the reaction. Hence, a tobacco genotype that produces local lesion in response to TMV infection would carry a resistance N gene to TMV while a genotype with systemic mosaic is lacking the N gene.

SAR is an induced, systemic defence response caused by a wide range of pathogens producing necrosis in the leaf tissues. As a result of SAR, subsequent pathogen infections develop decreased symptoms compared with the situation not involving SAR. The SAR-induced increased resistance acts against a wide range of pathogens, and it lasts several weeks or even months after the primary infection.

Induction of SAR in tobacco (*Nicotiana tabacum* var. Samsun) was carried out in this study via infecting different lines of tobacco with *Tobacco mosaic virus* (TMV). The two studied varieties differ in respect to only one gene, the N gene. Hence, if induction of systemic acquired resistance (SAR) is by pathogen and a defence response signal is via PR gene expression, it should come only from infected leaves of TMV-infected tobacco var. Samsun carrying dominant NN alleles and not from either susceptible var. Samsun carrying recessive nn alleles or non-infected upper leaves of the resistant plants. The objective of this study was to demonstrate the onset of defence response, spread of SAR in tobacco plants infected with TMV and detection of expression of the marker gene, PR protein gene (PR1).

2. Materials and Methods

2.1. Plant Materials

Seeds of tobacco (*Nicotiana tabacum*) var. Samsun NN and nn were sown on potted soil media (20 x 30 cm^2) in insect-proof greenhouse at the Department of Applied Biology, University of Helsinki, Finland in December, 2006. One plant per pot was maintained for each category in duplicate. Seven weeks old plants were used for inoculation as well as non-inoculated control.

2.2. Inoculation Procedures and Maintenance of Control

Tobacco seedlings in two categories were used for different inoculations at four-leaf stage. Mock inoculation was performed both for plants of tobacco var. Samsun NN and nn by dusting (Figure 1) the two lowest leaves with carborundum followed by rubbing with sterile water. A TMV-infected leaf of *N. benthamiana* was ground in distilled water (1 g leaf material/ 5ml water) as indicated in Figure 2. Inoculations of both tobacco var. Samsun NN and nn plants were performed by rubbing TMV inoculum sap on each of the two carborundum-dusted leaves (Figure 3).

Figure 1. Dusting tobacco leaf with carborundum.

Figure 2. Tobacco leaf sap preparation for inoculation from infected and uninfected tobacco leaf.

Figure 3. Rubbing tobacco leaf with tobacco leaf sap.

2.3. Sample Collection, RNA Extraction, Purification and Quantification

A total of fourteen leaf samples were collected for RNA extraction. Initial collection of leaf samples (Pre-inoculation) were performed by picking one lower leaf from one NN plant (Sample 1) and one lower leaf from one nn plant (Sample 2) while the second collection of leaf samples (3 days post-inoculation, dpi) were performed by picking one inoculated leaf from one inoculated NN plant (Sample 3) and one inoculated leaf from one inoculated nn plant (Sample 4). Final collection of leaf samples (10 days post-inoculation, dpi) were done by picking one inoculated leaf from each of two inoculated NN plants (Samples 5 and 6), one inoculated leaf from each of two inoculated nn plants (Samples 7 and 8), one upper leaf from each of two inoculated NN plants (Samples 9 and 10), one upper leaf from each of two inoculated nn plants (Samples 11 and 12), one upper leaf from one mock-inoculated NN plant (Sample 13), and one upper leaf from one mock-inoculated nn plant (Sample 14).

In each case, the picked leaf was immediately wrapped with tinfoil and put into liquid nitrogen awaiting RNA extraction. Each leaf sample was ground in a separate mortar with liquid nitrogen followed by putting 0.2 - 0.5 g of the ground sample into 2 ml Eppendorf tube. Total RNA was extracted and purified for each sample following the specific Trizol-extraction protocol (Caldo *et al.*, 2004) and precipitated by LiCl over night.

2.4. Detection and Quantification of RNA Level

The concentration of RNA was determined by measuring the absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) in a spectrophotometer for a 1:100 dilution of RNA in water: 1 µl of RNA sample in 99 µl distilled water (1/100 dilution). A range of 1.9-2.2 of ratio between A260/*A*280 was used as significant reading reference for pure RNA values.

RNA samples were first separated by size using electrophoresis in an agarose gel under denaturing conditions. The RNA was then transferred to a membrane, crosslinked and hybridized with a labelled probe. Northern blot analysis and real-time PCR were used as methods to evaluate the level of expression of PR 1 gene.

3. Result and Discussion

Localized necrosis was observed at 3 and 10 dpi only in TMV-inoculated NN plants (Figure 4 and 6) in contrast to absence of similar localized symptom in the corresponding inoculated nn plants except systemic yellowing at 10 dpi (Figure 5 and 7).

Strong RNA bands were observed from Northern blot x-ray film (Figure 8) for samples 3, 5 and 6 all of which were TMV infected resistant tobacco var. Samsun carrying dominant NN alleles. This is in contrast to absence of any bands for mock inoculated samples 13 and 14 as well as non-inoculated samples 1 and 2. Also some other samples (4, 10 and 12) did not show any bands. There is a weak signal in sample 9. Some RNA bands appeared in samples 7, 8 and 11 which were all TMV-infected tobacco var. Samsun carrying susceptible recessive nn alleles (Figure 8). The reason for the PR1 gene expression in the non-resistant plants is unknown, but could be due to other pathways such as exposure to light and likes which need further study. Data were analyzed using Microsoft Office Excel 2003 and presented using real-time PCR graph (Figure 9), which shows the level of expression of PR 1. Both the data generated by northern blot analysis and real-time PCR showed variation among experimental treatments with respect to PR1 gene expression as presented in Figures 8 and 9. Northern blotting and real-time PCR gave similar results as indicated in Figures 8 and 9. PR1 was expressed both in inoculated lower (sample 5 and 6) as well as non-inoculated upper leaves (sample 8 and 9) of NN plants but it was at low level for sample 9. Difference was observed for sample 10 in the result for northern blotting and real-time PCR, most probably signifying high precision and sensitivity of real-time PCR in indicating PR1 expression compared to northern blotting. The RNA ladder was not visible in agarose gel and did not enable to observe the actual result. This confirmed the importance of proceeding to northern blotting.

Figure 4. Necrosis in NN (resistance gene) plant (3 dpi).

Figure 5. No necrosis in nn (resistance gene lacking) plants (3 dpi).

Figure 6. Necrosis in NN (Resistance gene) (10dpi) plant.

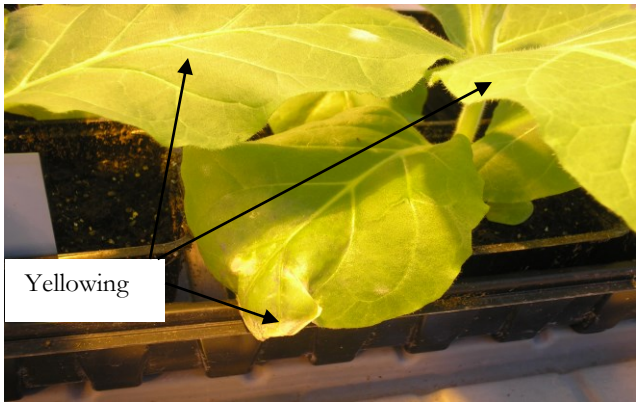

Figure 7. Yellowing in nn (resistance gene lacking) plants (10 dpi).

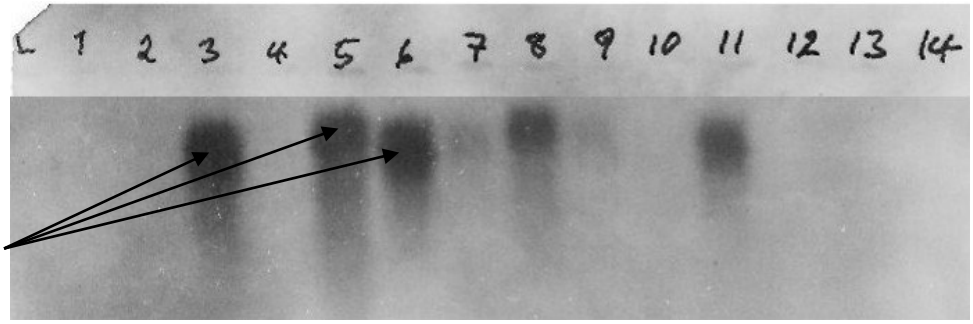

Figure 8. Northern blotting x-ray film.

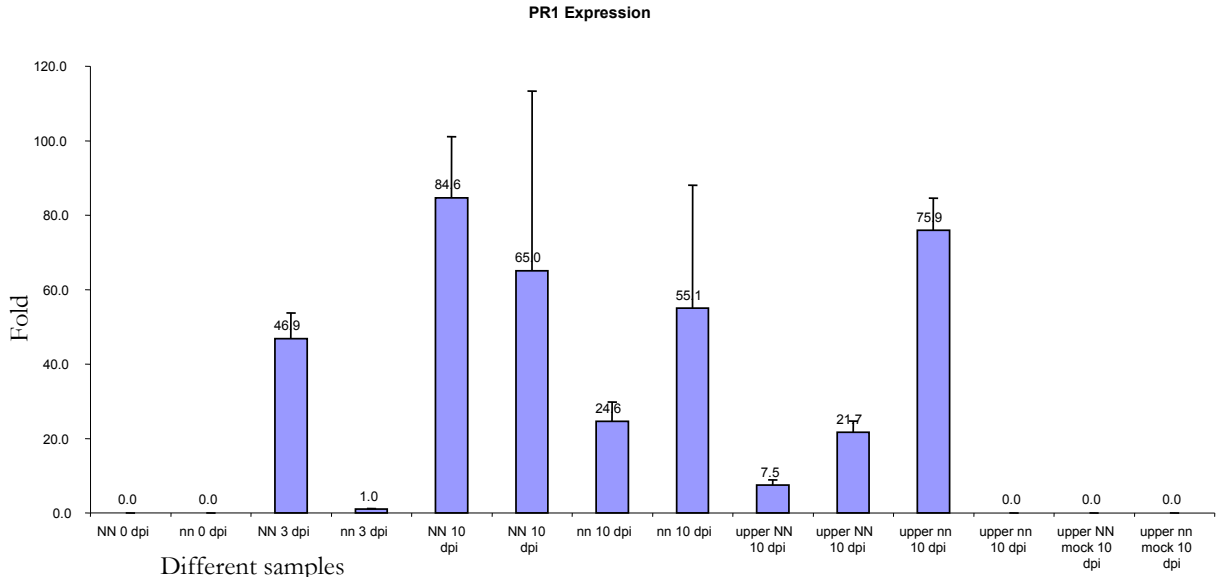

Figure 9. Real-time PCR graph, expression of PR 1 proteins.

The observed localized necrosis indicated the hypersensitive response programmed cell death in resistant contrast to systemic infection (yellowing) in susceptible genotypes.

The appearance of bands for samples obtained by taking inoculated lower leaves of TMV infected tobacco var. Samsun NN both at 3 dpi as well as 10 dpi confirmed the expression of pathogen-related gene (PR1) after inoculation with TMV. Further samples taken 10 dpi from inoculated tobacco var. Samsun NN showed accumulation of PR transcripts (PR1) in both treated and systemic leaves. SAR responses triggered by hypersensitive responses are due to interaction between N protein of the resistant host and the corresponding avirulence protein of TMV. According to Nicola (1997), the N' gene, a single dominant gene originating from

Nicotiana sylvestris, is associated with a hypersensitive reaction (HR) directed against most strains of tobamoviruses. The involvement of the coat protein sequence in the induction of the N' was first demonstrated by Saito *et al.* (1989). This work has indicated the restriction of TMV to the leaf part directly adjoining the induced necrotic lesions. The absence of any necrotic lesions in the plants of infected tobacco var. Samsun (nn) is the result of lack of recognition of the virus avirulence factor. In general, the result of this study indicated localized induction of necrotic lesions in infected tobacco var. Samsun (NN) as indicator for the HR programmed cell death and PRl expression as indicator for SAR in systemic leaves. However, all plants of nn variety are susceptible and showed only systemic mosaic symptoms instead of localized lesions due to lack of resistance factor.

The result of this study is in accordance with the theoretical or hypothetic response in recognition of pathogen Avr-gene product and resistance gene product R which cause expression of PR1 in resistant lines and resulted SAR that gives increased resistance throughout the plant.

4. Conclusion

The result of this study is in agreement with the theory of gene-for-gene interaction in plant defence responses in that the NN gene in TMV-infected tobacco var. Samsun carrying N allele recognized the corresponding avirulence gene of the virus inducing the SAR in systemic leaves as indicated via expression of PR1 protein and HR in infected leaves as visualized by localized necrosis in infected leaves. Data obtained from northern blot analysis and real-time PCR suggested the product of the resistance N gene in the recognition process to be the pathogen related proteins, PR protein. The N gene encodes the N protein. Activation of the N protein by TMV results in the production of PR1.

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Registration of Adu and Barkume: Improved Sweet Potato (*Ipomoea batatas*) Varieties for Eastern Ethiopia

Tekalign Tsegaw* and Nigussie Dechassa

Haramaya University, Department of Plant Sciences, P O Box 138, Dire Dawa, Ethiopia

Abstract: Two improved sweet potato (*Ipomoea batatas*) varieties, namely, Adu (Cuba-2) and Barkume (TIS-8250-2) were developed by Root and Tuber Crops Improvement Program and approved by the National Variety Releasing Committee in 2007. The performances of the varieties were evaluated at four locations in the eastern part of Ethiopia from 2003 to 2006. Adu and Barkume produced 49% and 89% more fresh root yield, respectively than Becule (local check). Although both of them were stable for fresh root yield, they exhibited high sensitivity to environmental changes and adaptation to environments favoring high yields. Adu is an early maturing and has a yellowish cream flesh colour indicating that it is a variety rich in beta carotene and the taste of its cooked tubers was classed as very good by farmers. Barkume is medium maturing and with uniform medium sized roots preferred by farmers. The taste of its cooked tubers was classed as good by farmers. Both varieties are recommended for production in eastern Ethiopia with altitudes ranging from 1650-2000 meters above sea level.

1. Introduction

Sweet potato (*Ipomoea batatas*) is native to South America and belongs to the family of convolvulaceae. It plays an essential role in Ethiopia in general and in the eastern part of the country in particular as a food security crop because of its tolerance to water stress and marginal soils. It is also valued for its nutritional importance as it has high content of carbohydrates, protein, minerals, and vitamins particularly vitamin A. It is also used as animal feed. It is grown under a wider range of environmental conditions and performs well on marginal land with low inputs.

The Root and Tuber Crops Improvement Program of Haramaya University has been introducing sweet potato germplasm with a wider genetic base from the Asian Vegetable Research and Development Center (AVRDC), International Potato Center (CIP), and International Institute of Tropical Agriculture (IITA) and has been undertaking multi-locational yield trials to identify high yielding, widely adaptable variety (ies) with good resistance to biotic and abiotc stresses. Special emphasis was given to yellowish and orange-fleshed sweet potato varieties which are rich in beta carotene to address Vitamin A deficiency, a serious health problem in the eastern part of the country. After a number of trials, for many years the program managed to release two high-yielding sweet potato varieties (Adu and Barkume) with the consent of the National Varity Releasing Committee.

2. Origin and Pedigree

The varieies Adu (Cuba-2) and Barkume (TIS-8250-2) were introduced from Cuba and the Asian Vegetable Research and Development Center (AVRDC, Taiwan), respectively in the 1980s and they were subjected to multi-locational trials in the eastern part of the country.

3. Yield Performance and Stability

Both varieties including local check (Becule) were tested at Babile, Haramaya, Hirna and Alberekete from 2003 to 2006. Disease free terminal vine cuttings of the varieties were used as a planting material throughout the trials and they were planted at a spacing of 80 cm by 50 cm on ridges. At all sites, the trials were conducted without fertilizer and supplemental irrigation to simulate farmers practice. No major disease and insect pest were observed during the trials. Adu and Barkume had 49% and 89% more fresh root yield, respectively over Becule which is a widely cultivated local variety (Table 1).

Table 1. Fresh root yield (t/ha) of three sweet potato varieties tested at four locations.

	Locations					
Variety	Babile	Haramaya	Hirna	Arbarakate	Mean*	Over the check
Adu (Cuba-2)	10.64	12.03	27.54	13.37	15.90	49%
Barkume(TIS-8250-2)	11.02	14.84	35.68	16.34	19.47	89%
Bercule (check)	6.91	12.40	14.73	8.65	10.66	

** Mean yield of 2003, 2004 and 2005*

Eberhart and Russell (1966) defined a stable variety has a mean higher than the mean of a group, unity regression coefficient ($\beta_i = 1$) and deviation from regression as small as possible ($S^2d_i = 0$). Both of the varieties exhibited regression coefficients significantly higher than one suggesting high sensitivity to environmental change and adaptation to high-yielding environments (Table 2). The G x E interaction study indicated that both varieties are not stable for root yield.

* Corresponding author. E-mail: tekalignsegaw@yahoo.com

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Table 2. Estimates of stability parameters for root yield (t ha^{-1}) of Adu and Barkume sweet potato varieties.

Genotype	Mean root yield (ton/ha)	Regression coefficient (β_i)	Deviation from regression (S^2d_i)
1. Adu (Cuba-2)	15.90	1.120**	2.828×
2. Barkume (TIS-8250-2)	19.47	1.514**	27.855××

** = *Significantly different from unity at 1 % probability levels.*
×× = *Significantly different from zero at 1 % probability levels.*

4. Quality Attributes

Taste and physical characteristics of boiled roots of Adu and Barkume are presented in Table 3. Adu is an early maturing and high yielding variety having root size and shape favored by farmers. It has yellowish cream flesh colour indicating that it is a variety rich in beta carotene which is a precursor of vitamin A. Thus, it could play a significant role in reducing Vitamin A deficiency, a serious problem in the region. The taste of the cooked tubers was classed as very good by farmers around Haramaya area. Barkume is medium maturing and high yielding with uniform medium sized roots often preferred by farmers. The taste of cooked tubers was classed as good by farmers.

Table 3. Taste and physical characteristics of boiled root.

			Flesh			
Variety	Cooking Ability	Peeling Ability	Color	Texture	Test	Palatability
Adu (Cuba-2)	Quick	Easy	Yellowish cream	Dry	Very sweet	Very good
Barkume (TIS-8250-2)	Quick	Easy	Cream	Slightly moist	Sweet	Very good

5. Adaptation

Both of the varieties are recommended for lowlands, mid and high altitude area of eastern and Western Hararghe with altitudes ranging from 1650-2000 meters above sea levels.

6. Acknowledgment

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7. Reference

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Appendix I. Vine characteristics, leaf characters and storage root characteristics of Adu and Barkume.

Characteristic	**Adu (Cuba-2)**	**Barkume (**TIS-8250-2)
1. Vine characters		
Twining habit	Moderately twining	Moderately twining
Plant type	Semi-compact (75-150 cm)	Semi-compact (75-150 cm)
Vine internode diameter	Thin (4-6 mm)	Very thick (> 12 mm)
Vine internode Length	Very short (< 3cm)	Very short (< 3cm)
Vine Pigmentation		
Predominant colour	Green with few purple spots	Green with few purple spots
Secondary colour	Purple nodes	Purple nodes
Vine tip pubescence	Sparse	Sparse
2. Leaf characters		
Mature leaf Shape		
General outline	Lobed	Lobed
Type of leaf lobe	Deep	Slight
Number of lobe	Five	Three
Shape of central lobe	Elliptic	Semi-elliptic
Mature leaf size	Medium (8-15 cm)	Medium (8-15 cm)
Abaxial vein pigmentation	Main rib partially purple	All veins partially purple
Mature leaf colour	Green with purple edge	Green
Immature leaf colour	Light green	Light green
Petiole Pigmentation	Green with purple near leaf	Green with purple near leaf
Petiole length	Short (10-20 cm)	Short (10-20 cm)
3. Storage root characters		
Storage root Shape	Ovate	Irregular elliptic slightly cured
Storage defects	Shallow longitudinal grooves	Shallow longitudinal grooves
Storage root cortex thickness	Very thin (1 mm or less)	Very thin (1 mm or less)
Storage root skin colour		
Predominant colour	White	Pink
Intensity	Dark	Pale
Secondary colour	Cream	Absence
Storage root flesh colour		
Predominant colour	Cream	White
Secondary colour	Absence	Absence
Distribution of colour	Absence	Absence
Storage roots arrangement:	Disperse	Disperse
Mean storage root number per hill	3.7	10.6

Registration of Food Barley (*Hordeum vulgare* L.) Variety HB 1307 for Mid and High Altitude Areas of Ethiopia

Birhanu Bekele and Berhane Lakew

Holetta Agricultural Research Center, P O Box 2003, Addis Ababa, Ethiopia

Abstract: Six-rowed food type barley, HB 1307, was developed by Holetta Agricultural Research Center (HARC) from a cross between a landrace line and exotic germplasm (Awra gebs-1 x IBON93/91) and released in 2006 for mid and high altitude areas. The three consecutive years' (2002-2004) tests proved its superiority in grain yield performance, stability, and wide adaptation. It has good physical grain quality, resistance to leaf rust and scald, moderate resistance to net and spot blotch, lodging tolerance, and good biomass yield. The variety's agronomic and quality merits and better performance than the checks makes it dependable for similar agro-ecologies considered in the study.

1. Origin and Pedigree

HB 1307 is derived from a cross between a landrace line (Awra gebs-1) and an exotic germplasm from ICARDA (IBON 93/91). The local farmers' cultivar, Awra gebs, was collected from North Gonder, Ethiopia, in 1994. IBON 93/91 is a line introduced from ICARDA through an International Barley Observation Nursery in 1991 with a pedigree of GLORIA "S"/COME "S"//ESC.II.72.607. 14E.9E.6E/3/SHYRI CMB87-489-L-5Y-3B-1Y-OM. The cross (Awra gebs-1 x IBON 93/91) was made in the off-season of 1995 at HARC.

2. Breeding Methodology

Bulk method was employed, where the segregating materials were bulk harvested and advanced twice a year, using off-season irrigation and the main season rains till the fifth filial generation (F_5) and a plant was selected from space-grown F_6 plants to develop the line.

3. Agronomic and Morphological Characteristics

The agronomic and morphological characteristics of HB 1307 are given in Appendix I.

4. Grain Yield Performance and Stability

Twelve food barley genotypes, including one recently releaseed standard check, Dimtu, and one major local cultivar of the respective locations were tested during the main seasons of 2002 through to 2004 at 13 locations in the southeastern, central and northern parts of Ethiopia, and complete data was secured at 6 locations: Holetta, Bekoji, Kofele, Debre-Tabor, Ambo, and Hosaina. The grain yield performance and stability parameters of HB 1307 and the checks are as summarized in Table 1. The mean yield of the new variety was significantly higher than the checks. The grain yield advantages of the new variety over the standard check, Dimtu, and the local cultivars of the respective locations, were 28.3% and 34.4%, respectively, with better lodging tolerance and good biomass yield (Table 3). Moreover, for verification (2005/06 cropping season), the new variety was compared in 14 on-station and on-farm sites that are located between 2300 m and 3020 m altitudes in the southeastern, central and northern highlands of the country. Complete grain yield data and secured at 12 verification sites, and the new variety (HB 1307) was superior in 8 sites or 67% of the sites compared to the checks (Table 2). Severe waterlogged conditions at two verification sites in Galessa resulted in complete crop failure and poor performance, the only survivors and better performers being HB 1307 and the local cultivar, indicating the tolerance of the new variety to the this particulars stress.

Absence of significant genotype x location and the greater size of the G x E variance component (334%) relative to the genotypic variance component justified assessing the variety's merit with respect to stability (FAO, 2002).

Table 1. Mean actual on-station and on-farm grain yields (t/ha), Kataoka's yield reliability indices (I_E) at P=0.95 (t/ha), regression coefficients (b), deviations from regression (S_{dij}^2), and environmental variances (S_i^2) of the new and the check varieties tested through 2002 to 2004 in 18 environments.

No.	Variety	Grain yield (t/ha)			b [c]	S_{dij}^2	S_i^2 [d]
		On-station [a]	On-farm [a]	I_E [b]			
1	HB 1307	4.78 a	3.54 a	2.901 a	0.928	0.839	0.10 a
2	Dimtu	3.73 b	2.30 c	2.340 b	0.762 *	-0.817	0.05 a
3	Local check	3.56 c	2.84 b	1.524 c	0.937	-0.198	0.09 a

NOTE: All statistical comparisons are based on the analyses done on the complete genotypes (12 varieties) grain yield data.

[a] *= figures with different letters significantly differ at* $lsd_{0.05}$.

[b] *= figures with different letters significantly differ at* $P \leq 0.10$ *based on Dunnett's one-tailed test.*

[c] *= figures with * significantly differ from unity at* $P \leq 0.05$.

[d] *= figures with the same letter are not different from the most stable variety, 'Dimtu', at* $P \leq 0.05$ *and* $P \leq 0.01$ *according to Ekbohm's test.*

* Corresponding author. E-mail: birhanuale@yahoo.com

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Partitioning the G x E interaction effect, based on a joint linear regression method (Eberhart and Russel, 1996), indicated that the regression coefficient (*b*) of the new variety was not different from unity and its deviation from regression (S_{dij}^2) was insignificant. In additions, its environmental variance (S_i^2) was not significantly different from the most stable variety, Dimtu. Kataoka's yield reliability index (I_E) when computed, based on S_i^2 at P = 0.95, showed the significant superiority of the new variety over the standard and the local checks (Table 1). Out of 18 environments considered for analysis from the three consecutive years' multi-location data, the new variety won in 13 (87%) environments.

Table 2. Grain yield (t/ha) results of HB 1307 and the check varieties from 12 verification sites conducted during 2005/06 cropping season.

Locations	Altitude (m.a.s.l)	Varieties		
		HB 1307	Dimtu	Local cultivar
Ambo (On-station)	2300	1.57	1.37	0.80
Wolmera	2400	2.02	1.46	1.25
Holetta (On-station)	2400	2.82	1.81	0.76
Bekoji Chefa	2600	2.61	1.18	0.90
Altufa	2725	0.73	0.87	0.67
Bekoji (On-station)	2780	4.84	2.97	2.19
Anokere I	2970	2.31	2.03	1.69
Anokere II	2970	1.47	1.72	1.12
Galessa I [a]	3000	2.10	1.02	1.67
Galessa II [a]	3000	1.11	*	1.35
Gaint (On-station)	3020	2.14	1.66	1.26
Gaint	3020	1.48	0.78	0.84
Mean		2.10	1.53	1.21

NOTE: Figures in bold indicate the best performing variety at each site
** = Complete crop failure due to severe water logging*
[a] = sites with severe water logging

5. Reaction to the Major Leaf Diseases and Quality Traits

The mean reactions of the varieties to the major foliar diseases of barley are as shown in Table 3. The resistance level of the new variety was better than the standard and the local checks for scald and leaf rust and comparable for net and spot blotch. The physical grain quality of HB 1307 is superior to the checks since its grains are white colored and heavier in hectoliter and thousand grain weight unlike the gray and light seeded standard and local checks (Table 3).

Table 3. Agronomic performance and disease scores of HB 1307 and the check varieties tested in food barley national variety trial through 2002 to 2004 averaged across 6 locations.

No.	Variety	DH	PH	BY	Lodging %	Sc	Nb	Sb	Lr [a]	HW	TGW
1	HB 1307	83	106	16.4	9	1	3	3	2 R	65.3	43.9
2	Dimtu	88	118	13.2	28	2	2	2	30 MR	64.7	41.2
3	Local check	84	114	14.0	40	2	3	2	47 S	62.8	38.1
	Mean	85	113	14.5	26	2	3	2	26	64.3	41.1

NOTE: DH=Days to heading, PH=Plant height (cm), BY = Biomas yield (t/ha), Sc=Scald (0-9), Nb = Net blotch (0-9), Sb = Spot blotch (0-9), Lr = Leaf rust (%), HW=Hectoliter weight (kg/hl), and TKW = Thousand kernel weight (g).
[a] = R=resistant, MR=moderately resistant, and S=susceptible

6. Conclusions

HB 1307 was superior in grain yield performance in most environments with satisfactory grain yield stability. It is more resistant to scald and leaf rust than the checks and comparable for net and spot blotches. It has better agronomic characteristics, particularly lodging tolerance with good biomass yields and moderate tolerance to water logging. The superiority of its physical grain quality compared to the standard and the local check was also among its important merits. Therefore, the cultivation of the new variety in mid-and high-altitudes of the major barley growing areas is strongly advised.

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8. References

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FAO (Food and Agricultural Organization). 2002. Genotype x Environment Interactions: Challenges and Opportunities for Plant Breeding and Cultivar Recommendations. Plant Production and Protection Paper No. 174. Rome, Italy.

Appendix I. Description of variety HB 1307

1. Variety: HB 1307 (Cross number EH 1700/F_7.B1.63.70)
2. Agronomic and morphological characteristics
   - 2.1. Adaptation area: Highlands of Shewa, highlands of Arsi, Hosaina, South Gonder and Similar areas.
     - Altitude (m.a.s.l.): 2000 – 3000
     - Rainfall (mm): 700 – 1000
   - 2.2. Seed rate (kg/ha): 85 for drilling and 125 for broadcasting
   - 2.3. Planting date: Optimum dates for the different localities ranging from late May to end of June.
   - 2.4. Fertilizer rate (kg/ha):
     - ♦ N 46
     - ♦ P_2O_5 41
   - 2.5. Days to heading: 83
   - 2.6. Days to maturity: 137
   - 2.7. Plant height (cm): 106
   - 2.8. Growth habit: Intermediate
   - 2.9. 1000 seed weight (g): 43.9
   - 2.10. Test weight (kg/hl): 65.3
   - 2.11. Seed color: White
   - 2.12. Row type: 6
   - 2.13. Spike orientation: Drooping (at maturity)
   - 2.14. Glume appendage: Awnleted on all rows
   - 2.15. Glume awnlet length: Less than glume
   - 2.16. Glume awnlet texture: Rough
   - 2.17. Lemma awn texture: Rough
   - 2.18. Lemma awn size: Long
   - 2.19. Lodging tendency: Resistant
   - 2.20. Water logging tolerance: Moderate
   - 2.21. Crop pest reaction: Resistant to leaf rust and scald, and moderately resistant to net and spot blotches.
   - 2.22. Grain yield (t/ha):
     - ♦ Research field: 4.78
     - ♦ Farmers' field: 3.54
3. Year of release: 2006
4. Breeder/Maintainer: HARC/EIAR