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African Journal of Plant Science

*Full Length Research Paper*

# Control of some *Penicillium* and *Aspergillus* rots of *Discorea alata* Poir and *Discorea rotundata* L. using extracts of *Xylopia aethiopica* (Dunal.) Linn. and *Syzygium aromaticum* (Linn.) Merr.

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**The antimicrobial activities of *Xylopia aethiopica* and *Syzygium aromaticum* extracts on fungi associated with rotting white and water yam was investigated. Diseased and healthy yam species of *Dioscorea* spp. were obtained from some markets. Fungal isolation was done from the samples using standard procedures. Leaves and fruits of *X. aethiopica* and *S. aromaticum* were obtained from the botanical garden, University of Ibadan, Ibadan. Crude aqueous and ethanol extracts of the plants were obtained using standard procedures. After pathogenicity tests, the isolated fungi were cultured on acidified potato dextrose agar (APDA) that were impregnated separately with the leaves and fruits of *X. aethiopica* and fruits of *S. aromaticum* extracts at specific concentrations for 10 days. Experimental design was completely randomized design (CRD) with three replicates. Mycelial extension of the fungi was measured daily using a meter rule. Data were subjected to statistical analysis using SAS software. Means separation was done using LSD (DMRT) at P≤0.05. The isolated fungi were identified as *Aspergillus niger, Aspergillus fumigatus,* and *Penicillium chrysogenum*. Pathogenicity test showed that the three fungi caused rotting in the yams. Growth inhibition of the fungi was significantly (P≤0.05) higher with ethanol extracts than aqueous extract. Highest mycelial growth inhibitory effect was recorded in the *S. aromaticum* fruit ethanol extracts on all the organisms. Likewise, *X. aethiopica* leaf aqueous extract showed high mycelial growth inhibition on *A. fumigatus* at 50 and 75% concentrations while *X. aethiopica* fruit ethanol and aqueous extracts was noted to have inhibitory effects on the growth of *A. niger* and *P. chrysogenum* at 50 and 75% concentrations respectively. The *in vitro* result underscores the antifungal abilities of these plant extracts and is also suggestive of their promising potential in vivo. Further works are underway to examine their antimicrobial potentials in the field.**

**Key words:** *Dioscorea alata*, *Dioscorea rotundata,* postharvest rot, *Syzygium aromaticum*, *Xylopia Aethiopica*.

## INTRODUCTION

Yam belongs to Dioscorea family and is rated as one of the most important staple food crops in most parts of West Africa especially Nigeria (Olayemi and Ajaiyeoba, 2007). Yams are root tuber bearing plants grown and harvested annually with over 600 species out of which, six are economically and socially important as regards export purposes, medicine and food (IITA, 2009). The six edible species of yam are; white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata*), bitter yam (*Dioscorea dumetorum*), aerial yam (*Dioscorea bulbifera*),

Chinese yam, (*Dioscorea esculenta*), yellow yam (*Dioscorea cayenensis*) (Zaknayiba and Tanko, 2013; Lawal et al., 2014; Princewill-Ogbonna and Ibeji, 2015). The variation in taste of yam inspires it's processing in different forms. Some are eaten as cooked starchy vegetables, some are boiled and mashed, and some are baked, roasted, fried, or pounded into thick paste after boiling and eaten with soup (Frank and Kingsley, 2014). Also, some yam tubers can be sliced and used as herbal medicine in China (Lee et al., 2003).

The crop plays an encouraging role as a guarantee for household food security. Nigeria is the largest producer of yam in the world followed by Ghana, Cote d' Ivoire, Benin and Togo with a total global output of 67% and an annual yam production estimated at 44.11 metric tonnes out of 65.94 metric tonnes total global production in 2016 (FAO, 2013). Farmers engage in yam production for household production, production of planting materials for private uses, income from sale of yams and surplus seed yams. The superstition and ritual often associated with yam in West Africa is an indication of the antiquity of this crop (Frank and Kingsley, 2014).

The steady rise in demand and supply of yam over the years has not been zealously met as farmers encounter various major constraints in the production, harvesting and marketing of yam. Studies by Zaknayiba and Tanko (2013) revealed inadequate storage facilities, poor producers, prices, incidences of pests and diseases, lack of access to farm inputs and finances are the negative constraints faced by farmers in yam production. Many tuber crops especially yams in Nigeria are labor intensive as the high cost of labour constrains small farm holders from enhancing productivity (Ayanwuyi et al., 2011).

Most of the labour costs in yam production are mostly felt during the planting process and to cut costs, family members are duly engaged from the production to the marketing of the yam produce (Zaknayiba and Tanko, 2013). In 2015, Nigeria had a total decline in yam production of about 3.4% (IITA, 2009; Ike and Noni, 2006). The reason was attributed to the various constraints like pests and diseases, inadequate storage and processing facilities, inadequate preservations, marketing and access to markets. Diseases and pests related issues have been identified as a major menace in yam production. These include; fungi such as *Aspergillus niger, Penicillium chrysogenum, Botryodiplodia theobromae, Fusarium oxysporum, Aspergillus fumigatus* etc. and symptoms which includes leaf spot, tuber rots; insects such as tuber and leaf beetles and parasitic nematodes (Asante et al., 2007; IITA, 2009; Zaknayiba and Tanko, 2013; Bongiorno et al., 2016).

Several methods have been adopted for controlling losses due to post harvest disease of yam. These include the use of chemicals, biological method of control, and the use of natural plant extracts, as reported by Amusa et al. (2003). Because of the low capital income of farmers in Nigeria and lack of expertise in the safe handling of chemicals, farmers resorted to the method of crop rotation, fallowing, planting of healthy material and destruction of infected crop cultivars in controlling the diseases of yam tubers, and most times, these are done poorly (Nwakiti, 1982). Chemical method of control has helped to reduce the rate of storage losses and also increases yield obtained. But the problem arising with the use of chemicals is that it is expensive, can cause environmental pollution and may also induce pathogen resistance. Biological control method has been preferred in some cases because it is selective with no side effect and cheap. Resistance to biological control is rare and biological control agents are self-propagative and self-perpetuating (Okigbo and Ikediugwu, 2000). Some plants are known to synthesize phytochemicals with antimicrobial activities and are used successfully in the control of diseases in humans and crops like yam, cowpea, rice, etc. (Bediako et al., 2007).

There had been increased attention on management of plant diseases using biological control measures (Okigbo and Nmeka, 2005). The extracts of *Xylopia aethiopica* and *Syzygium aromaticum* have been reported to have high antimicrobial activity against several plant pathogens. Therefore the objectives of this work were to: Isolate and identify fungi associated with post-harvest rot of *D. rotundata* (white yam) and *D. alata* (water yam), to evaluate the effectiveness of extracts of *X. aethiopica* and *S. aromaticum.*

On growth of the isolated rot pathogens *in-vitro, t*o examine impact of concentration on the effectiveness of the extracts, to evaluate the effectiveness of *X. aethiopica* and *S. aromaticum* extracts (*in vitro*) on the mycelia growth of the rot pathogens and to compare the effectiveness of *X. aethiopica* (Linn) and *S. aromaticum* plant parts extracts on the isolated fungi.

## METHODOLOGY

Diseased yam tubers (*D. alata* and *D. rotundata*) were obtained from Bodija market in Ibadan, Oyo state, Nigeria. Leaf and fruits of *X. aethiopica* and *S. aromaticum* were collected from the Botanical garden, University of Ibadan, Oyo state. Pieces of diseased white and water yam obtained from different markets in Ibadan were surfaced sterilized and cultured on acidified petri plates of potato dextrose agar (APDA) following standard procedures. Incubation at room temperature was done for 7 days. After pathogenicity tests and preparation of the plant extracts (leaf and fruits of *X. aethiopica* and *S. aromaticum*), their antifungal assay was examined at three different concentrations viz; 35, 50 and 75% following standard procedures (Sobowale et al., 2015). There were two controls; 0%

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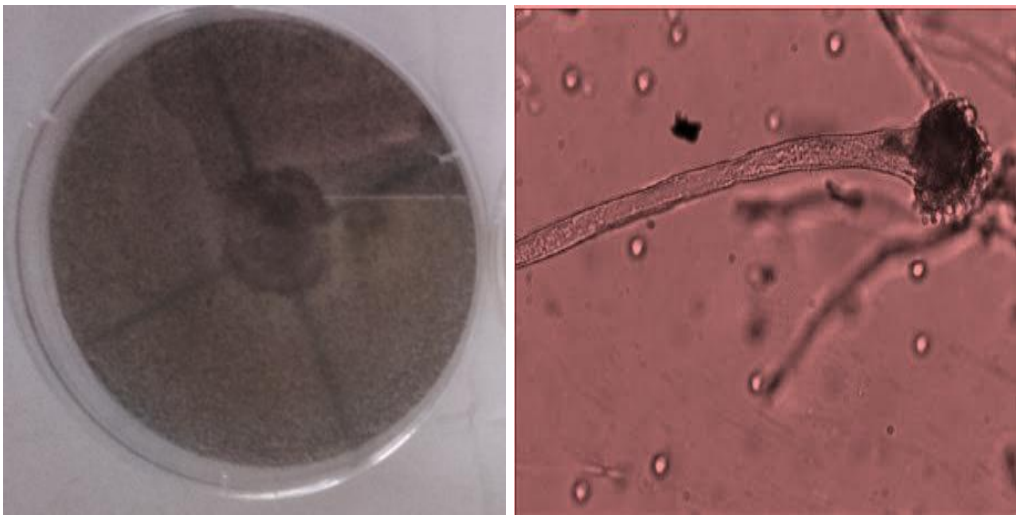
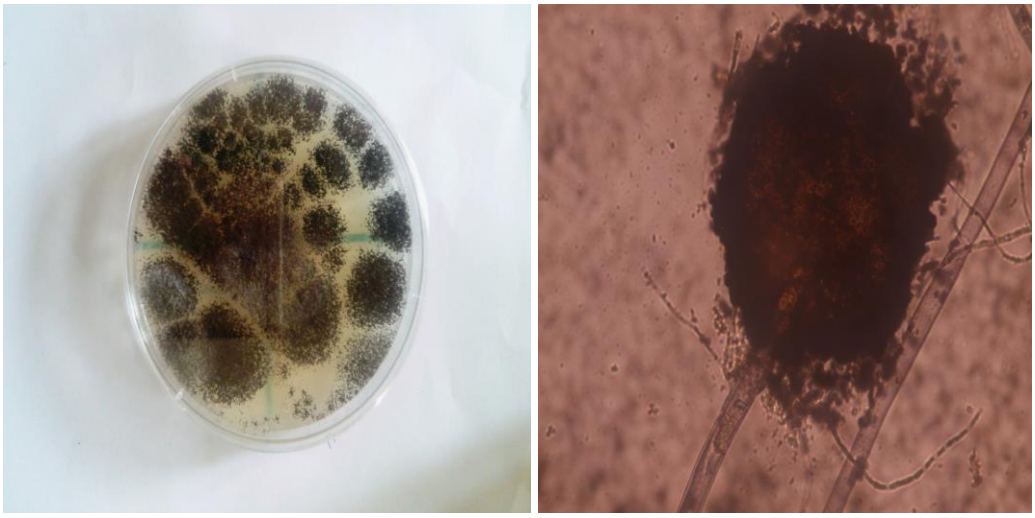


a b

**Plate 1.** Pure culture (a) and Photomicrograph (b) of *A. fumigatus.*



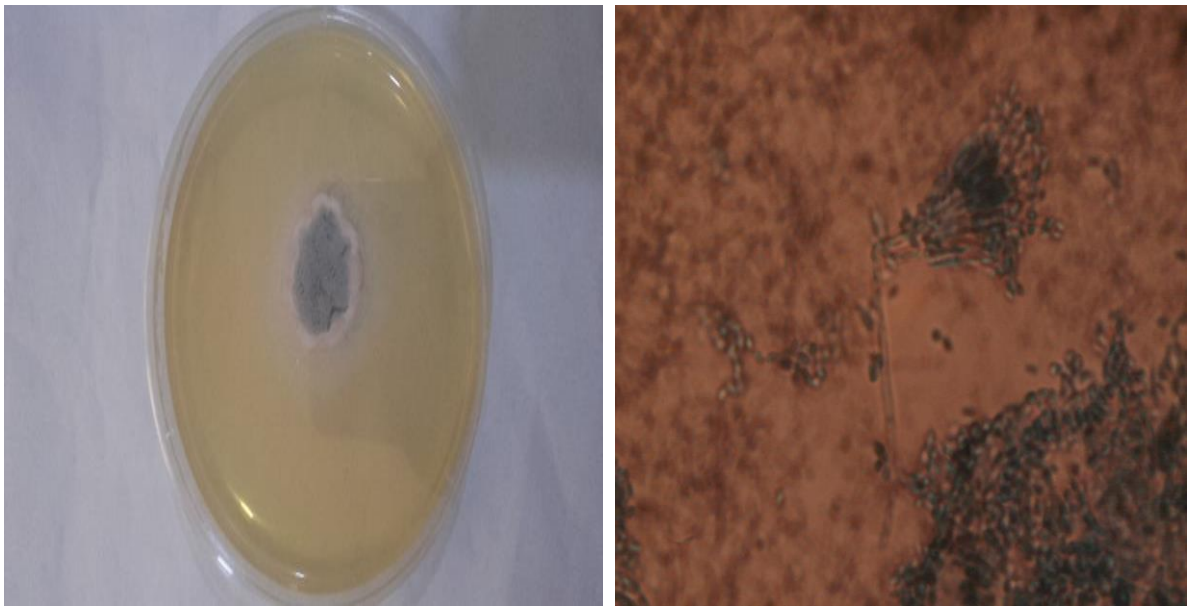
a b

**Plate 2.** Pure culture (a) and Photomicrograph (b) of *A. niger.*

with agar and 0% with ethanol. The experiment was conducted in a completely randomized design (CRD). All experiments were done in triplicates. Incubation was done at 28°C and diametric growths of the fungi were measured at 24 h interval using meter rule and recorded (Sobowale et al., 2015). The data collected were subjected to analysis of variance (ANOVA) using generalized linear model (GLM) procedure of SAS (version 9.2). Means were separated using Duncan's multiple range test (DMRT) at P≤0.05.

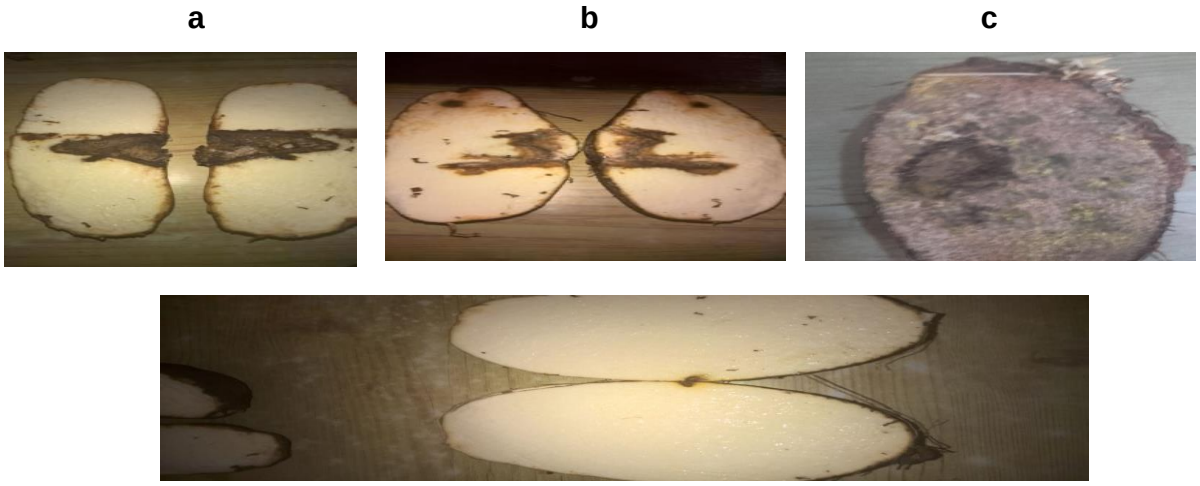
## RESULTS

The fungi isolated from the rotting white and water yam tubers include; *A. fumigatus* (Plate 1), *A. niger* (Plate 2), and *P. chrysogenum* (Plate 3). The pathogenicity test conducted showed that *A. niger*, *A. fumigatus* and *P. chrysogenum* caused rotting on the water yam (Plate 4) and white yam (Plate 5) tubers in storage. The result showed that *P. chrysogenum* was more virulent on both yam tubers while the other fungi strains were not as virulent. Growth inhibition of the fungi by leaf and fruit extracts of *X. aethiopica* was significantly higher with ethanol extracts than aqueous extract as shown in Table 1. Growth reduction by fruit extract was better than that of leaf with significant differences on certain days after



a b

**Plate 3.** Pure culture (a) and Photomicrograph (b) of *P. chrysogenum.*



a b c

d

**Plate 4.** Pathogenicity test for *A. niger* (a), *A. fumigatus* (b) and *P. chrysogenum* (c) on water yam; d = control.

inoculation. Growth inhibition of *A. niger* was generally more than that of other two fungi with significant differences on days 5 to 10. Inhibition at all concentrations was significantly better than that in aqueous control. Inhibition at 75% concentration was significantly better than those at other concentrations as seen in Table 1.

Growth inhibition of the fungi by fruit extracts of *S. aromaticum* was significantly higher with ethanol extracts than aqueous extract as seen in Table 2. Generally, inhibition of *P. chrysogenum* by the fruit extract was significantly better than that of the other two fungi. However, the impact of *S. aromaticum* extracts on growth of *A. niger* was significantly higher than that of *X. aethiopica* while the converse is true for *P. chrysogenum* as shown in Figure 1. Growth inhibitions of *A. fumigatus* by aqueous leaf extracts of *X. aethiopica* at all concentrations were significantly better than that in the

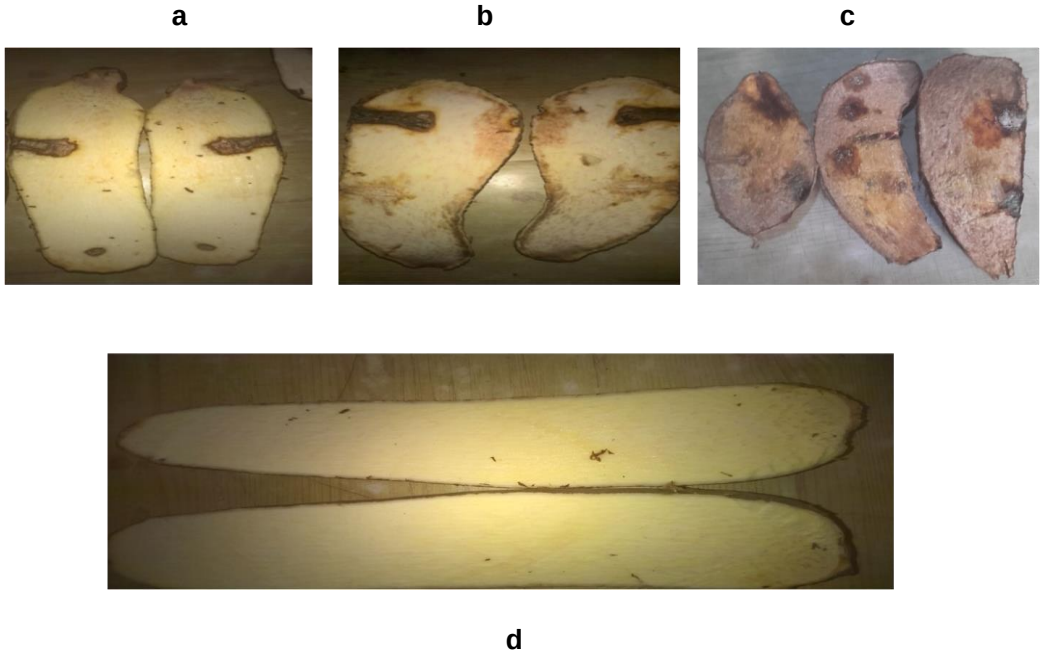



**Plate 5.** Pathogenicity test for *A. niger* (a), *A. fumigatus* (b) and *P. chrysogenum* (c) on white yam; (d= control).

**Table 1.** Growth inhibition of the isolated fungi by *X. aethiopica (*leaf and fruit) extracts at days after incubation.

| Parameters | Variables | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Solvents | Ethanol | 0.22a | 0.54a | 0.80a | 1.01a | 1.27b | 1.45b | 1.63b | 1.85b | 2.01a | 2.28a |
| | Aqueous | 0.04a | 0.56a | 0.82a | 1.13a | 1.60a | 1.65a | 1.78a | 2.03a | 2.23a | 2.43a |
| | LSD | 0.06 | 0.14 | 0.18 | 0.19 | 0.24 | 0.25 | 0.24 | 0.25 | 0.23 | 0.25 |
| | Leaf | 0.19a | 0.61a | 0.89a | 1.13a | 1.59a | 1.67a | 1.78a | 2.03a | 2.15a | 2.36a |
| | Fruit | 0.06b | 0.50a | 0.73a | 1.01a | 1.27b | 1.44b | 1.63a | 1.85a | 2.08a | 2.34a |
| Plant Part | LSD | 0.06 | 0.14 | 0.18 | 0.19 | 0.24 | 0.25 | 0.24 | 0.25 | 0.23 | 0.25 |
| | A. niger | 0.14a | 0.54a | 0.80a | 0.95a | 1.22b | 1.33b | 1.42b | 1.56b | 1.67b | 1.85b |
| | A.fumigatus | 0.19a | 0.55a | 0.83a | 1.11a | 1.46a | 1.61a | 1.85a | 2.11a | 2.29a | 2.50a |
| | P.chrysogenum | 0.06b | 0.56a | 0.80a | 1.16a | 1.61a | 1.71a | 1.85a | 2.16a | 2.39a | 2.71a |
| Fungi | LSD | 0.07 | 0.17 | 0.23 | 0.23 | 0.30 | 0.30 | 0.29 | 0.30 | 0.29 | 0.30 |
| Concentration | 35% | 0.25a | 0.96a | 1.27a | 1.58a | 2.09a | 2.27a | 2.46a | 2.65b | 2.92a | 3.16a |
| | 50% | 0.13b | 0.67b | 1.00a | 1.20b | 1.71b | 1.76b | 1.94b | 2.11c | 2.27b | 2.48c |
| | 75% | 0.03c | 0.38c | 0.69b | 0.87c | 1.18c | 1.25c | 1.38c | 1.63d | 1.69c | 1.90d |
| | C1(Agar) | 0.23a | 0.74b | 1.06a | 1.67a | 2.13a | 2.41a | 2.69a | 3.04a | 3.28a | 3.61a |
| | C2(Ethanol) | 0.00c | 0.01d | 0.03c | 0.03d | 0.05d | 0.05d | 0.05d | 0.28e | 0.43d | 0.63e |
| | LSD | 0.09 | 0.21 | 0.29 | 0.0 | 0.38 | 0.39 | 0.38 | 0.39 | 0.37 | 0.39 |

Means with different letters in a column are significantly different ($p \leq 0.05$).

controls as seen in Plate 6. Growth inhibitions of *A. niger* by ethanol fruit extracts of *X. aethiopica* at all concentrations was significantly better than that in the controls as shown in Plate 7. Growth inhibitions of *P. chrysogenum* by ethanol fruit extracts of *X. aethiopica* at all concentrations were significantly better than that in the

**Table 2.** Inhibition of the fungi by extracts of *S. aromaticum* fruit at days after incubation.

| Parameters | Variables | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Solvents | Ethanol | 0.10a | 0.15b | 0.39a | 0.35b | 0.43b | 0.53b | 0.61b | 0.76b | 0.87b | 1.06b |
| | Aqueous | 0.08a | 0.23a | 0.26a | 0.51a | 0.71a | 0.87a | 1.10a | 1.21a | 1.35a | 1.66a |
| | LSD | 0.06 | 0.09 | 0.14 | 0.16 | 0.15 | 0.17 | 0.17 | 0.23 | 0.22 | 0.24 |
| Fungi | *A. niger* | 0.15a | 0.42a | 0.65a | 0.77a | 0.85a | 0.99a | 1.09a | 1.34A | 1.41A | 1.59A |
| | *A.fumigatus* | 0.12a | 0.15b | 0.25b | 0.35b | 0.63b | 0.72b | 0.85b | 1.04b | 1.16a | 1.46a |
| | *P.chrysogenum* | 0.00b | 0.08b | 0.09b | 0.17b | 0.30c | 0.40c | 0.47c | 0.56c | 0.75b | 1.02b |
| | LSD | 0.07 | 0.11 | 0.17 | 0.19 | 0.19 | 0.21 | 0.21 | 0.28 | 0.27 | 0.30 |
| Concentration | 35% | 0.00b | 0.12c | 1.27a | 1.58a | 2.09a | 2.27a | 2.46a | 2.65b | 2.92a | 3.16a |
| | 50% | 0.13b | 0.67b | 1.00a | 1.20b | 1.71b | 1.76b | 1.94b | 2.11c | 2.27b | 2.48c |
| | 75% | 0.03c | 0.38c | 0.69b | 0.87c | 1.18c | 1.25c | 1.38c | 1.63d | 1.69c | 1.90d |
| | C1(Agar) | 0.23a | 0.74b | 1.06a | 1.67a | 2.13a | 2.41a | 2.69a | 3.04a | 3.28a | 3.61a |
| | C2(Ethanol) | 0.00c | 0.01d | 0.03c | 0.03d | 0.05d | 0.05d | 0.05d | 0.28e | 0.43d | 0.63e |
| | LSD | 0.09 | 0.21 | 0.29 | 0.0 | 0.38 | 0.39 | 0.38 | 0.39 | 0.37 | 0.39 |

Means with different letters in a column are significantly different ($p \leq 0.05$).

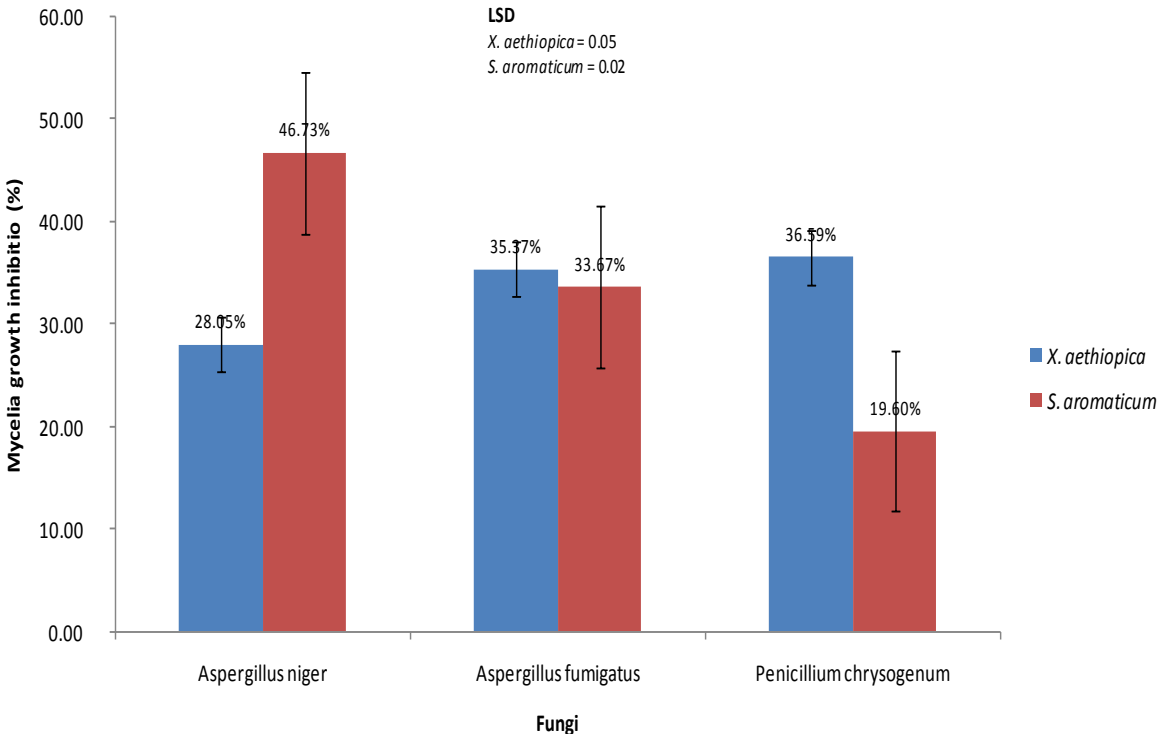
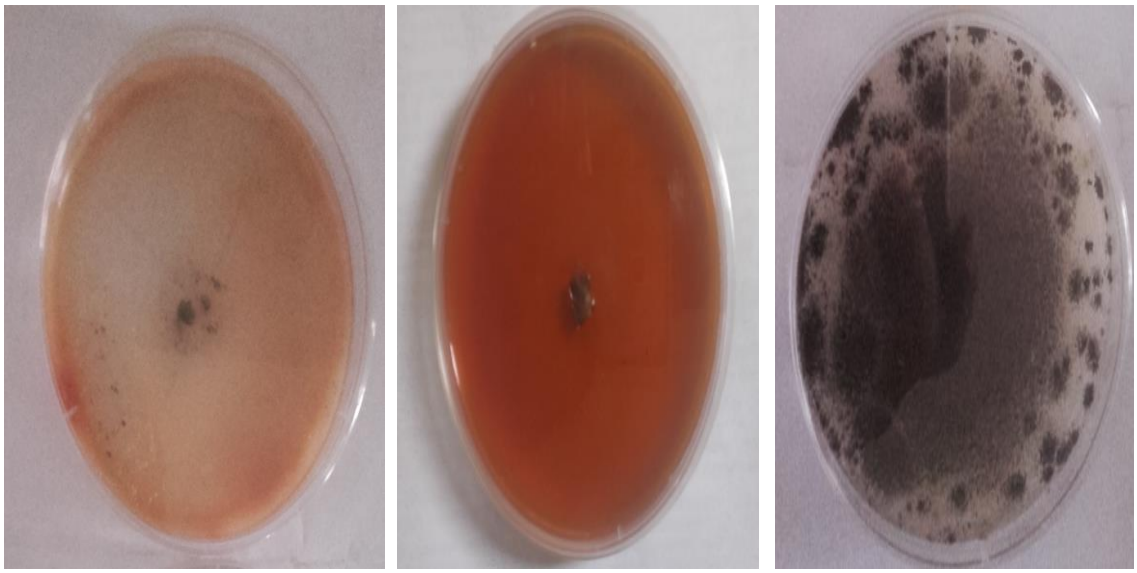



**Figure 1.** Pooled effect of *Xylopia aethiopica and Syzygium aromaticum* extracts on the isolated fungi.

controls as shown in Plate 8. Growth inhibitions of *P. chrysogenum* by ethanol fruit extracts of *S. aromaticum* at all concentrations were significantly better than that in the controls as seen in Plate 9. Inhibitions at 75% concentration were significantly better than that at other concentrations as seen in Table 2. The F values for the model, concentration, fungi, plant part, solvent and days were all highly (P>0.0001) significant for the antifungal

a b c

**Plate 6.** Inhibition of *A. fumigatus by X. aethiopica* aqueous leaf extracts at 50% (a) and 75% concentrations (b) with control (c).



a b c

**Plate 7.** Inhibition of *A. niger by X. aethiopica* ethanol fruit extracts at 35% (a) and 75% concentrations (b) with control (c).

activities of both *X. aethiopica* and *S. aromaticum*. Different interactions among the variables were also highly significant (P>0.0001) as shown in Tables 3 and 4.

## DISCUSSION

The antimicrobial potentials of *X. aethiopica* and *S. aromaticum* evaluated on *A. niger*, *A. fumigatus* and *P. chrysogenum* obtained from rotting yam tubers (*D. rotundata and D. alata*) showed inhibitory potentials on the mycelial growth of the fungi. *A. niger, A. fumigatus* and *P. chrysogenum* amongst others have been reported to be the causal agents of post-harvest rot of yam tubers in storage (Okigbo and Nmeka, 2005). The extracts of *X. aethiopica* and *S. aromaticum* have been reported to have anti-microbial and anti-fungal properties of which their derivatives are of great importance in public health, cosmetics, medicine and agriculture (Coyne et al, 2012).

The results obtained with fruit and leaf extracts of *X.*

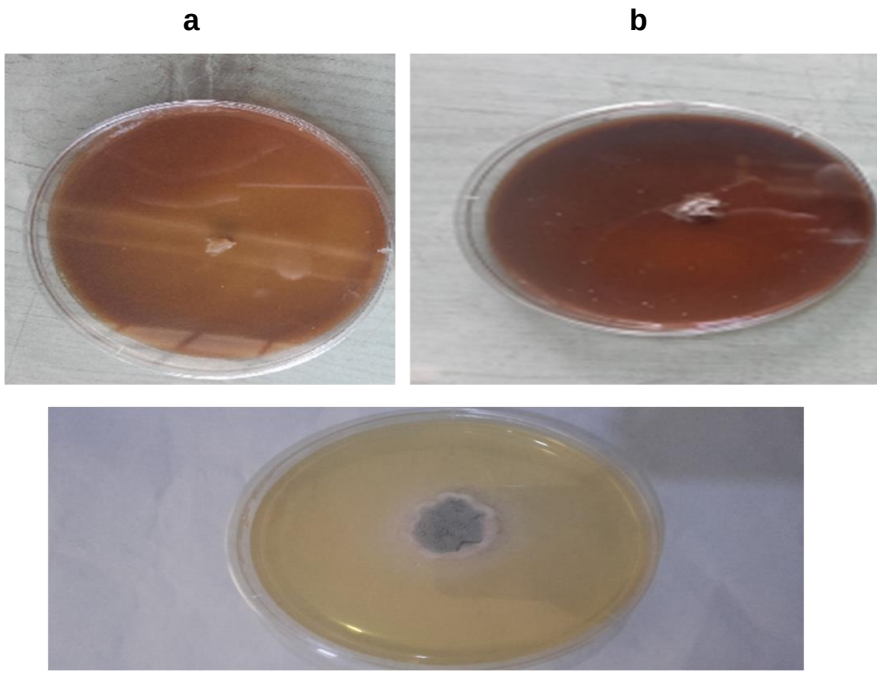



**Plate 8.** Inhibition of *P. chrysogenum by X. aethiopica* ethanol fruit extracts of at 50% (a) 75% concentrations (b) with control (c).

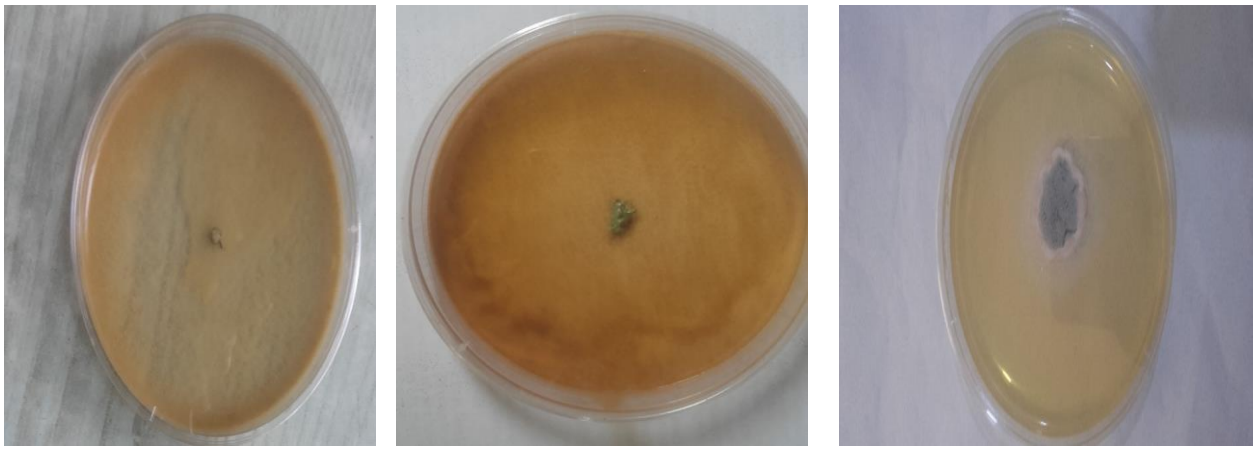



**Plate 9.** Growth inhibition of *P. chrysogenum* by *S. aromaticum* fruit ethanol extracts at 50% (a) and aqueous 75% concentrations (b) with control (c).

*aethiopica* are suggestive of higher antifungal potency of the former than the latter. It may thus be advisable to pay more attention on the fruit extract when field experiment is to be done. Extract concentration is also a key consideration for such a field experiment. The highly significant F values (P>0.0001) for the models in all the experiments show their appropriateness or 'goodness of fit'. This means effective growth inhibitions of the three fungi depend to a large extent on the fungi, plant part, concentration and interactions amongst them.

The highly significant F value for concentration, fungi, plant part, solvent, days as well as the various interactions among them in the case of both *X. aethiopica* and *S. aromaticum* are suggestive of the significant impacts

**Table 3.** ANOVA table for antifungal activity of *X. aethiopica* on the fungi isolated from rotting *Dioscorea* spp.

| Source | Df | SS | MS | F value | Pr < f |
|---|---|---|---|---|---|
| M | 321 | 2528.77 | 7.88 | 34.92 | 0.0001** |
| C | 4 | 875.52 | 218.88 | 970.34 | 0.0001** |
| F | 2 | 43.6 | 21.8 | 96.65 | 0.0001** |
| P | 1 | 6.44 | 6.44 | 28.57 | 0.0001** |
| D | 9 | 831.67 | 92.41 | 409.66 | 0.0001** |
| S | 1 | 6.62 | 6.62 | 29.37 | 0.0001** |
| F *C | 8 | 104.12 | 13.02 | 57.7 | 0.0001** |
| P *C | 4 | 11.75 | 2.94 | 13.02 | 0.0001** |
| C *D | 36 | 182.77 | 5.08 | 22.51 | 0.0001** |
| S *C | 4 | 11.01 | 2.75 | 12.2 | 0.0001** |
| P *F | 2 | 6.99 | 3.49 | 15.49 | 0.0001** |
| F *D | 18 | 30.35 | 1.69 | 7.47 | 0.0001** |
| S *F | 2 | 8.65 | 4.33 | 19.18 | 0.0001** |
| P *D | 9 | 6.42 | 0.71 | 3.16 | 0.0009** |
| S *P | 1 | 56.22 | 56.22 | 249.21 | 0.0001** |
| S *D | 9 | 7.85 | 0.87 | 3.86 | 0.0001** |
| P *F*C | 8 | 3.71 | 0.46 | 2.05 | 0.0374* |
| F*C*D | 72 | 33.24 | 0.46 | 2.05 | 0.0001** |
| S*F*C | 8 | 28.19 | 3.52 | 15.62 | 0.0001** |
| P *C*D | 36 | 6.7 | 0.19 | 0.83 | 0.7595 |
| S*P*C | 4 | 32.29 | 8.07 | 35.79 | 0.0001** |
| S*C*D | 36 | 18.17 | 0.5 | 2.24 | 0.0001** |
| P*F *D | 18 | 2.61 | 0.14 | 0.64 | 0.8687 |
| S*P*F | 2 | 200.33 | 100.16 | 444.04 | 0.0001** |
| S*F*P | 18 | 6.14 | 0.34 | 1.51 | 0.0766 |
| S*P*D | 9 | 7.41 | 0.82 | 3.65 | 0.0002** |
| Error | 1478 | 333.39 | 0.23 | | |
| Corrected total | 1799 | 2862.16 | | | |
| $R^2$ | 0.88 | | | | |

Significant = *: Highly significant= **
Key: M- Model, C- Concentration, F-Fungi, P-Plant part, S-Solvent. D –Days.

played by these factors on the antifungal activities of the plant parts. It means the same plant part will most likely exert different antifungal effect on different fungi. This is also corroborated by the results obtained in the pooled effect of *X. aethiopica and S. aromaticum* extracts on the isolated fungi. This agrees with the works of Suleiman and Falaiye (2013) who reported that extracts from different plant parts are used in controlling different fungi. The highly significant F values (P>0.0001) for plant parts may also be suggesting that the different plant parts employed might contain certain phytochemicals that are capable of inhibiting the growth of several fungal pathogens. The highly significant F values (P>0.0001) for fungi shows that the different fungi had significantly different growth responses in the presence of extracts of *S. aromaticum*.

The significant F values (P>0.0001) for days means that the growth inhibitory effects of the *S. aromaticum* on *A. niger, A. fumigatus* and *P. chrysogenum* among incubation days differed significantly. This is thus suggesting that contact period between plant extracts and the fungi is also critical for effective inhibition. The highly significant F value (P>0.0001) for solvent, indicates that method of extraction can also impact on the effectiveness of extracts against the fungal growth. This agrees with the work of Azwanida (2015) who reported that different plant parts require certain extraction methods in order that their antifungal potentials could be obtained. The results obtained with the aqueous and ethanol extracts showed that both solvents are good for extraction of extracts from *X. aethiopica* and *S. aromaticum*.

The highly significant F value (P>0.0001) for interaction between fungi and concentration means that any particular concentration of extract did not impact similar antifungal effect on any two fungi. This means the antifungal effect of the extracts at any particular concentration differed significantly from one fungus to the

**Table 4.** ANOVA table for antifungal activity of *S. aromaticum* extracts on the isolated fungi.

| Source | Df | SS | MS | F value | P < f |
|---|---|---|---|---|---|
| M | 227 | 998.84 | 4.4 | 218.29 | 0.0001** |
| C | 4 | 524.07 | 131.02 | 6499.53 | 0.0001** |
| F | 2 | 43.93 | 21.97 | 1089.77 | 0.0001** |
| D | 9 | 135.6 | 15.07 | 747.45 | 0.0001** |
| S | 1 | 19.94 | 19.94 | 989.01 | 0.0001** |
| F*C | 8 | 23.6 | 2.95 | 146.35 | 0.0001** |
| C*D | 36 | 139.23 | 3.87 | 191.87 | 0.0001** |
| S* C | 4 | 31.95 | 7.99 | 396.24 | 0.0001** |
| F*D | 18 | 5.9 | 0.33 | 16.27 | 0.0001** |
| S*F | 2 | 19.58 | 9.79 | 485.72 | 0.0001** |
| S*D | 9 | 7.42 | 0.82 | 40.87 | 0.0001** |
| F*C*D | 72 | 10.75 | 0.15 | 7.4 | 0.0001** |
| S*F*C | 8 | 22.23 | 2.78 | 137.82 | 0.0001** |
| S*C*D | 36 | 9.11 | 0.25 | 12.56 | 0.0001** |
| S*F*D | 18 | 5.54 | 0.31 | 15.26 | 0.0001** |
| Error | 672 | 13.55 | 0.02 | | |
| Corrected total | 899 | 1012.39 | | | |
| R Square | 0.99 | | | | |

Highly significant= **, Key: M- Model, C- Concentration, F-Fungi, S-Solvent, D-Days.

other. The highly significant F value (P>0.0001) for plant part and concentration (P>0.0001) means that any particular extract concentration of any particular plant part exerted significantly different antifungal effect on two different fungi. In other words the antifungal effect of extract of any particular concentration differed significantly from one fungus to the other. It can thus be said that appreciable growth reduction of the isolated fungi is dependent amongst other factors on the type of extract engaged as well as the concentration of the extracts. Onuh et al., (2015) reported that the higher the concentration, the more effective the plant extract on mycelial growth inhibition.

The highly significant F value (P>0.0001) for plant part and fungi means that extract from any particular plant part will most likely exert significantly different antifungal effect on two different fungi. The highly significant F value (P>0.0001) for concentration and day means that two different concentrations of the same extract did not exert similar antifungal impact at the same incubation day. It thus means that the antifungal effects of two different extract concentrations on the same incubation day differed significantly. The highly significant F value (P>0.0001) for plant part and day means that the antifungal impact of extract from any particular plant part differed significantly from one incubation day to the other. This suggests that length of time or contact period between extract and fungus will most likely be the key to effective fungal control in field experiment. The highly significant F value (P>0.0001) for solvents and fungi means that extracts by different solvents exerted significantly different antifungal impact on the same fungus. Solvent for extraction should therefore be carefully considered for plant extract to be used for antifungal purposes. The significant F value (P>0.0374) for interactions among plant part, fungi and concentration is suggestive. This means effectiveness of any particular concentration of extract of any particular plant part on growth of any fungus does not mean effectiveness on another fungus. Thus the 75% concentration of *X. aethiopica* extract which was most effective against *P. chrysogenum, A. niger and A. fumigatus* may not necessarily be effective against other fungi

The highly significant F values (P>0.0001) for interactions among fungi, concentration and days means exposure period of any of the three fungi to any specific extract concentration played a key role in the effectiveness of such extract (of both *S. aromaticum* and *X. aethiopica*). This fact was also validated by the highly significant F value (P>0.0001) for interactions among solvent, fungi and days in the case of *S. aromaticum*. It means at any incubation day, a specific extract concentration (of *S. aromaticum* or *X. aethiopica*) exerted significantly different impact on the three isolated fungi.

The highly significant F value (P>0.0001) for interactions among solvent, plant part and concentration shows that the antifungal effectiveness of any particular concentration of a specific *X. aethiopica* plant part was not the same among extraction solvents. It means 75% aqueous and ethanol extracts, for instance, of the same plant part (either fruit or leaf) will most likely have significantly different antifungal activities. The highly significant F

value (P>0.0001) for interactions among solvent, plant part and fungi shows that *X. aethiopica* extracts of the same part but of different extraction solvent significantly differed in effectiveness on the three fungi. The highly significant F value (P>0.0002) for interactions among solvent, plant part and days means that extract from a specific part of *X. aethiopica* and of a specific extraction solvent exerted different antifungal activity on different days of incubation.

The highly significant F values (P>0.0001) for interactions among solvent, fungi and concentration means that the same concentration of *S. aromaticum* extract of the same extraction solvent had significantly different effectiveness against the three fungi. The antifungal potentials of both *S. aromaticum* and *X. aethiopica* might not be unconnected with certain phytochemicals like tannins, alkaloids, flavonoids, phenols and glycosides contained in them. Volatile compound known as eugenol which occurred in large quantities in certain fruits has been reported to have antimicrobial activity against some pathogens (Ayoola et al., 2008; Mishra et al., 2014). Fleischer (2003) submitted that the fruit of certain plants contains higher amounts of flavonoids than the leaves and that it was responsible for the antimicrobial activity of the fruit.

## CONCLUSION AND RECOMMENDATIONS

This study has shown that the leaves and fruits of *X. aethiopica* and fruits of *S. aromaticum* have the antimicrobial potentials against fungi associated with rotting in white and water yam tubers especially rot caused by *A. niger, A. fumigatus and P. chrysogenum.* Highest antifungal activity was obtained with *S. aromaticum* fruit ethanol extracts. Similarly, leaf aqueous extract of *X. aethiopica* at 50% and 75% concentrations gave significant growth inhibition of *A. fumigatus.* The same concentrations of ethanol and aqueous extracts of *X. aethiopica* fruit significantly inhibited growth of *A. niger* and *P. chrysogenum.* However, there is need for further study on the phytochemicals of these plants to ascertain those associated with their antimicrobial capabilities before embarking on field experiments.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Combining ability of highland adapted maize (*Zea mays L.*) inbred lines for grain yield and yield related traits under optimum and low nitrogen conditions

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**Maize is staple cereal crop in Ethiopia despite the fact that its production is constrained by nitrogen deficiency due to the high cost of fertilizer, and risks of drought. Therefore, development of maize varieties for low nitrogen might be one of the options to overcome the problem. The objective of this study was to estimate combining ability of highland maize inbred lines for yield and yield related traits under low nitrogen (low N) stress and non-stress conditions. Twenty-six inbred lines (two heterotic testers and twenty-four lines) were crossed using line × tester mating design, which generated 48 $F_1$ hybrids and along with two hybrid checks (AMH853 and AMH 851) that were evaluated using alpha lattice design with two replications for grain yield and yield related traits within 2017 cropping season at Ambo under low and optimum nitrogen. Analyses of variances showed significant mean squares due to crosses for all traits under both low N stress and non-stress conditions, except for ear per plant under low N stress condition. The mean squares for general (GCA) and specific (SCA) combining abilities were significant for most of the traits under both conditions. Generally, the study indicated the importance of both additive and non-additive gene effects in most cases, while non-additive gene effects are less important under low-N stress. Inbred lines L1, L2, L9 and L20 were found as good combiners for grain yield at optimum N environment, whereas L5 and L14 were good general combiners under low N stress condition. L20 were good combiner for grain yield in combine analysis across environments and hence were promising parents for hybrid cultivars development. Based on SCA effects and per se performance, L5×T2 and L7×T2 were identified as promising hybrids for majority of traits studied in combined analysis across environments.**

**Key words:** General combining ability, gene action, heterotic group, specific combining ability.

## INTRODUCTION

Maize (*Zea mays* L) is an important cereal in the world, belonging to the tribe Maydeae of the grass family Poaceae (Acquaah, 2007). It is an important stable food crop for many people around the world. As the cultivation of early maize spread to different geographical regions from Mexico and Central America, where maize is widely

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believed to have originated, there was a rapid evolution of many races adapted to a wide variety growing conditions. Maize is a popular and widely cultivated food crop in Africa since its introduction to the continent around 1500 A.D. by Portuguese traders (McCann, 2005) and then arrived in Ethiopia slightly later, around the late 17th century (Huffnagel, 1961).

Ethiopia is the fifth largest producer of maize in Africa and smallholder farmers make up 94% of the crop production (http.//ethioagp.org, 2017). Maize ranks second after *teff* in area coverage and first in total production (CSA, 2017). It is the most widely consumed grain. According to CSA data, 80% of maize production is used for household consumption, 10% is sold on the open market, the remainder is used for seed, wages in kind, and animal feed (USDA, 2015). Maize research in Ethiopia started in 1950's with the evaluation of introduced materials focusing mainly on grain yield, early maturity, decreased plant height, lower ear placement and resistance to major biotic stresses (Benti, 1992). Since then, the research system has developed and released a number of improved varieties with their accompanying agronomic practices and plant protection technologies for all maize growing agro-ecologies of the country.

Average yield of maize in Ethiopia is 3.67 t $ha^{-1}$ (CSA, 2017) which is lower than the world average 5.65 t $ha^{-1}$ (USDA, 2018). The wide yield gap is attributed to an array of abiotic and biotic stresses. In spite of its wide adaptation and efforts made to develop improved maize technologies for different maize agro-ecological zones, many biotic and abiotic constraints still, limit maize production and productivity in different maize producing area of Ethiopia (Abate et al., 2017). The major abiotic stresses in the highland zones are frost, hail and water-logging (on Vertisols). Soils are characterized by undulating terrain, low fertility, and the region is characterized by wide variations in climatic conditions (Twumasi et al., 2002). Low soil fertility is mainly due to low soil nitrogen (N); N deficiency is common where N is applied at below optimal levels because of high cost of mineral fertilizer relative to the economic returns, or when there are significant risks of drought (Lafitte and Edmeades, 1994).

The high altitude, sub-humid maize agro ecology (1800-2400 m.a.s.l.) in Ethiopia is estimated to cover 20% of the land devoted to annual maize cultivation. Adoption of maize is increasing in the highland agro-ecology (Demissew et al., 2013). To meet the needs of increasing maize production in the highlands of Ethiopia, the Highland Maize Breeding Program was established in collaboration with the International Maize and Wheat Improvement Center (CIMMYT), in 1998. Since 1999, the breeding program has released seven superior highland maize cultivars for wide production.

The use of cultivars that utilize nitrogen more efficiently could greatly improve maize productivity in maize-based cropping systems. So far, combining ability effects in maize inbred lines has been extensively studied under non-stressed conditions for different sets of new maize inbred lines developed/introduced and adapted at different times (Amare et al., 2016; Ziggiju et al., 2016; Abakemal et al., 2016; Assefa et al., 2017). Even though CIMMYT has made significant progress in developing maize germplasm tolerant to low N (Banziger and Lafitte, 1997; Banziger et al., 1997; Worku et al., 2008; Dagne, 2008; Mohamed et al., 2014; Mafouasson et al., 2017). Information is still limited regarding combining ability of maize inbred lines and choosing the best testers to use when developing stress tolerant single and three-way cross hybrids for the highlands.

Understanding the relative importance of general and specific combing ability effects for different traits for newly developed and/or introduced inbred lines is of paramount importance to design future breeding strategies for the development of hybrid and/or synthetic varieties. In the current study, therefore, an attempt was made to identify high yielding hybrids tolerant to low N soil, determine the combining abilities and mode of gene action of elite maize inbred lines for hybrid development under low and optimum nitrogen conditions.

## MATERIALS AND METHODS

### Experimental site

The field experiments were conducted at Ambo Agricultural Research Centers during the 2017 main cropping season. Geographically, Ambo is located at 8°57'N latitude, 38°7'E longitude and at an altitude of 2225 m.a.s.l with average annual rainfall of 1110 mm, maximum and minimum temperature of 26 and 11°C, respectively. The soil type of the experimental field is vertisols (http.//www.eiar.gov.et/index.php/research-centers). The total precipitation during the growing season (May to December 2017) was 864.1 mm, and the mean minimum and maximum temperatures were 10.51 and 24.1°C, respectively (Ambo Agricultural Research Centers Meteorological Station, Unpublished Data).

### Experimental design

The hybrids were planted in alpha-lattice design (Patterson and Williams, 1976) with two replicates. Design and randomization of the trials were generated using CIMMYT's software known as field book (Banziger and Vivek, 2007). One-row plots of 5.25 m length and 75 × 25 cm spacing between rows and plants were used to achieve 53,333 plants/ha. Two seeds were hand planted per hill and later thinned out to have one plant per hill after seedlings established well.

The hybrids were evaluated under optimal and low N conditions in adjacent fields with the same soil type in 2017. The experiment under low N-stress condition was laid in a field that had been depleted of N by continuous cropping of maize for several seasons and removing the crop residues after each season. No additional N fertilizer was applied. Under non-stress N conditions, the recommended rate of diammonium phosphate (DAP) fertilizer was applied once at planting using a rate of 100 kg $ha^{-1}$ while 200 kg $ha^{-1}$ of Urea was applied in split at planting, knee height and flowering

**Table 1.** Soil properties at two depths of the experimental fields at Ambo, 2017.

| Field | | Depth (cm) | pH | Available P (ppm) | N (%) | OC (%) | OM (%) |
|---|---|---|---|---|---|---|---|
| Ambo | Optimum N field | 0-30 | 6.7 | 49.6 | 0.13 | 1.64 | 2.8 |
| | | 30-60 | 6.9 | 37.8 | 0.09 | 1.49 | 2.6 |
| | Low N field | 0-30 | 7.2 | 10.5 | 0.11 | 1.41 | 2.4 |
| | | 30-60 | 7.4 | 7.4 | 0.08 | 1.31 | 2.3 |

OC=Organic carbon, OM=organic matter.

stages of the crop. Other crop management practices were carried out as recommended for the location.

**Soil sampling and analysis**

Soil samples from the experimental sites were taken before planting. First, one representative composite soil sample was taken from ploughed and leveled field at three places diagonally across the plot (in zigzag method) with auger. Samples were taken from 0 to 30 cm and 30 to 60 cm depth of top soil and composited to make one representative soil sample for each depth before planting. The composited soil samples were subjected to analysis before planting. Results of the soil analysis are shown in Table 1.

**Experimental materials**

The experiment consisted of 48 test crosses produced by crossing 24 inbred lines to two testers in line × tester mating design, and two standard checks (AMH851 and AMH853). The inbred lines were introduced from CIMMYT-Zimbabwe. The two testers, FS59 (Tester 1) and FS67 (Tester 2) are adapted lines locally developed at Ambo. FS59 is heterotic group B while FS67 is heterotic group A. The lines × tester crosses were made by highland maize breeding program during the main season of 2016. AMH851 and AMH853 are commercial hybrid checks released for and produced in the highland agro-ecologies of Ethiopia. The list and pedigrees of the inbred lines and testers used for the study are shown in Table 2.

**Data recorded**

Data on grain yield and other agronomic traits were collected on plot and individual plant basis. Anthesis date (AD) and silking date (SD) were recorded as 'number of days after planting', when 50% of plants were shedding pollen and silking, respectively. The anthesis-silking interval (ASI) was calculated as silking date minus anthesis date. Leaf senescence (SEN) was scored 10 and 12 weeks after planting on a scale from 0 to 10, dividing the percentage of the estimated total leaf area below the ear that is dead by 10. A score of 1 = less than 10% dead leaf and 10 = more than 90% dead leaf. Plant height (PH) was measured as the average height of five randomly selected plants measured in cm from base of the plant to the first tassel branch. Ear height (EH) was measured as the average height of five randomly selected plants measured in cm from base of the plant to the node bearing the upper most ear of the same plants used to measure plant height. At harvest, the number of ears per plant (EPP) was computed as the total number of harvested ears in each plot divided by the stand count at harvest. Number of kernels per row (NKR) was recorded by counting kernels in each row from five randomly taken ears and the average value was recorded as kernels per row. Number of kernel rows per ear (KRE) this was measured as total number of kernel rows of the ear was counted from five randomly taken ears and the average value was used as kernel rows per ear. Thousand kernels weight (TKWT) was recorded as the weight in grams of 1000 random kernels was weighed from each plot using sensitive balance and was adjusted to 12.5% moisture level. Grain yield (t $ha^{-1}$) was measured as the total grain yield in kg per plot and adjusted to 12.5% moisture level was used to calculate grain yield per hectare.

**Data analysis**

Prior to data analysis, anthesis-silking interval (ASI) was normalized using lnSQRT (*ASI* +10) as suggested by Bolaños and Edmeades (1996). Analysis of variance per environment was conducted with the PROC MIXED procedure in SAS (2002) considering genotypes as fixed effects and replications and blocks within replications as random. Relative reductions in grain yield and agronomic traits under low N was calculated as (1 - MV low N / MV optimum N), where MV low N are mean traits values obtained in experiment under low N and MV optimum N are mean traits values obtained in experiment under optimum N (Banziger et al., 1997). Combined analysis across environments also computed using PROC GLM in SAS software version 9.0 (SAS, 2002). The combined analysis was done for the significant trait in individual location analysis after testing the homogeneity of error variances through the application of the F-test (Gomez and Gomez, 1984).

Further analysis was done according to the line × tester analysis (Kempthorne, 1957) to partition the mean square due to crosses into lines, tester and line by tester effects (Dabholkar, 1999) using SAS computer program (SAS, 2002) for traits that shows significant differences among crosses. General combining abilities of lines and testers, and specific combining abilities of lines by testers were computed for the characters that show significant differences among crosses in the ANOVA.

## RESULTS AND DISCUSSION

Hybrids exhibited highly significant (P<0.01) differences in most traits under low and optimum N conditions at Ambo except number of kernels per row under low N (Table 3). Combined analyses were performed for the traits that showed significant genotypic mean squares for individual location analysis and homogenous error variance analyzed using F-test (Gomez and Gomez, 1984). Combined analysis of variance across environments revealed that al traits exhibited highly significant (P<0.01) differences among the hybrids (Table 4). Significant differences observed among hybrids for individual and across environments indicated the existence of a high

**Table 2.** The pedigree and source of the lines and testers used in the study.

| Line code | Pedigree | Source |
|---|---|---|
| L1 | (LPSC7-F96-1-2-1-1-B-B-B*/OFP9)-3-1-1-1-1-B-B-# | CIMMYT/AHMBP |
| L2 | (LPSC7-F96-1-2-1-1-B-B-B*/OFP39)-6-1-1-1-1-B-B-# | CIMMYT/AHMBP |
| L3 | (LPSC7-F71-1-2-1-2-B-B-B*/OFP1)-B-14-4-1-B-B-B-# | CIMMYT/AHMBP |
| L4 | (LPSC7-F71-1-2-1-2-B-B-B*/OFP2)-B-1-3-1-B-B-B-# | CIMMYT/AHMBP |
| L5 | (LPSC7-F71-1-2-1-2-B-B-B*/OFP3)-B-18-1-1-B-B-B-# | CIMMYT/AHMBP |
| L6 | CML539-B-# | CIMMYT/AHMBP |
| L7 | (CML539*/OFP9)-4-1-1-2-1-B-B-# | CIMMYT/AHMBP |
| L8 | (CML539*/OFP27)-2-1-2-1-1-B-B-# | CIMMYT/AHMBP |
| L9 | (CML539*/OFP14)-2-1-1-2-1-B-B-# | CIMMYT/AHMBP |
| L10 | (CML539*/OFP14)-2-1-3-1-2-B-B-# | CIMMYT/AHMBP |
| L11 | CML539*/OFP1)-B-6-1-1-B-B-B-# | CIMMYT/AHMBP |
| L12 | CML539*/OFP1)-B-11-2-1-B-B-B-# | CIMMYT/AHMBP |
| L13 | (CML539*/OFP4)-B-12-1-1-B-B-B-# | CIMMYT/AHMBP |
| L14 | CML442-# | CIMMYT/AHMBP |
| L15 | (CML442*/OFP1)-B-14-4-2-B-B-B-# | CIMMYT/AHMBP |
| L16 | (CML442*/OFP1)-B-18-2-2-B-B-B-# | CIMMYT/AHMBP |
| L17 | (CML442*/OFP4)-B-4-1-2-B-B-B-# | CIMMYT/AHMBP |
| L18 | (CML442*/OFP4)-B-17-3-2-B-B-B-# | CIMMYT/AHMBP |
| L19 | (CML395*/OFP105)-1-2-3-1-2-B-B-# | CIMMYT/AHMBP |
| L20 | (CML444*/OFP23)-6-3-1-1-2-B-B-# | CIMMYT/AHMBP |
| L21 | ([CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-BBBB]-1-5-1-1-1-BBB-B-B-B*/OFP106)-1-2-2-2-2-B-B-# | CIMMYT/AHMBP |
| L23 | (CML495*/OFP6)-B-3-3-3-B-B-# | CIMMYT/AHMBP |
| L24 | (CML495*/OFP6)-B-27-1-1-B-# | CIMMYT/AHMBP |
| | **TESTER** | |
| T1 | FS59 | AMBO |
| T2 | FS67 | AMBO |
| | **CHECKS** | |
| 1 | KOLBA (AMH853) | AMBO |
| 2 | JIBAT (AMH 851) | AMBO |

AHMBP=Ambo Highland Maize Breeding Program.

level of variation for various characteristics, which makes selection possible for improved grain yield and agronomic traits under low N stress and non-stress conditions. Similar results have been reported (Dagne, 2008; Amare et al., 2016; Abakemal et al., 2016; Bullo and Dagne, 2016; Keno et al., 2017; Mafouasson et al., 2017).

## Mean performance of genotypes

The mean grain yield for hybrids tested under optimum N ranged from 5.62 to 10.76 t $ha^{-1}$ with a mean value of 7.73 t $ha^{-1}$ (Table 5). Among the crosses, 17 crosses showed significantly higher yield than the hybrid check Jibat and one cross-revealed significantly higher yield than the check hybrid Kolba. Under low N experiment, mean grain yield for all hybrids were 3.23 t $ha^{-1}$ ranging from 2.13 to 4.61 t $ha^{-1}$ (Table 5). Worku et al. (2008), Mohamed et al. (2014), Mafouasson et al. (2017), Talukder et al. (2016) and Assefa et al. (2017) in their studies reported that experimental varieties showed better performance than the best check for most yields and other traits.

Mean relative grain yield loss under low N was 58.2%.

Mean relative loss of days to anthesis, days to silking and anthesis-silking interval increased by 18.1, 18. 5 and 0.8%, respectively. Plant height, ear height and ears per plant decreased by 16.3, 16.5 and 33.1, respectively

**Table 3.** Line × tester analysis of variance for grain yield and yield related traits under low and optimum N conditions at Ambo in 2017.

| Trait | | Hybrid df=49 | Cross df=47 | GCAL df=23 | GCAT df=1 | $SCA_{LxT}$ df=23 | Error | % contr. GCA | % contr. SCA |
|---|---|---|---|---|---|---|---|---|---|
| GYF (t $ha^{-1}$) | Low N | 0.54* | 0.65* | 0.69* | 1.11$^{ns}$ | 0.60$^{ns}$ | 0.39 | 55 | 45 |
| | Opt. N | 3.91** | 3.83** | 5.06** | 1.85$^{ns}$ | 2.69* | 0.91 | 66 | 34 |
| DA (days) | Low N | 7.6** | 9.34** | 15.22** | 1.76$^{ns}$ | 3.78* | 2.1 | 80 | 20 |
| | Opt. N | 11.57** | 14.42** | 24.04** | 44.01** | 3.51$^{ns}$ | 4.45 | 88 | 12 |
| DS (days) | Low N | 18.6** | 21.08** | 16.95** | 263.3** | 14.69* | 6.71 | 66 | 34 |
| | Opt. N | 11.79** | 13.02** | 19.48** | 45.38** | 5.16$^{ns}$ | 5.64 | 81 | 19 |
| ASI (days) | Low N | 0.03** | 0.03** | 0.02* | 0.48** | 0.03** | 0.01 | 57 | 43 |
| | Opt. N | 0.01** | 0.01** | 0.01** | 0.00$^{ns}$ | 0.01** | 0.004 | 47 | 53 |
| PH (cm) | Low N | 413.4** | 790.91** | 1045.22** | 5192.04** | 345.26$^{ns}$ | 374.12 | 79 | 21 |
| | Opt. N | 992.19** | 1195.9** | 1049.21** | 25905.51** | 268.27** | 113.79 | 89 | 11 |
| EH (cm) | Low N | 227.87** | 442.68** | 415.78** | 6402.7** | 210.45$^{ns}$ | 169.44 | 77 | 23 |
| | Opt. N | 438.16** | 558.76** | 521.65** | 11331.76** | 127.48** | 66.19 | 89 | 11 |
| EPP(#) | Low N | 0.03* | 0.03$^{ns}$ | 0.03$^{ns}$ | 0.13* | 0.03$^{ns}$ | 0.03 | 58 | 42 |
| | Opt. N | 0.01** | 0.11** | 0.13** | 0.10$^{ns}$ | 0.09* | 0.04 | 60 | 40 |
| SEN (scale) | Low N | 1.13** | 1.43** | 1.28** | 20.17** | 0.78* | 0.38 | 74 | 26 |
| NRPE (#) | low N | 1.07** | 1.11** | 0.80$^{ns}$ | 25.32* | 0.38$^{ns}$ | 0.47 | 66 | 34 |
| | Opt. N | 1.52* | 1.70** | 2.02** | 13.72** | 0.85$^{ns}$ | 1.72 | 75 | 25 |
| NKPR (#) | Low N | 10.81$^{ns}$ | | | | | | | |
| | Opt. N | 16.08* | 16.62** | 15.28* | 43.16* | 16.81* | 8.32 | 51 | 49 |
| TKW (g) | Low N | 2503.16** | 2910.92** | 1586.84$^{ns}$ | 53756.9** | 2024.32$^{ns}$ | 1213.1 | 83 | 17 |
| | Opt. N | 2636.28** | 4140.33** | 4124.85** | 60324.9** | 1713.00* | 703.59 | 80 | 20 |

*, **Significant at 0.05 and 0.01, GYF=Grain yield; DA= days to anthesis; DS= days to silking; ASI=anthesis silking interval; PH=plant height; EH= ear height; EPP=ear per plant; NRPE=number of rows per ear; NKPR= number of kernels per row; TKW= thousand kernel weight; number.

(Table 5). The level of yield loss between low and high N varied depending on the degree of N depletion in the soil (Banziger and Lafitte, 1997). Banziger and Lafitte (1997) reported a significant reduction in plant height (27.1%), ears per plant (11.2%), grains per ear (47.8%) and grain weight (30.7%) under low N. Presterl et al. (2003) and Worku et al. (2008) reported that low-N stress reduced grain yield by 37 and 64%, respectively.

## Combining ability analyses

The partitioning of significant crosses mean squares into general combining ability (GCA) and specific combining ability (SCA) showed that SCA mean squares were significantly different for grain yield under optimum N condition (Table 3). Line GCA means squares were significantly different for grain yield at Ambo under optimum and low condition (Table 3). In combined analysis across environments, significant GCA and SCA mean squares were observed for grain yield (Table 4) which implied that importance of both additive and non-additive gene actions in governing grain yield. In agreement with the present study, Tamirat et al. (2014), Girma et al. (2015), Bullo and Dagne (2016) and Amare et al. (2016) were reported the importance of both additive and non-additive gene actions in governing grain yield in maize.

GCA sums of squares were larger than SCA sums of squares for grain yield under low, optimum N and across

**Table 4.** Mean squares from line x tester analysis of variance for yield and yield related traits over two location Ambo under optimum and low N-conditions.

| Source of variation | DF | GYF (t ha$^{-1}$) | DA (days) | DS (days) | PH (cm) | EH (cm) | EPP (#) | NRPE (#) | TKW (g) |
|---|---|---|---|---|---|---|---|---|---|
| Location (Loc) | 1 | 374** | 2818** | 6259** | 21863** | 4947** | 12** | 1.6$^{ns}$ | 395$^{ns}$ |
| Replication (Loc) | 2 | 0.3$^{ns}$ | 0.2$^{ns}$ | 2.5$^{ns}$ | 264.8$^{ns}$ | 91.7$^{ns}$ | 0.0$^{ns}$ | 5.2** | 33.6$^{ns}$ |
| Hybrid | 49 | 1.73** | 16.44** | 21.04** | 1095** | 558.1** | 0.07** | 1.99** | 4501.6** |
| Crosses (Cr) | 47 | 2.1** | 17.1** | 24.8** | 1304.1** | 663.3** | 0.1** | 2.3** | 5229.8** |
| Hybrid × Loc | 49 | 1.7$^{ns}$ | 2.6$^{ns}$ | 6.96$^{ns}$ | 244.5$^{ns}$ | 93.08$^{ns}$ | 0.05$^{ns}$ | 0.4$^{ns}$ | 1035.8* |
| Cr × Loc | 47 | 1.7$^{**}$ | 2.9$^{ns}$ | 6.6$^{ns}$ | 251.1$^{ns}$ | 91.5$^{ns}$ | 0.1$^{*}$ | 0.5$^{ns}$ | 1188.8$^{ns}$ |
| GCAL | 23 | 2.8** | 30.4** | 26.5** | 1394.5** | 604.0** | 0.1* | 2.3** | 3850.2** |
| GCAT | 1 | 2.0$^{ns}$ | 5.6$^{ns}$ | 257** | 20974** | 13581** | 0.2* | 38.1** | 107024** |
| SCA L × T | 23 | 1.4* | 4.3* | 13.0** | 358.6* | 160.7** | 0.1** | 0.9$^{ns}$ | 2183.7** |
| GCAL × Loc | 23 | 2.2** | 3.2$^{ns}$ | 6.1$^{ns}$ | 213.6$^{ns}$ | 109.5$^{ns}$ | 0.1* | 0.6$^{ns}$ | 1209.4$^{ns}$ |
| GCAT × Loc | 1 | 0.0$^{ns}$ | 21.2** | 60.5** | 4523.9** | 430.3* | 0.0$^{ns}$ | 0.9$^{ns}$ | 233.3$^{ns}$ |
| SCALXT × Loc | 23 | 1.4* | 1.7$^{ns}$ | 4.7$^{ns}$ | 102.8$^{ns}$ | 58.8$^{ns}$ | 0.0$^{ns}$ | 0.4$^{ns}$ | 1209.8$^{ns}$ |
| error | 68 | 0.7 | 2.2 | 4.9 | 171.4 | 74.0 | 0.0 | 0.6 | 869.4 |
| % contr. GCA | - | 67.4 | 87.6 | 74.4 | 86.5 | 88.1 | 52.0 | 82.1 | 79.6 |
| % contr. SCA | - | 32.6 | 12.4 | 25.6 | 13.5 | 11.9 | 48.0 | 17.9 | 20.4 |

*, **Significant at 0.05 and 0.01; GYF= grain yield; DA= days to anthesis; DS= days to silking; EH= ear height; PH= plant height; EPP= ears per plant; NRPE= number of rows per ear; TKW= thousand kernel weight; number.

environments 55, 66 and 67.4%, respectively (Tables 3 and 4). The predominance of GCA sums of squares to SCA sums of squares for grain yield indicated the relative importance of additive gene action to non-additive gene action for this trait (Beck et al., 1990). In line with this study, Tamirat et al. (2014) reported the preponderance of additive gene action in the inheritance of grain yield while in contrast to these findings, Kanagarasu et al. (2010) and Melkamu (2013) reported the dominant role of SCA gene action in the grain yield of maize.

In combined analysis, significant GCA and SCA mean squares were observed for anthesis and silking date (Table 4) that implied the importance of both additive and non-additive gene actions in governing these traits. Results of this study are in accordance with the findings of Melkamu (2013), Shushay et al. (2013), Tamirat et al. (2014) and Girma et al. (2015) who reported significant mean squares due to GCA and SCA for days to anthesis and silking.

Mean squares due to crosses for plant and ear height were highly significant ($P$ < 0.01). Combining ability analysis revealed that highly significant GCA effects of lines and testers for plant and ear height under both environments. SCA mean squares were highly significant under optimum N condition but non-significant under low N (Table 3). In combined analysis across environments, significant GCA and SCA mean squares were observed for plant and ear height (Table 4). In line with these findings, Worku et al. (2008) reported high mean square due to GCA, SCA effects under high N and also Demissew et al. (2011) found significant GCA and SCA mean squares for plant and ear height, in contrast to these finding. Gudeta (2007) reported significant GCA and non-significant SCA mean squares for plant height.

Mean squares due to crosses for number of ears per plant were highly significant ($P$ < 0.01) at Ambo optimum N condition while it is non-significant under low N condition. Significant GCA of lines and SCA mean squares were observed under optimum nitrogen environment. In contrast to these findings, Abakemal et al. (2016) reported non-significant mean square due to line GCA and significant SCA mean squares. GCA sums of squares were larger than SCA sums of squares for number of ears per plant under both conditions (Table 3). Highly significant differences were observed among crosses and tester GCA for thousand-kernel weight under both N condition (Table 3). In combine analysis across environments, significant GCA and SCA mean squares were observed for the trait (Table 4). In agreement with the present results, Kanagarasu et al. (2010) and Abakemal et al. (2016) reported significant mean squares due to GCA and SCA for thousand-kernel weight.

## Estimates general combining ability effects

The estimates of line GCA effects for grain yield and yield related traits under optimum N at Ambo are shown in Table 6. The inbred lines varied significantly in GCA for all traits. Line GCA for grain yield varied from -1.91 to 1.26 t ha$^{-1}$. Even though a total of 13 lines showed positive GCA effects for grain yield only four inbred lines

**Table 5.** Mean values, coefficient of variation (CV) and range of grain yield and yield related of testcrosses evaluated at Ambo under Optimum and low nitrogen stress conditions, 2017.

| Statistics | | GYF (t ha$^{-1}$) | DA (days) | DS (days) | ASI (days) | PH (cm) | EH (cm) | EPP (#) | SEN (scale) | NRPE (#) | NKPR (#) | TKW (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Minimum | 5.62 | 89 | 90 | 1.04 | 197 | 100.5 | 1.05 | - | 11.34 | 29.33 | 210.46 |
| | Maximum | 10.76 | 99 | 101 | 1.34 | 299 | 175.6 | 2.01 | - | 16.17 | 44.84 | 410.46 |
| | Cross mean | 7.67 | 94 | 95 | 1.22 | 249 | 131.4 | 1.51 | - | 12.82 | 34.77 | 309.83 |
| Optimum N | Check mean | 9.1 | 91 | 92 | 1.21 | 266 | 145.5 | 1.46 | - | 13.5 | 34.34 | 366.63 |
| | Grand mean | 7.73 | 94 | 95 | 1.22 | 250 | 131.9 | 1.51 | - | 12.85 | 34.75 | 312.1 |
| | CV (%) | 16.07 | 1.8 | 2.1 | 3.25 | 3.35 | 4.43 | 13.4 | - | 6.67 | 8.08 | 7.42 |
| | LSD (5%) | 2.52 | 3.5 | 4 | 0.06 | 17.1 | 11.88 | 0.15 | - | 1.75 | 5.7 | 61.51 |
| | Minimum | 2.13 | 107 | 108 | 1.01 | 164.1 | 83.4 | 0.69 | 3.1 | 11 | 27.4 | 242.4 |
| | Maximum | 4.61 | 115.6 | 120 | 1.5 | 237.6 | 134 | 1.28 | 6.2 | 14.34 | 38 | 377.2 |
| | Cross mean | 3.24 | 110.7 | 112.7 | 1.22 | 208.73 | 109.8 | 1.01 | 4.83 | 12.64 | 32.94 | 305.49 |
| Low N | Check mean | 2.83 | 109 | 113.2 | 1.3 | 226 | 119.3 | 0.95 | 4.5 | 12.5 | 31.25 | 301.33 |
| | Grand mean | 3.23 | 110.7 | 112.8 | 1.23 | 209.4 | 110.2 | 1.01 | 4.82 | 12.63 | 32.87 | 305.33 |
| | CV (%) | 15.6 | 1.15 | 2.1 | 7.8 | 6.18 | 6.17 | 16.04 | 11.17 | 5.3 | 8.2 | 9.92 |
| | LSD (5%) | 1.02 | 2.59 | 4.81 | 0.2 | 26.28 | 13.81 | 0.35 | 1.09 | 1.36 | ns | 61.51 |
| | Relative reduction | 58.2 | -18.1 | -18.4 | -0.82 | 16.25 | 16.49 | 33.11 | - | 1.71 | 5.41 | 2.17 |

Percent relative reduction due to low N stress (1 - MV low N/Optimum N); GYF=Grain yield; DA=days to anthesis; DS=days to silking; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPP=ears per plant; SEN=leaf senescence; NRPE=number of rows per ear; NKPR=number of kernels per row; TKW=thousand kernel weight, #=number.

L1, L2, L9 and L20 showed highly significant positive GCA effects for grain yield. In contrast, L4, L13, L15, L16 and L21 had significant negative GCA effects for grain yield. Among the testers, none of them showed significant GCA effects for grain yield per hectare. Line GCA effects for days to anthesis ranged between -4.55 and 3.45. The female parents L9, L10, L14, L18 and L20, revealed positive and significant GCA effects contributed to late maturity. The female parents L3, L7, L8, L12, and L20 were the best general combiners for days to anthesis, which exhibited negative and significant GCA effects. The GCA estimates of parental lines ranged from -4.1 to 3.4 for days to silking. The female parents L3, L7, L8, L12 and L23 were negative and significant difference of GCA effects. Result of the current study are in accordance with the findings of Melkamu (2013), Girma et al. (2015), and Amare et al. (2016) who reported significant positive and negative GCA effects for these traits in their combining ability study.

General combining ability estimate of lines for plant and ear height ranged from -29.66 to 36.34 cm and -23.11 to 24.14 cm, respectively. Inbred lines L7, L11, L12, L19 and L20 showed significant negative line GCA effects for plant height and inbred lines L8, L14, L15, L16 and L24 showed highly significant negative line GCA effects for ear height. In maize, shorter plant height is desirable for lodging resistance. L1, L2, L18 and L23 had significantly high positive line GCA effects for ears per plant while L15 showed significantly high negative GCA effect. Kanagarasu et al. (2010) and Shushay et al. (2013) reported similar results for these traits.

Inbred lines L12 and L16 showed highly significant ($p<0.01$) positive line GCA effects for number of rows per ear while inbred lines L18 and L20 showed highly significant ($p<0.01$) positive line GCA effects for number of kernels per row. For thousand-kernel weight, significant positive line GCA effects were observed for L7, L11, L12 and L19 while significant negative line GCA effects were observed for L13, L14, and L22. Both positive and negative GCA effects were reported in maize by several investigators (Tamirat et al., 2014; Chandel and Mankotia, 2014).

**Table 6.** General combining ability effects of 24 inbred lines and 2 testers for grain yield and yield related traits under optimum N conditions at Ambo, 2017.

| Line | GYF (t ha$^{-1}$) | AD (days) | SD (days) | ASI (days) | PH (cm) | EH (cm) | EPP (#) | NRPE (#) | TKW (g) |
|---|---|---|---|---|---|---|---|---|---|
| L1 | 1.41* | -0.8 | -1.1 | -0.01 | 3.34 | 11.1** | 0.24* | 0.84 | 14.87 |
| L2 | 2.2** | -1.55 | -1.35 | 0.01 | 0.34 | 2.89 | 0.27* | 0.68 | -25.12 |
| L3 | 0.26 | -4.1** | -4.1** | 0 | -7.41 | -1.86 | 0.2 | -1.2* | -36.51* |
| L4 | -1.91** | 1.2 | 0.4 | -0.04 | -12.6* | -8.86* | 0.08 | -0.82 | -75.4** |
| L5 | 0.45 | 1.45 | 1.9 | 0.02 | -10.16* | -5.86 | -0.12 | 0.51 | 27.57 |
| L6 | 0.01 | -0.05 | 0.4 | 0.02 | 6.59 | 0.89 | -0.03 | 0.34 | -7.37 |
| L7 | 0.1 | -4.30** | -3.35** | 0.04 | 22.34** | 6.14 | -0.15 | -0.66 | 31.08* |
| L8 | 0.78 | -3.80** | -3.85** | 0 | -20.66** | -17.36** | 0.12 | -0.16 | 16.03 |
| L9 | 1.62** | 2.95** | 3.40** | 0.02 | 7.34 | 8.14** | 0.12 | -0.32 | -24.02 |
| L10 | 0.09 | 3.45** | 2.90** | -0.02 | -2.91 | 8.64** | -0.09 | 0.34 | 34.14* |
| L11 | 0.89 | 0.2 | -0.1 | -0.01 | 36.34** | 17.14** | -0.03 | -0.16 | 8.64 |
| L12 | -0.27 | -2.55* | -2.85* | -0.01 | 10.34* | 1.39 | -0.2 | 1.34** | 45.96** |
| L13 | -1.27* | 1.2 | 0.4 | -0.03 | -8.41 | -8.86* | -0.03 | -0.66 | -21.89 |
| L14 | -0.24 | 2.20* | 2.15 | 0 | -17.91** | -10.11** | -0.01 | -0.66 | 9.72 |
| L15 | -1.21* | -0.55 | 1.15 | 0.07 | -25.41** | -11.86** | -0.28** | -0.82 | -10.37 |
| L16 | -1.35* | -1.8 | -0.85 | 0.04 | -29.66** | -23.11** | -0.06 | 1.26** | -15.84 |
| L17 | -0.56 | 1.95 | 2.15 | 0.01 | 0.84 | 5.39 | -0.13 | 0.84 | -10.11 |
| L18 | 0.72 | 3.20** | 2.65* | -0.02 | 2.34 | 14.64** | 0.21* | 0.18 | -21.32 |
| L19 | -1.04 | 1.2 | 1.15 | 0 | 13.09** | 5.64 | -0.32 | -0.32 | 81.30** |
| L20 | 2.08** | 2.20* | 2.40* | 0.01 | 30.84** | 24.14** | -0.14 | 0.84 | 18.92 |
| L21 | -1.54* | 1.95 | -0.1 | -0.1** | -8.66 | 1.14 | -0.19 | -0.32 | 4.92 |
| L22 | -0.38 | 0.2 | 0.15 | 0 | 9.34 | 2.39 | 0.16 | -0.32 | -34.96* |
| L23 | 0.05 | -4.55** | -2.85* | 0.07** | 4.59 | -7.86* | 0.29** | -0.82 | 2.6 |
| L24 | -0.83 | 0.7 | -0.61 | -0.07** | -3.91 | -13.86** | 0.11 | 0.01 | -12.83 |
| S.E. (gi) | 0.57 | 1.02 | 1.07 | 0.02 | 4.79 | 3.47 | 0.1 | 0.45 | 13.89 |
| SE ($g_i$-$g_j$) | 0.81 | 1.44 | 1.51 | 0.03 | 6.77 | 4.91 | 0.15 | 0.64 | 19.65 |
| **Tester** | | | | | | | | | |
| T1 | -0.14 | 0.68* | 0.69* | 0.001 | 16.34** | 10.86** | -0.03 | 0.38** | -25.07** |
| T2 | 0.14 | -0.68* | -0.69* | 0 | -16.43** | -10.86** | 0.03 | -0.38** | 25.07** |
| S.E. (gi) | 0.16 | 0.29 | 0.31 | 0.01 | 1.38 | 1 | 0.03 | 0.13 | 4.01 |
| SE ($g_i$-$g_j$) | 0.23 | 0.42 | 0.44 | 0.01 | 1.95 | 1.42 | 0.04 | 0.18 | 5.67 |

*P< 0.05; ** P< 0.01; GYF=Grain yield; DA= days to anthesis; DS= days to silking; ASI=anthesis silking interval; PH=plant height (cm); EH= ear height; EPP=ear per plant; NRPE=number of rows per ear; NKPR= number of kernels per row; TKW= thousand kernel weight; #= number.

The estimates of line GCA effects of the inbred lines for various traits under low N conditions at Ambo are shown in Table 7. Inbred lines L5 and L14 had significant positive line GCA effects for grain yield while none of inbred line had significant negative line GCA effects. L3, L7, L8 and L14 had highly significant negative line GCA effects for days to anthesis while inbred lines L9, L10, L18, L20 and L21 showed highly significant positive line GCA effects for this trait. The female parents L3 and L23 were negative and highly significant difference (P<0.01) GCA effect for days to silking. The result of this study is in accordance with Mafouasson et al. (2017), who found desirable GCA effects for these traits in combining ability and gene action of tropical maize inbred lines under low and high nitrogen conditions.

Significant negative line GCA effects for plant and ear height were observed for L3, L4, L16, and L24 while significant positive GCA effects were observed for L11. Inbred lines L2, L5 and L23 showed highly significant negative line GCA effects for leaf senescence while L9, L11 and L20 had significant positive GCA effects for this trait. Inbred lines L20 had positive highly significant GCA effects for number of rows per ear but negative and significant for L15. For thousand-kernel weight, L5 and L14 showed significant positive GCA effects. Worku et al. (2008) reported similar results for these traits.

**Table 7.** General combining ability effects (GCA) of 24 inbred lines and two testers for grain yield and yield related traits under low N conditions at Ambo, 2017.

| Line | GYF (t ha$^{-1}$) | AD (days) | SD (days) | ASI (days) | PH (cm) | EH (cm) | SEN (scale) | NRPE (#) | TKW (g) |
|---|---|---|---|---|---|---|---|---|---|
| L1 | -0.32 | -1.47* | -1.49 | 0.02 | -7.73 | -4.79 | 0.17 | 0.19 | -6.69 |
| L2 | 0.23 | -1.22 | -2.99* | -0.07 | 10.77 | 15.71* | -0.83** | 0.53 | -15.05 |
| L3 | 0.21 | -2.72** | -3.74** | -0.03 | -28.23** | -16.54* | -0.08 | -0.31 | -5.53 |
| L4 | -0.58 | 1.28 | 1.51 | 0.01 | -21.73* | -13.29* | 0.42 | -0.31 | -30.16 |
| L5 | 1.11** | 1.78* | 0.01 | -0.05 | -3.48 | 2.46 | -0.83** | 0.53 | 36.30* |
| L6 | 0.08 | -1.47* | -0.24 | 0.04 | 15.02 | 6.21 | 0.42 | 0.2 | -12.09 |
| L7 | -0.09 | -2.72** | 0.26 | 0.12* | 15.52 | 0.71 | 0.17 | 0.03 | -8.96 |
| L8 | -0.19 | -2.22** | -1.49 | 0.05 | -5.48 | -4.04 | -0.58 | 0.36 | 9.31 |
| L9 | -0.49 | 3.03** | 2.26 | -0.03 | 7.02 | 5.46 | 0.67* | -0.64 | -16.86 |
| L10 | 0.12 | 3.78** | 3.51* | 0 | 4.02 | 11.71 | -0.33 | -0.14 | -11.71 |
| L11 | -0.16 | -0.72 | -1.24 | 0 | 19.52* | 16.96* | 0.92** | -0.14 | -11.78 |
| L12 | -0.35 | -0.22 | 2.51 | 0.1* | 17.02 | 8.21 | 0.17 | 0.53 | -0.63 |
| L13 | -0.34 | 0.78 | 0.51 | -0.01 | -5.48 | -1.04 | 0.42 | -0.3 | -8.51 |
| L14 | 0.77* | -2.47** | -1.99 | 0.03 | 8.27 | 0.96 | -0.33 | 0.19 | 35.04* |
| L15 | 0.15 | -1.72* | -1.24 | 0.03 | -12.23 | -5.79 | -0.33 | -0.80* | 29.6 |
| L16 | 0.08 | -1.72* | -1.24 | 0.03 | -25.73* | -15.04* | -0.33* | 0.36 | -1.55 |
| L17 | -0.27 | 0.03 | 2.76* | 0.09 | -24.23* | -8.29 | 0.67 | 0.03 | -30.25 |
| L18 | -0.31 | 3.03** | 2.51 | -0.03 | -13.23 | 1.71 | 0.42 | -0.31 | 1.87 |
| L19 | 0.2 | 0.03 | 1.51 | 0.07 | 14.52 | 6.46 | 0.42 | 0.36 | 34.49 |
| L20 | 0.38 | 2.53** | 0.76 | -0.08 | 26.27** | 8.71 | 0.67* | 1.03** | 14.64 |
| L21 | -0.53 | 2.03** | 0.26 | -0.07 | 5.77 | 8.46 | 0.17 | -0.47 | 1.86 |
| L22 | -0.04 | 0.78 | -0.24 | -0.06 | 17.27 | 5.46 | -0.33 | 0.03 | -22.7 |
| L23 | 0.58 | -0.47 | -3.99** | -0.16** | 6.02 | -9.04 | -1.33** | -0.64 | 25.72 |
| L24 | -0.24 | 0.03 | 1.51 | 0.04 | -19.48* | -21.29** | -0.33 | -0.3 | -6.32 |
| S.E. (gi) | 0.31 | 0.72 | 1.3 | 0.05 | 9.67 | 6.51 | 0.31 | 0.34 | 17.41 |
| SE (gi-gj) | 0.44 | 1.02 | 1.83 | 0.07 | 13.68 | 9.2 | 0.44 | 0.49 | 24.63 |
| **Tester** | | | | | | | | | |
| T1 | -0.11 | -0.14 | 1.66** | 0.07** | 7.35* | 8.17** | 0.46** | 0.51** | -23.66** |
| T2 | 0.11 | 0.14 | -1.66** | -0.07** | -7.35* | -8.17** | -0.46** | -0.51** | 23.66** |
| S.E. (gi-gj) | 0.09 | 0.21 | 0.37 | 0.01 | 2.79 | 1.88 | 0.09 | 0.1 | 5.03 |
| SE(d) | 0.13 | 0.3 | 0.53 | 0.02 | 3.95 | 2.66 | 0.13 | 0.14 | 7.11 |

*P<0.05; **P<0.01; GYF=Grain yield; DA= days to anthesis; DS= days to silking; ASI=anthesis silking interval; PH=plant height (cm); EH= ear height SEN= leaf senescence; NRPE=number of rows per ear; TKW= thousand kernel weight (g), #= number.

Across environments combined analysis, fourteen inbred lines showed positive GCA effects for grain yield only one inbred line L20 (1.21 tha$^{-1}$) showed positive and significant GCA effects indicating the potential advantage of the inbred lines for the development of high-yielding hybrids. Among the testers (males), none of them showed significant GCA effects for grain yield per hectare (Table 9). Results of the current study are in accordance with the findings of Shushay et al. (2013), Kamara et al. (2014), Tamirat et al. (2014), Amare et al. (2016), Abakemal et al. (2016) and Assefa et al. (2017) who reported significant positive and negative GCA effects for grain yield in maize germplasm. Lines with positive GCA effects for grain yield can be extensively used in hybridization program as they contribute favorable alleles in the development of high yielding varieties.

## Estimates of specific combining ability effects

The specific combining ability effects at individual and across locations were computed for traits that showed significant SCA mean squares in combining ability analysis. At Ambo optimum N, 50% of the crosses showed positive SCA effects for grain yield out of which two crosses, namely; L3 × T1 and L7 × T2 (Table 8)

**Table 8.** Estimates of specific combining ability (SCA) of line x testers crosses for grain yield and yield related traits under low and optimum N conditions at Ambo, 2017.

| Line | Optimum N | | | | | | low N | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | GYF (t ha$^{-1}$) | ASI (days) | PH (cm) | EH (cm) | EPP (#) | NKPR (#) | TKW (g) | AD (days) | SD (days) | ASI (days) | SEN (scale) |
| | T1 | T1 | T1 | T1 | T1 | T1 | T1 | T1 | T1 | T1 | T1 |
| L1 | 0.83 | -0.01 | 3.82 | 5.64 | -0.04 | -3.34 | 15.19 | 0.89 | 0.09 | -0.03 | -0.46 |
| L2 | 1.06 | -0.01 | 3.82 | 0.89 | 0.01 | -1.84 | 19.92 | -0.86 | -0.41 | 0.04 | 0.04 |
| L3 | 1.90* | 0.02 | 3.57 | -0.36 | 0.24 | 1.75 | 28.42 | 1.14 | -1.66 | -0.12 | 0.79 |
| L4 | 0.28 | 0.04 | 16.32* | 9.64 | 0.27 | -0.42 | 1.13 | -0.86 | 0.59 | 0.07 | 0.29 |
| L5 | -1.63 | -0.06 | -6.18 | -2.36 | -0.12 | -0.42 | 4.34 | 0.64 | 3.59 | 0.1 | 0.04 |
| L6 | -0.04 | 0.02 | 0.57 | 1.39 | -0.13 | -1.17 | 11.88 | 0.39 | 0.84 | 0 | -0.21 |
| L7 | -1.67* | 0.06 | -6.68 | -0.86 | -0.28 | 1.5 | -0.79 | 0.64 | 2.34 | 0.05 | 0.04 |
| L8 | -0.21 | 0 | -3.68 | -1.36 | -0.19 | 0.58 | 19.87 | 0.14 | -1.41 | -0.06 | -0.21 |
| L9 | -0.44 | 0.02 | -1.68 | -0.36 | -0.01 | -0.67 | -18.68 | -0.61 | -0.16 | 0.04 | -0.46 |
| L10 | -1.01 | -0.02 | 4.57 | 1.64 | 0.21 | 1.49 | -29.02 | 1.64 | 1.59 | 0 | -0.46 |
| L11 | -0.58 | -0.01 | -3.18 | 0.64 | 0.05 | 0 | -0.43 | -0.36 | -1.66 | -0.05 | -0.21 |
| L12 | -0.29 | -0.01 | 5.82 | 4.39 | 0.15 | 2.5 | 28.56 | -1.36 | 0.09 | 0.04 | 0.54 |
| L13 | 0.68 | -0.03 | -2.93 | -3.36 | -0.07 | 0.66 | 12.3 | 0.64 | 2.09 | 0.07 | 0.29 |
| L14 | 0.26 | -0.02 | 14.57* | 4.39 | -0.02 | 2.33 | -43.62* | 0.89 | -0.41 | -0.05 | 0.04 |
| L15 | 0.34 | 0.05 | 6.57 | 1.64 | 0.04 | -1.01 | -12.19 | 0.14 | 0.34 | 0.01 | 0.54 |
| L16 | 0.41 | 0.04 | 6.32 | -3.11 | 0.05 | -1.67 | -25.75 | 0.14 | 0.84 | 0.03 | 0.54 |
| L17 | -0.41 | 0.05 | -0.68 | -0.11 | -0.16 | -0.59 | -4.74 | -0.61 | 2.84 | 0.12 | 0.04 |
| L18 | 0.7 | -0.02 | 1.32 | 0.14 | 0.18 | 5.91** | -1.89 | -0.11 | -1.91 | -0.09 | 0.29 |
| L19 | 0.46 | 0.02 | 2.57 | 3.14 | -0.02 | 0.08 | 6.07 | -0.61 | 0.09 | 0.02 | 0.29 |
| L20 | 0.17 | -0.01 | -0.68 | 8.14 | 0.02 | -2.59 | -27.42 | 0.39 | 1.34 | 0.07 | 0.04 |
| L21 | -0.31 | 0.05 | 1.82 | 5.14 | 0.11 | 0.5 | 16.35 | -2.11* | -2.16 | 0.01 | 0.04 |
| L22 | -0.57 | -0.02 | -12.68 | -11.11* | -0.18 | 1.33 | -13.97 | -1.86 | -0.16 | 0.09 | 0.04 |
| L23 | -0.58 | -0.03 | -12.43 | -8.36 | -0.18 | -2.34 | -21.06 | 0.39 | -1.41 | -0.08 | -0.96* |
| L24 | 0.64 | -0.11** | -20.9** | -15.36** | 0.06** | -2.59 | 35.55 | 1.39 | -5.4** | -0.3** | -0.96* |
| SE | 0.81 | 0.03 | 6.77 | 4.91 | 0.15 | 2.02 | 19.65 | 1.02 | 1.83 | 0.07 | 0.44 |
| SE ($S_{ji}$-$S_{kl}$) | 1.14 | 0.05 | 9.57 | 6.94 | 0.21 | 2.86 | 27.79 | 1.45 | 2.59 | 0.09 | 0.62 |

*P<0.05; **P<0.01; GYF=Grain yield; DA= days to anthesis; DS= days to silking ASI=anthesis silking interval; PH=plant height (cm); EH=ear height; EPP= ear per plant; SEN= leaf senescence; NKPR= number of kernels per row; TKW= thousand kernel weight, #= number

showed positive and significant SCA effects for grain yield with SCA values of 1.9 and 1.67 t/ha, respectively, indicating that these crosses were good specific combinations for grain yield. In combined analysis across environments two crosses, L7×T2 and L5×T2 (Table 9) showed positive and significant SCA effects for the trait. Crosses with the higher value of SCA effect also showed higher values of mean grain yield performance, indicating good correspondence between SCA effects and mean grain yield. Hence, such cross combinations could effectively be exploited in hybrid breeding program in maize research. On the other hand, two cross combinations L3 × T2 and L7 × T1 expressed negative and significant SCA effects for grain yield under optimum condition, which are undesirable as these crosses showed a tendency to reduce grain yield performance. The finding of the current study is in agreement with that of Mohamed et al. (2014) who reported significant positive and negative SCA effects for grain yield in line × tester analysis experiment under two nitrogen fertilizer levels. Mafouasson et al. (2017) who reported significant positive and negative SCA effects for grain yield in study 42 tropical maize inbred lines for grain yield and yield related traits under low and optimal N conditions.

At Ambo low N, cross L21×T2 (-2.11) showed significantly negative SCA effects for days to anthesis and cross L24×T1 (-5.41) showed highly significantly negative SCA effects for days to silking and Anthesis silking interval. Which were desirable for earliness (Table 8). For plant height, three crosses (L24 × T1, L4×T2 and L14×T1) under optimum N condition (Table 8) showed negative and significant SCA effects for the trait, indicating that these crosses had good specific combination for shorter plant stature. For ear height,

**Table 9.** Specific combining ability effects of crosses for grain yield and GCA effects of lines and testers across environments.

| Line | Testers | | GCA lines |
|---|---|---|---|
| | T1 | T2 | |
| L1 | 0.28 | -0.29 | 0.58 |
| L2 | 0.73 | -0.74 | 1.11 |
| L3 | 0.75 | -0.75 | 0.30 |
| L4 | -0.04 | 0.03 | -1.16 |
| L5 | -1.05* | 1.04* | 0.73 |
| L6 | -0.41 | 0.40 | 0.12 |
| L7 | -1.10* | 1.09* | 0.05 |
| L8 | -0.002 | -0.01 | 0.17 |
| L9 | -0.04 | 0.03 | 0.53 |
| L10 | -0.26 | 0.26 | 0.17 |
| L11 | -0.26 | 0.25 | 0.34 |
| L12 | 0.06 | -0.07 | -0.33 |
| L13 | 0.25 | -0.26 | -0.74 |
| L14 | 0.00 | -0.01 | 0.26 |
| L15 | 0.23 | -0.24 | -0.65 |
| L16 | 0.28 | -0.29 | -0.67 |
| L17 | -0.19 | 0.18 | -0.42 |
| L18 | 0.43 | -0.44 | 0.27 |
| L19 | 0.12 | -0.13 | -0.47 |
| L20 | 0.01 | -0.02 | 1.21* |
| L21 | 0.07 | -0.08 | -1.03 |
| L22 | -0.35 | 0.34 | -0.08 |
| L23 | -0.26 | 0.25 | 0.34 |
| L24 | 0.63 | 0.25 | -0.50 |
| SE (SCA effects) | 0.41 | | 0.58 |
| Tester | - | - | Tester |
| 1 | - | - | -0.11 |
| 2 | - | - | 0.12 |
| SE( SCA effects) | - | - | 0.1 |

crosses L24 × T1 and L22 × T1 under optimum N condition (Table 8) showed negative and significant SCA effects for the trait. For thousand-kernel weight, one cross (L14×T2) with SCA values of 43.62 at Ambo (optimum N) was good combinations for thousand-kernel weight as they showed positive and significant SCA effects for this trait. In line with the present results (Melkamu, 2013). Shushay et al. (2013) and Tolera et al. (2017) found significantly positive and negative SCA effects for plant and ear height.

## Conclusion

The current study was conducted with the objective of estimating the general and specific combining abilities of highland maize inbred lines and mode of gene action using line × tester mating design. Fifty maize hybrids including 48 testcrosses developed by crossing 24 elite maize inbred lines with two testers and two standard checks were planted at Ambo (low and optimum N) during the 2017 cropping season in alpha lattice design replicated twice. Data were recorded on grain yield and agronomic traits. Analysis of variance indicated hybrids exhibited highly significant ($P<0.01$) differences in most traits under low and optimum N conditions except number of kernels per row under low N. Mean squares due to crosses were significant for all traits in both environments except ear per plant under low N stress condition. Among the crosses, L2 × T1 (10.76) t $ha^{-1}$ showed high yield at Ambo (optimum N). At Ambo low N, L5 × T2 (4.61 t $ha^{-1}$), L6 × T2 (4.37 t $ha^{-1}$), L14 × T2 (4.31 t $ha^{-1}$), L20 × T2 (4.14 t $ha^{-1}$), and L23 × T2 (4.11 t $ha^{-1}$) were crosses with high yield.

Combining ability analysis is important in identifying the best parents or parental combinations for a hybridization

program. Line GCA and SCA mean squares where significant for most traits at each and across environments except SCA at low N stress environment, which is concluded that non-additive gene effects are less important for the inheritance of characters under low N stress condition. Significant GCA and SCA mean squares for most traits measured indicated that both additive and non-additive gene actions are important in determining the inheritance of these traits. General combining ability sum of squares component was greater than SCA sum of squares for all of the studied traits, suggesting that variations among crosses were mainly due to additive rather than non-additive gene effects; and hence, selection would be effective in improving grain yield and other agronomic traits. Based on combining ability analysis, L20, L2, L1 and L9 were found to be the best general combiners for grain yield at optimum N environments whereas L5 and L14 were best general combiners under low N stress condition. Inbred lines with a high GCA effect for grain yield are desirable for synthetic and open pollinated varieties development as well as for inclusion in breeding program. For grain yield, crosses L3×T1 and L7×T2 had good specific combining ability for optimum N location. These hybrids could be included for further studies for the improvement of grain yield and related traits.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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