African Journal of
Plant Science

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African Journal of Plant Science

Full Length Research Paper

Meta-analysis of the effects of *Rhizobia* inoculants and phosphorus fertilizer on soybean nodulation in Africa

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Soybean has the potential to bring significant benefits in diversified cropping systems, which could help restructuring soil fertility and allow smallholders to increase grain yield. *Rhizobium* inoculation improves the biological nitrogen fixation (BNF) in legume crops and assists resource-poor farmers to increase grain yield at lower financial costs. The efficacy of symbiotic bacteria on legumes can also be improved through supplementation of phosphorus fertilizer. In this work, a meta-analysis of 29 peer-reviewed studies was performed to understand the effects of various *Rhizobium* strains and phosphate fertilizer application on soybean nodules. Results showed that *Rhizobium* inoculation was highly effective in increasing the number of soybean nodules, nodule dry weight, and shoot dry weight. Application of phosphorus fertilizer increased the overall nodule number due to improved BNF processes by *Rhizobia*. The main effects of both *Rhizobium* inoculation and phosphate fertilizer resulted in moving grain yields to 1.67 t ha^{-1} and 1.95 t ha^{-1}, respectively. Furthermore, the interaction of *Rhizobium* inoculants and phosphorus led to relatively higher grain yield (2.51 t ha^{-1}). Therefore, African smallholders were advised to adopt *Rhizobium* inoculation in soybean fields concomitantly to phosphate fertilizer application, to improve soybean productivity at lower costs.

Key words: Phosphorus application, nodule number, nodule dry weight, shoots dry weight, grain yield.

INTRODUCTION

The African population was expected to double in the next 40 years (Cleland, 2013), raising food insecurity especially in the sub-Saharan region where 239 million people are experiencing dire undernourishment (FAO, 2020). Sustainable intensification and integrated approaches are therefore needed to increase the

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agricultural productivity of smallholders, improve household food security, and reduce poverty at the country level (Peoples et al., 1995; McNamara, 2009). Integrating legume crops, especially soybean, is an important approach in many cropping systems, as they can perform biological N_2 fixation (BNF), thus reducing N fertilizer requirements and improving grain yield (Peoples et al., 1995; Giller, 2001). Soybean was first domesticated in China and has been grown in other Asian countries like Japan and Korea for more than 3000 years as a primary source of vital proteins and vegetable oil (Giller, 2001; Herridge et al., 2008; Nishinari et al., 2014). Globally, soybean (*Glycine max* (L.) Merr.) is the world's largest grown legume crop (Giller, 2001), accounting for 50% of the worldwide legume crop area and 68% of global crop production (Herridge et al., 2008). However, there is no clear evidence of when soybean was first introduced to Africa (Mpepereki et al., 2000), but its nodulation with indigenous *Rhizobia* in African soils was first ascertained by Corby (1967).

The ability of symbiotic rhizobial bacteria to fix atmospheric nitrogen in legume plants can improve grain yield without applying nitrogen fertilizer (van Heerwaarden et al., 2017). Herridge et al. (2008) reported that soybean can fix more than 16 million tons of N annually, which is 77% of the N fixed by legume crops. Soybean has been reported to fix 80% of its nitrogen requirements (Smaling et al., 2008). *Bradyrhizobium* strains are commonly used in soybean inoculation worldwide (Chianu et al., 2011; Chen et al., 2015). In Africa, *Rhizobia* inoculants have been used to control the effects of debilitating soil fertility and high fertilizer costs incurred certainly by smallholders. They became an affordable and effective agronomic approach in improving yield and promoting sustainable agriculture (Dakora and Keya, 1997; Paynel et al., 2008). Ronner et al. (2016) found that *Rhizobia* inoculants increase soybean yield at a lower financial cost compared to chemical N fertilizers, thus benefiting the resource-poor farmers. Despite a rapid expansion of soybean production in many African countries (Mpepereki et al., 2000) and wide use of inoculants, legume yields in the smallholder farming sector generally remain far below their potential (Ronner et al., 2016). The effectiveness of *Rhizobia* inoculants can be affected by factors like soil nutrient status, organic matter content, pH, salinity, temperature, drought, and managerial practices (Thilakarathna and Raizada, 2017). However, Ronner et al. (2016) mentioned that the soybean yields in Africa could be improved through the use of adaptive technologies like phosphate fertilizer and improved varieties to aid the *Rhizobium* inoculation approach. Phosphorus (P) is the second most important macronutrient required by the legume plants in the BNF among other crucial processes (Uchida, 2000). Symbiotic *Rhizobium* bacteria need P as the energy storage and transfer component (adenosine diphosphate (ATP) and

adenosine triphosphate (ATP) for the conversion of free N_2 to ammonium (NH_4), a N usable form by legumes (Dakora and Keya, 1997). Furthermore, P increases nodule number and size, and it promotes general root growth. Legumes need optimum P levels for maximum nitrogen fixation and to achieve high grain yield (Bashir et al., 2011). Since 1980, no meta-analysis was conducted to determine the extent to which *Rhizobium* inoculation and phosphorus fertilizer technologies have influenced soybean productivity under field conditions in Africa. This study aimed to review various researches conducted in Africa to understand the effectiveness of rhizobial inoculants, P-fertilizer, and their interaction on soybean performances. The following conceptual model (Figure 1) was suggested to predict the effects of *Rhizobium* inoculation, phosphate fertilizer, and other adaptive technologies that could further improve soybean yield.

METHODOLOGY

Data collection

An extensive literature synthesis was performed based on robust published research articles in 1980-2020 downloaded from the ScienceDirect databases (https://www.sciencedirect.com) and Web of Science (http://apps-webofknowledge-com.vpn.cau.edu.cn). The search terms used as the main topics in both databases were *Rhizobium*, Phosphorus, Soybean OR *Glycine max*, Nodulation, and Grain yield. A total of 441 research articles were obtained from ScienceDirect and 170 from Web of Science. Google Scholar provided additional articles. Only 86 articles were retained after excluding duplicates and exploitation of the titles and abstracts' relevance to the subject of the work for further screening. A study had to meet six requirements for its consideration in the dataset. They included: being conducted under rainfed or irrigated field conditions; assessing the effect of any strain of commercial *Rhizobium* inoculant or chemical phosphorus fertilizer, and/or both on nodulation characteristics and grain yield; presence of a control to either *Rhizobium* inoculation or phosphorus application; having every treatment being repeated at least three times; being conducted in an African country; having been published between 1980 and 2020.

The different characteristics of the nodulation consisted of nodule number, nodule dry weight, and shoot dry weight. A database with 396 data points extracted from 29 qualified peer-reviewed articles based on the aforementioned criteria was compiled. Studies with a sample size of less than 2 were excluded from analyses because they would have resulted in small size effect (Viechtbauer, 2010). 26 out of the 29 researches retained were conducted in the sub-Saharan-Africa region (SSA) and the other 3 in the Saharan region (Figure 2). Treatment variances, standard deviations, or standard errors were disregarded as they were only presented in a few studies. In fact, only treatment mean values of nodule number, nodule dry weight, shoot dry weight, and grain yield were collected. Experiment details recorded include location/country, latitude, longitude, annual mean temperature and rainfall, soil type, pH, organic matter content, total available nitrogen, available phosphorus, inoculant strain, nodule number, nodule dry weight, shoot dry weight, grain yield, phosphorus application rates, to name a few. Control and experimental treatments' data were recorded as well as data on the interaction effect that was assessed in 4 studies only. The available data showed that the minimum and maximum
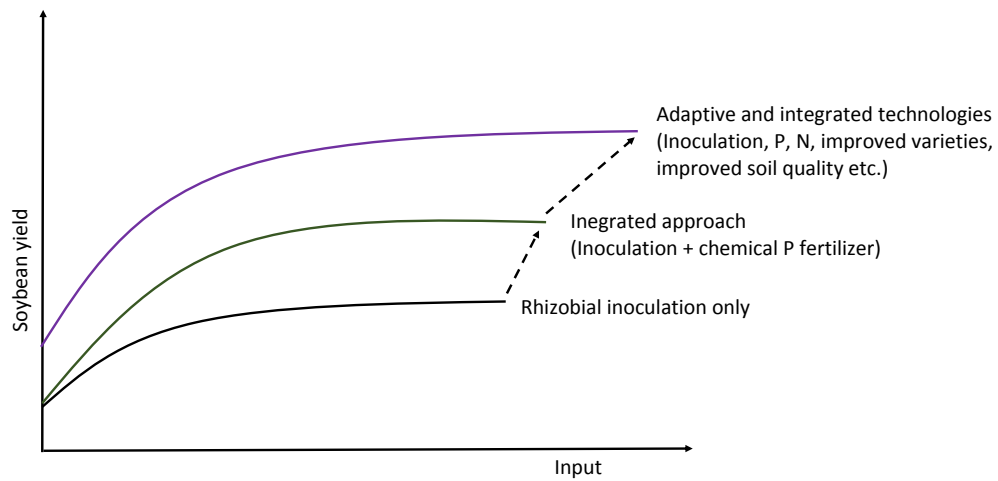
Figure 1. A conceptual model for anticipating the effect of integrating *Rhizobium* inoculation with phosphorus fertilizer and other adaptive technologies.
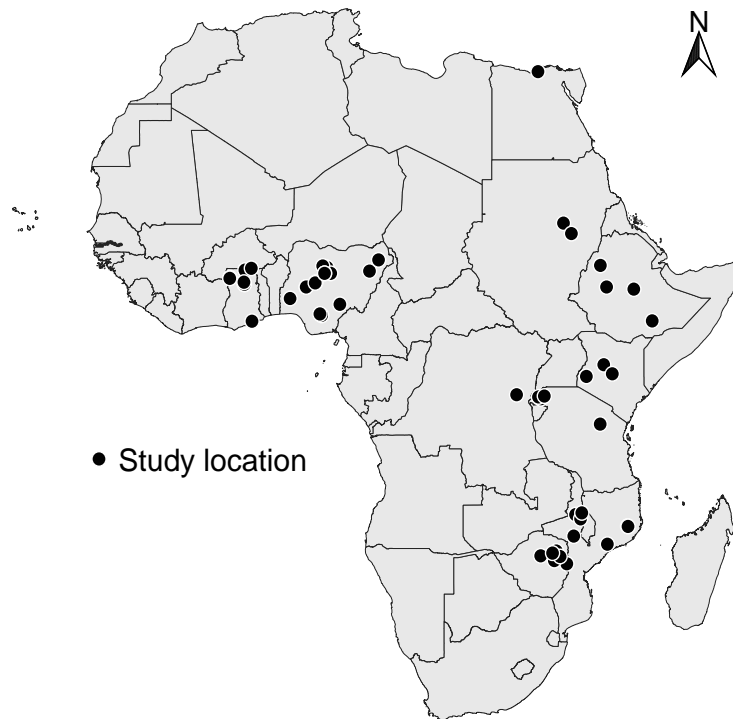
Figure 2. Distribution of the experimental locations of the studies included in the meta-analysis.

mean annual temperatures were 22 and 29°C, minimum and maximum mean annual rainfall were 552 mm and 1300 mm, respectively. Data reported in one study but conducted in more than one country/location or in different years were considered derived from different studies. Tabular and graphical data were collected and in the latter case, Engauge Digitizer software version 12.1 was used for data extraction. Detailed information of the selected peer-reviewed studies is given in Table 1.

Data processing

Data on nodule number, nodule dry weight, shoot dry weight, and grain yield were pooled on per variable basis. Means, and standard

deviations (STDEV.P) were calculated using Microsoft Excel package and the data were exported to R software for statistical analyses. The author's name, publication year, and sample sizes for each study were recorded for corresponding means and STDEV.P.

Statistical analyses

The effects of *Rhizobium* inoculation and phosphorus fertilizer on soybean performances were estimated using the random-effects model (REM) described by Viechtbauer (2010). The statistical analyses in this study were all performed in R version 4.0.2 (R Core Team, 2020), using the R program Metafor (Viechtbauer, 2010; Schwarzer et al., 2015). The *Escalc()* function contributed to calculate the standardized mean differences (SMD), which measure effect sizes that allowed comparing treatments to the controls (Viechtbauer, 2010). The REM was used to estimate the average true effect and the total amount of heterogeneity among the true effects (Viechtbauer, 2010; Schwarzer et al., 2015). Results were presented in form of forest plots with the *Forest()* function (Lewis and Clarke, 2001) and boxplots using the *Plot()* function (Viechtbauer, 2010). Heterogeneities within-study and between-study were assessed using the I^2 statistic (Higgins and Thompson, 2002). The presence of publication bias and/or heterogeneity was determined by creating Funnel plots for both the inoculation and phosphorus variables (Sterne et al., 2001; Rothstein et al., 2006). A Funnel plot is a simple scatter plot for the study's estimated treatment effects (x-axis) against the measure of study size on the y-axis (Sterne et al.,, 2001). Trim and fill method using the *Trimfill()* function (Viechtbauer, 2010; Schwarzer et al., 2015) was applied on asymmetrical Funnel plots to determine the effect of missing studies on the overall outcome. The standardized mean difference for the study was plotted on the horizontal axis against the standard error on the vertical axis. A box plot using the *Plot()* function was realized to show the overall effects of rhizobial inoculation, phosphate fertilizer, and the interaction on soybean grain yield (Figure 15). Bootstrapping was iterated 1000 times (95% CI) with R package Mosaic (Pruim et al., 2015) to improve the probability that the confidence interval was calculated around the relative yield mean. The frequency distribution plots were then plotted with the *Ggplot*() function.

RESULTS

Effects of *Rhizobium* inoculation on soybean

Response of nodule dry weight to inoculation

The effects of inoculation on nodule dry weight are shown in Figure 3. The heterogeneity (I^2) of the combined studies was 92% (P=0.0001). The study by Argaw (2014) clearly showed that inoculation significantly favored nodulation contrasting seven other studies, which had 95% confidence interval (CI) lines touching or crossing no effect line, indicating that inoculation did not influence nodulation. The position of the diamond symbol on the graph, which showed the overall effect of inoculation, testified that inoculation significantly favored nodulation. Furthermore, the overall effect of *Rhizobium* inoculation had 95% CI of -2.02[-2.95, -1.08] (Figure 3). The purpose of the funnel plot was to indicate the level of bias in this

study. Twenty studies showed an asymmetric distribution pattern, indicating the presence of bias (Figure 4). However, after trim and fill (white dots), the overall result was not significantly affected.

Soybean nodule number

The studies had an I^2 of 75 % (P=0.0001) indicating that they were heterogenous. *Rhizobium* inoculation favored high nodule numbers (Argaw, 2014) (Figure 5). The overall effect of soybean inoculation is shown by the diamond which is on the left side of the no effect line, indicating that inoculation significantly favored a high nodule number compared to no inoculation (Figure 5). The overall effect of inoculation had 95% CI of -1.62[-2.17, -1.07] (Figure 5). There was a little publication bias in 23 studies analyzed as shown by an asymmetric distribution pattern on the Funnel plot (Figure 6). Trim and fill did not result in a significant change to the overall result.

Shoot dry weight response to rhizobial inoculants

An I^2 of 54 % (P=0.0001) resulted from the 13 studies showing the presence of heterogeneity. Two studies clearly demonstrated that *Rhizobium* inoculation increased shoot weight (Pulver et al., 1982; Okereke et al., 2001) (Figure 7). The overall effect of soybean inoculation significantly increased shoot dry weight compared to the control treatment, according to the position of the diamond, which is on the left side of no effect line. A 95% CI of -1.31[-1.74, -0.88] was produced for the overall effect of inoculation. The studies showed a slightly asymmetric distribution pattern on the Funnel plot, indicating very limited bias in the 13 studies (Figure 8). Trim and fill of the missing studies did not bring meaningful change to the overall result.

Grain yield

The analyzed studies were heterogenous with an I^2 of 64 % (P=0.0001). *Rhizobium* inoculation significantly favored high grain yield compared to non-inoculated control with a symmetrical distribution pattern, indicating the absence of publication bias (Figure 10). a 95% CI of -1.05 [-1.39, 0.72] (Figure 9).

Studies within the inverted funnel of the Funnel plot had Response of soybean to phosphorus fertilizer application

Nodule number

The heterogeneity of the all the analyzed studies (I^2) was

Table 1. Summary of the 29 studies in the meta-analysis on the effects of *Rhizobium* inoculation and P-fertilizer on soybean.

References	Area/Country	Soil type	*Rhizobia* strains	Phosphorus (kg/ha)	Key findings
Dadson et al. (1984)	Legon, Ghana	Sandy-loam (0.05% TSN)	-*B. japonicum* (Nitragin S)	Triple superphosphate (TSP) -0 (control) - 30 kg P/ha - 60 kg P/ha - 90 kg P/ha	- Medium to high rates of phosphorus and *Rhizobia* treatments significantly increased nodule number, total dry matter, and grain yield compared to controls.
Okogun and Snginga (2003)	Fasola, Mokwa and Zaria, Nigeria	Sandy-loam (0.06% TSN)	- Control (local *Rhizobia*) - *Bradyrhizobium* isolate R25B - *B. japonicum* IRj 2180A + R25B		- The mixture of introduced R25B+IRj 2180A increased nodule number by 34%, while R25B formed only about 24% of the nodules but did not influence biomass yield. -Inoculation by foreign strains failed to significantly affect grain yield.
Ronner et al. (2016)	Kaduna and Kano, Nigeria	Luvisols	*B. japonicum* strain: - USDA 532c	Single Superphosphate (SSP, 18% P_2O_5) - 20 kg P/ha	- Phosphorus and *Rhizobia* inoculation increased soybean yields by 452 and 447 kg/ha respectively over control treatment. - The combined effect of phosphorus and inoculations resulted in highly significant yield averaging 777 kg/ha
Argaw (2014)	Shinille, Ethiopia	Sandy-clay (0.25% TSN)	- *B. japonicum* (TAL-379 isolate) - *Bradyrhizobium* sp. (UK-Isolate) - *Bradyrhizobium* sp. (local- isolate)		- Inoculation improved nodulation characteristics, plant growth and productivity over uninoculated treatment. - Local and UK isolates significantly increased grain yield (P=0.05) as compared to the control and TAL-379 treatments.
Okereke et al. (2000)	Awka, Nigeria	Sandy-loam (0.08-0.1% TSN)	*B. japonicum* strains -USDA 136 -USDA 138 -USDA 110 -USDA 122		- Nodule number and dry weight, shoot dry weight and grain yield significantly increased against uninoculated treatments.
Zengeni and Giller (2007)	Goromonzi, Zimbabwe	Sandy soil (0.05% TSN)	- Soybean isolates from nodules of the Magoye variety (M1-M5) - Soybean isolates from nodules of the Hernon variety (H1-H5) - Commercial inoculants (MAR 1491 and 1495)		- High variations in the nodule numbers and yields were considered unreliable indicators of effectiveness. - Different *Rhizobia* strains resulted in strong harvest index effects which directly related to grain yield.
Chowdhury et al. (1983)	Morogoro, Tanzania	Rhodustult (0.11% TSN)	*Rhizobia* strains: -IRj 2101 -IRj 2114 -IRj 2111 -IRj 2123		- Inoculation significantly increased nodule number and grain yields in the first year. - There was huge decrease in nodule number (about 10-folds) and grain yield in the subsequent years after inoculation evidenced by no significant differences between inoculated and uninoculated treatments.
Gyogluu et al. (2016)	Nampula, Ruace and Mutequelesse, Mozambique	-Sandy clay loam -Clayey loam	*B. japonicum* strain: -WB74		- *Rhizobia* inoculation effectively improved nodulation of TGx and non-TGx soybean varieties and yield was increased by 12% as compared to uninoculated control treatments.
Akpalu (2014)	Bolgatanga, Ghana	- 0.03% TSN	*Rhizobium* strain	Triple Superphosphate (TSP)	- *Rhizobia* inoculant plus phosphorus fertilizer treatment showed highly significant increase in

Table 1. Contd.

				-139.4 g P per 9 square meter	nodulation and root growth while phosphorus along failed to effect root growth. - Grain yield was significantly high in inoculant plus phosphorus fertilizer and phosphorus fertilizer only treatments (7.61 t/ha and 7.30 t/ha respectively) and lowest in *Rhizobia* inoculation only and control treatments (4.41 t/ha and 3.80 t/ha respectively).
Ahiabor et al. (2016)	Nyankpala, Ghana	Loamy-sand (0.50 g/kg TSN)	*Bradyrhizobium* strain: - USDA 532c	0, 22.5 and 45 kg P₂O₅/ha	- *Bradyrhizobium* inoculation had no effect on nodule number, nodule dry weight, shoot dry weight plant height and grain weight. - Phosphorus applied at 22.5 and 45 kg P₂O₅/ha significantly improved grain yield by 35.4 and 33.9% respectively and nitrogen fixation 49.39 and 69.82% respectively as compared to untreated control. - Application of inoculant plus phosphorus had no effect on the investigated parameters except phosphorus fertilizer which increased the growth and grain yield of soybeans.
Lamptey et al. (2014)	Nyankpala, Ghana	Loamy sand	*Rhizobium* strain	Triple Superphosphate (TSP) - 30 kg P/ha	- Both inoculation and phosphorus fertilizer significantly increased nodule number, nodule dry weight, shoot dry weight and grain yield.
van Heerwaarden et al., (2017)	DR Congo, Ethiopia, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Uganda and Zimbabwe	N/A	*Rhizobia* strains: - USDA 110 - 532c - WB74 - TAL379 - MAR1391		- Across all the countries, average yield of inoculated and uninoculated treatments was estimated at 1343 and 1227 kg/ha respectively. - Different varieties across different countries had no significant differences in uninoculated yields in contrast to high yield increase in inoculated soybean.
Fituma et al. (2018)	Metahara, Ethiopia	Calcaric Cambisols (0.12% TSN)	*Bradyrhizobia* strains: - SB6B1 - 532c (Legumefix)	Triple Superphosphate (TSP) - 0 (control) - 23 kg P/ha - 46 kg P/ha - 69 kg P/ha	- Nodule number, plant height, pods per plant, dry biomass yield and grain yield were significantly increased by SB6B1 inoculation over control. - Legumefix inoculation significantly increased nodule dry weight, nodulation rating and nodule volume. - Phosphorus applied at 69 kg P₂O₅/ha improved nodule number and nodule volume while 23 kg P₂O₅/ha increased 100-seeds weight. - Overall, inoculation with *Bradyrhizobia* strains significantly increased nodulation and grain yield.
Solomon et al. (2012)	Bako, Western Ethiopia	Nitisols (0.14% TSN)	*B. japonicum* strains: - TAL 378 - TAL 379		- Inoculation by *B. japonicum* strains increased all the nodulation characteristics (nodule number per plant, nodule dry weight, nodulation rating, and nodule volume per plant) compared to uninoculated soybean. - Yield was significantly higher (P ≤ 0.01) by 53.2% to the soybean inoculated by TAL 379 over uninoculated control.
Savala (2020)	Ntengo, Ruace and Muriaze, Mozambique	- 0.10% TSN - 0.12% TSN - 0.05% TSN	*B. japonicum* (USDA 110)	0 and 40 kg P₂O₅/ha	-*Bradyrhizobium* inoculation improved nodulation and yield ranging from 37% to 95% over control. The effect of phosphorus on nodulation was inconsistent across study locations and different varieties.
Kamara et al. (2014)	Miringa and Azir, Nigeria	Alfisols - Loamy		0, 20, and 40 kg P/ ha	- Phosphorus fertilizer increased soybean total dry weight and grain yield among other parameters

Table 1. Contd.

		(0.08% TSN)			
		- Clay loam (0.15% TSN)			tested though there was no significant difference between the effects of 20 and 40 kg P/ha rates.
Pulver et al. (1982)	Mokwa and Yandev, Nigeria Tanzania	Coarse-textured Paleustaff soils (low N soil) Sandy soil (low N)	*B. japonicum* strains - 110 - 110-M - 61A76 - SM-31 - SM-35 *Rhizobium* strain (Nitragin),		- Nodule mass increased significantly, but seldomly yield, in response to inoculation by several *R. japonicum* strains as compared to U.S. varieties. - Yield of U.S. varieties increased with inoculation than without inoculation. - The same results were noticed in Tanzania. - Overall, the response of nodule and yield characteristics were variety depended though inoculated performed better than uninoculated soybean.
Pule-Meulenberg et al. (2011)	Wa, Ghana	Ferric Luvisols	*B. japonicum* strain: - WB74		- *B. japonicum* strain WB74 significantly improved nodule number, shoot and whole plant dry weight, and nodule mass as compare to control treatments though it differed with soybean variety.
Okogun et al. (2005)	Kaya, Nigeria	N/A	*Bradyrhizobia* strains: - R25B - IRj 2180A		- Nodulation, shoot dry weight, percentage nitrogen derived from the air (%Ndfa), grain yield, and nutrient uptake varied across and within farmers' fields as affected by the variations in soil fertility and field management. - The shoot dry matter varied among farmers ranging from 2.4 to 166.3 g/plant with an average of 30.7 g/plant, and inoculated improved soybean variety (TGx 1448-2E outperformed uninoculated improved variety (Samsoy-2) though not statistically different. - Inoculation increased grain yields in both tested soybean varieties over uninoculated soybean.
Rurangwa et al. (2018)	Bugesera and Kamonyi and Kayonza, Rwanda	1.7-1.8 g/kg TSN	*B. japonicum* strain: - USDA 110	Triple Superphosphate (TSP): - 0 and 30 kg P/ha	- Inoculation, phosphorus and manure increased grain yield ranging from 1 to 3.8 t/ha in inoculated soybean plots as compared to 1 to 1.7 t/ha in untreated control plots.
Waswa et al. (2014)	Nyabeda, Kenya	Red clay loam (0.21% TSN)	- *B. japonicum* USDA 110 - *Rhizobia* isolates - NAK84 - NAK89 - NAK 115 - NAK 117 - NAK 128 - NAK 135		- NAK 128 outperformed USDA110 by 29% and 24% on both promiscuous and specific soybean varieties. - NAK 128 significantly increased nodule number by producing up to 2.4 million nodules (334 kg) per hectare more that USDA 110. - Overall, many *Rhizobia* isolates increased nodule number, nodule biomass and grain yield compared to uninoculated crop.
Muhammad (2010)	Minna, Nigeria	Alfisol (0.38% TSN)	*Rhizobia* strains: - R25B - IRj 2180A - IRc 46 - IRc291	Single Superphosphate (SSP): - 0, 25 and 50 kg P_2O_5/ha	- *Rhizobia* inoculation increased nodule number, shoot dry biomass and grain yield over uninoculated control. - Phosphorus also improved nodule number, shoot dry weight, and grain yield over control treatment.
Mulambula et al. (2019)	Meru South, Kenya	Clay (0.23% TSN)	*Rhizobia* strain rates: - 0, 100 and 200 g /ha	Triple Superphosphate (TSP): - 0, 20 and 30 kg P/ha	- *Rhizobia* inoculation and phosphorus fertilizer significantly (P=0.05) increased plant height, nodule number, nodule dry and fresh weight, mean number

Table 1. Contd.

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Reference	Location	Soil type	Strain	Fertilizer	Findings
					of branches and pods, shoot fresh and dry weight and seed weight averaging 29.35 cm and 26.79 cm, 38.71 and 35.14, 0.51 and 0.38, 5.5 g and 12.54g, 49.13 and 59.18, 77.65 and 90.91, 56.99 and 69.33, 168.9 and 148.13g for SB19 and SB24 soybean varieties respectively.
Rechiatu (2015)	Kpongu, Nyankpala and Manga, Ghana	Loamy sand (0.02 and 0.06% TSN) Sandy loam (0.04% TSN)	*Rhizobium* strain: - 532c (Legumefix)		- Soybean nodule dry weight responded significantly ($P<0.05$) to Legumefix inoculation over control, though varied with location. - Inoculation increased grain yield by 22.43% and 135.54% across two study locations, outperforming uninoculated control.
Tarekegn and Kibret (2017)	Pawe, Ethiopia	N/A	*B. japonicum* strain: - TAL-379	Triple Superphosphate (TSP): - 0, 23, 46 kg P_2O_5/ha	- Nodule number (80.26), fresh and dry weight (3.77 and 0.99 gm/plant respectively) were recorded following application of 46 kg P_2O_5/ha, *B. japonicum* and 11.5 kg N/ha. - Rhizobial inoculation and phosphorus fertilizer (46 kg P_2O_5/ha) increased seed yield by 11.91 gm/ plant and 15.97 gm/plant respectively. - Phosphorus applied at 23 kg P/ha resulted in highest plant biomass of 27.25 gm/plant. - 100-seed weight of 16.96 gm and grain yield of 3151.88 kg/ha were brought by the application of 46 kg P_2O_5/ ha, *B. japonicum* and 11.5 kg N/ha.
Okereke et al. (2001)	Igbariam, and Awka, Nigeria.	Loamy sand (Igbariam) and sandy loam (Awka) (0.14-0.18% TSN)	*B. japonicum* strains: -USDA136 - TAL 122		- *Bradyrhizobia* strains have increased nodule number, nodule dry weight, shoot dry weight, and grain yield compared to the uninoculated crop.
Khalid et al. (2011)	Shambat, Sudan	0.05% TSN	*Bradyrhizobium* - TAL 109		- Inoculation improved shoots and roots dry weight, nodulation, yield components and grain yield.
Mukhtar et al. (1987)	Gezira, Sudan	N/A	*R. japonicum* strains: - 2R-210-3A - 2R-210-2A - 2R-210-3 - PRC-201 - I 1110 Tn5 - 5PRC (ut) - (SR) RJ	Phosphorus fertilizer - 50 and 100 kg P_2O_5/ha	- Inoculation increased plant dry matter, nodule dry matter, yield and yield components and seed protein over control. - Applied phosphorus (50 and 100 P_2O_5/ha) plus starter nitrogen (10 kg N/ha) and inoculation gave unreliable results.
Youseif et al. (2014)	Giza, Egypt	Sandy loam (0.018% TSN)	*Rhizobia* strains: - NGB-SR3 - NGB-SR4 - NGB-SR7 - NGB-SR14		- Tested *Rhizobia* strains increased nodulation with the nodule masses of 265-362 mg/plant compared to 15-31 mg/plant of the uninoculated control. - Inoculants NGB-SR4 and NGB-SR7 out-performed other tested strains in terms of seed yield, N-yield and crude protein content.

N/A, not available; TSN, total soil nitrogen.

Author(s), Year	SMD [95%−CI]
Argaw, 2014	−7.43 [−8.57, −6.29]
Okereke et al., 2000	−1.37 [−2.53, −0.21]
Chowdhury et al., 1983	−0.64 [−1.23, −0.04]
Akpalu, 2014	−1.48 [−3.28, 0.33]
Ahiabor et al., 2016	1.12 [−0.21, 2.45]
Lamptey et al., 2014	−0.03 [−1.63, 1.57]
Fituma et al., 2018	−3.27 [−6.27, −0.27]
Solomon et al., 2012	−2.02 [−3.54, −0.50]
Youseif et al., 2014	−9.60 [−13.60, −5.59]
Savala, 2020	−2.44 [−3.82, −1.05]
Pulver et al., 1982a	−0.89 [−1.32, −0.47]
Pule−Meulenberg et al., 2011	−1.79 [−3.03, −0.55]
Osunde et al., 2003	−0.92 [−2.11, 0.27]
Mukhtar et al., 1987	−5.10 [−7.96, −2.24]
Waswa et al., 2014	−1.54 [−2.35, −0.72]
Mulambula et al., 2019	−2.60 [−4.48, −0.72]
Rechiatu et al., 2015	−1.74 [−3.06, −0.41]
Tarekegn et al., 2017	0.04 [−1.10, 1.17]
Okereke et al., 2001	−1.38 [−2.21, −0.56]
Pulver et al., 1982b	−2.19 [−3.94, −0.44]
RE Model, Heterogeneity: I^2=92%, p=0.0001	−2.02 [−2.95, −1.08]

Favors experimental   Favors control

−15   −10   −5   0   5

Standardized Mean Difference

Figure 3. The overall effect of soybean inoculation on nodule dry weight after doing a meta-analysis on 20 studies.
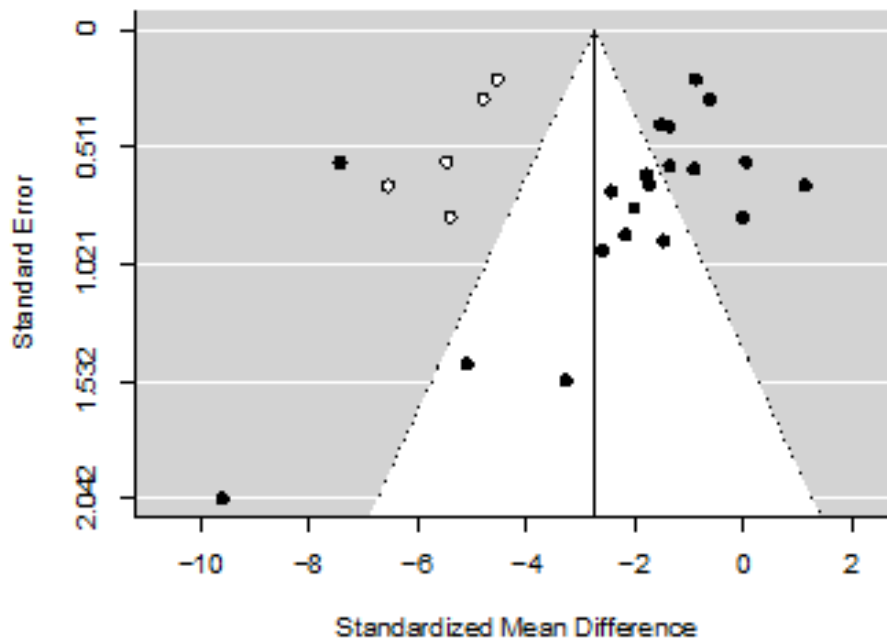
Figure 4. A funnel plot on nodule dry weight for 20 studies.

49 % (P=0.0001). The application of phosphorus fertilizer increased the number of nodules compared to controls (no phosphorus applications) (Figure 11). Phosphorus application resulted in a 95% CI of -1.73[-2.51, -0.94] as

Author(s), Year	SMD [95%−CI]
Okogun & Sanginga, 2003	−0.49 [−1.30, 0.33]
Argaw, 2014	−4.92 [−5.74, −4.11]
Okereke et al., 2000	−1.30 [−2.38, −0.22]
Zengeni & Giller, 2007	−1.53 [−2.65, −0.42]
Gyogluu et al., 2016	−0.80 [−1.89, 0.29]
Akpalu, 2014	−0.23 [−1.47, 1.01]
Ahiabor et al., 2016	0.28 [−0.97, 1.52]
Lamptey et al., 2014	−3.03 [−5.38, −0.69]
Fituma et al., 2018	−3.28 [−6.29, −0.28]
Solomon et al., 2012	−1.59 [−3.01, −0.17]
Savala, 2020	−1.34 [−2.59, −0.08]
Pulver et al., 1982a	−1.94 [−3.31, −0.57]
Pule−Meulenberg et al., 2011	−1.50 [−2.78, −0.22]
Osunde et al., 2003	−1.01 [−2.22, 0.19]
Rurangwa et al., 2018	−0.81 [−2.47, 0.86]
Okogun et al., 2005	−0.17 [−2.14, 1.79]
Waswa et al., 2014	−1.30 [−2.18, −0.42]
Muhammad, 2010	−5.09 [−7.95, −2.24]
Khalid et al., 2011	−0.67 [−1.84, 0.49]
Mulambula et al., 2019	−2.57 [−4.44, −0.70]
Tarekegn et al., 2017	−1.25 [−2.49, −0.01]
Okereke et al., 2001	−2.26 [−3.17, −1.34]
Pulver et al., 1982b	−4.16 [−6.63, −1.70]
RE Model, *Heterogeneity: I^2=75%, p=0.0001*	−1.62 [−2.17, −1.07]

Figure 5. The effect of soybean inoculation on nodule number.
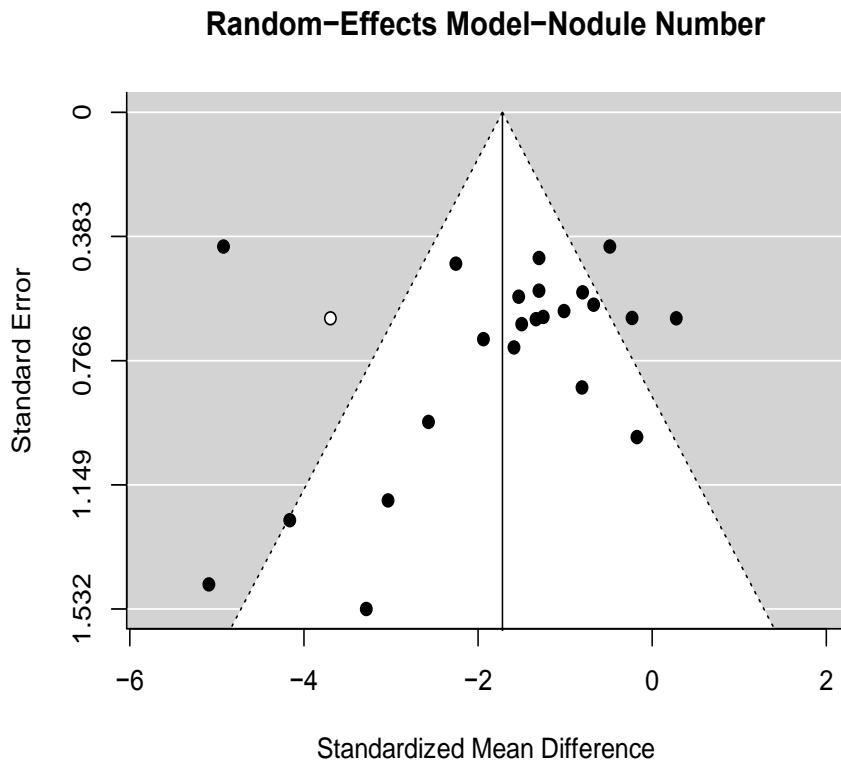
Figure 6. A funnel plot on nodule number response to inoculation for 23 studies.

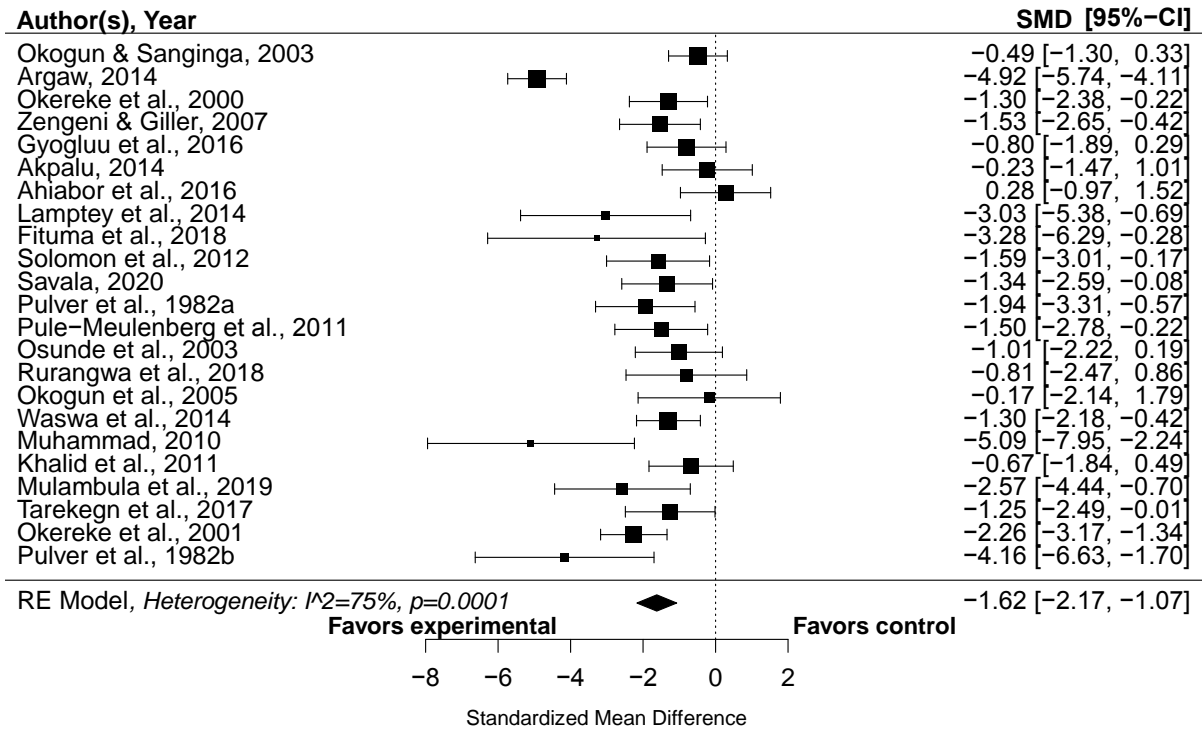| Author(s), Year		SMD [95%−CI]
Okogun & Sanginga, 2003		−0.30 [−1.10, 0.51]
Argaw, 2014		−2.73 [−4.95, −0.50]
Okereke et al., 2000		−1.10 [−2.16, −0.05]
Chowdhury et al., 1983		−1.02 [−1.87, −0.17]
Lamptey et al., 2014		−2.47 [−4.60, −0.35]
Solomon et al., 2012		−2.33 [−3.93, −0.72]
Youseif et al., 2014		−1.75 [−3.08, −0.42]
Pulver et al., 1982		−0.83 [−1.28, −0.38]
Pule−Meulenberg et al., 2011		−0.22 [−1.35, 0.92]
Osunde et al., 2003		−2.88 [−4.14, −1.63]
Mukhtar et al., 1987		−0.54 [−2.53, 1.46]
Muhammad et al., 2010		−0.67 [−2.09, 0.76]
Okogun et al., 2005		−0.83 [−2.88, 1.21]
Khalid et al., 2011		−0.98 [−2.18, 0.22]
Mulambula et al., 2019		−2.33 [−4.13, −0.54]
Okereke et al., 2001		−2.07 [−2.75, −1.38]
RE Model, *Heterogeneity: I^2=54%, p=0.0001*		−1.31 [−1.74, −0.88]

Favors experimental   Favors control

−6   −4   −2   0   2

Standardized Mean Difference

Figure 7. Shoot dry weight as affected by *Rhizobium* inoculation.

the total effect. The studies were asymmetrically distributed on the Funnel plot (Figure 12), showing the presence of bias. However, no significant changes were brought to the final result by trim and fill of the missing studies.

Grain yield
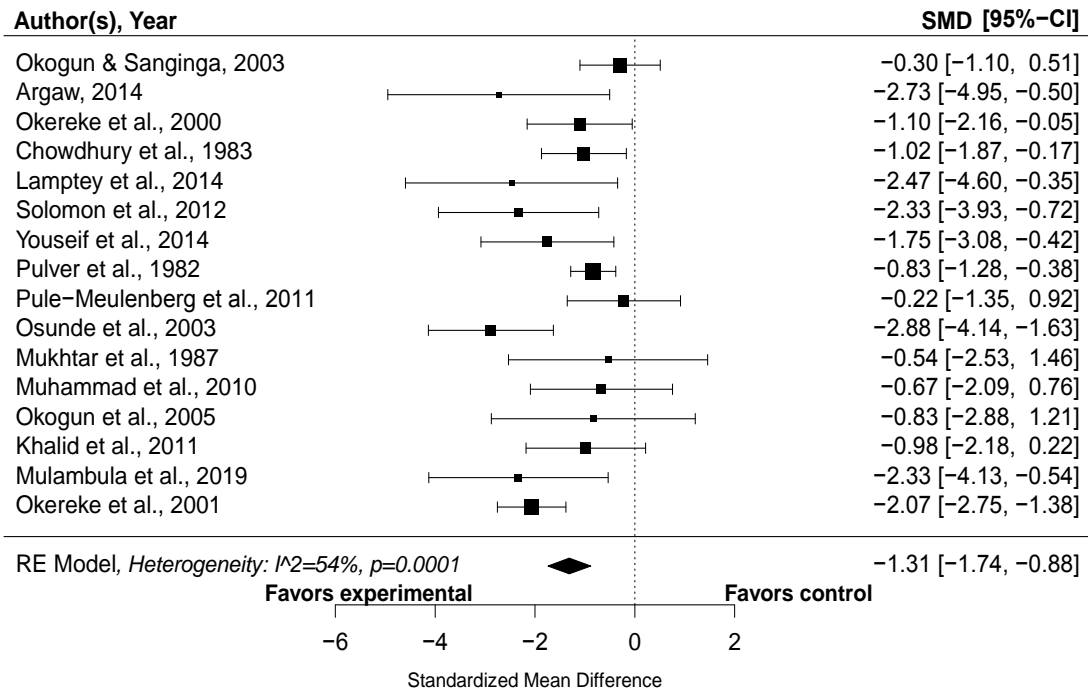
The 10 studies were less heterogenous (I^2 = 20%) (P=0.0001), and the overall effect of phosphorus application on standardized differences had 95% CI of -1.55[-2.14, -0.96] (Figure 13). The overall addition of phosphorus increased the grain yield compared to no phosphorus controls. The Funnel plot showed an asymmetrical distribution of the 10 studies (Figure 14), hence the presence of bias. The overall result remained unchanged after trim and fill.

Grain yield variations as influenced by *Rhizobium* inoculation and P fertilizer

The interaction of inoculation and phosphorus resulted in high grain yield (2.51 t ha^{-1}) compared to the main effects of inoculation and phosphorus (1.67 t ha^{-1} and 1.95 t ha^{-1}, respectively) (Figure 15). However, the main effects of phosphorus and inoculation also contributed to high grain yields.

Relative yield increase

The relative yield increase of the inoculated treatments over non-inoculated controls for the combined studies ranged from 74 to 87% (mean = 80%). The median, first and third quartiles were 80, 78.6, and 81.4%, respectively (Figure 16a). Phosphorus-treated plants had a mean relative yield increase of 73.4% over control treatments with median, first and third quartiles of 73, 71.5, and 75%, respectively (Figure 16b).

DISCUSSION

Response of soybean to rhizobial inoculation

The results of the meta-analysis confirmed that the inoculation of soybean with *Rhizobia* strains in African soils has a highly significant influence on nodule number, nodule dry weight, shoot dry weight, and yield. The performance of *Rhizobia* inoculants varies with strain species/isolates (*Bradyrhizobium/ Sinorhizobium*) and/or indigenous/introduced), soybean genotype, and soil underlying characteristics (pH, soil organic matter, nutrients, salinity, temperature) (Mapope and Dakora, 2016; Thilakarathna and Raizada, 2017). Many of the studies meta-analysed concluded that rhizobial inoculation effectively increased nodule number per plant. However, Thilakarathna and Raizada (2017) mentioned

Random−Effects Model−Shoot Dry Weight

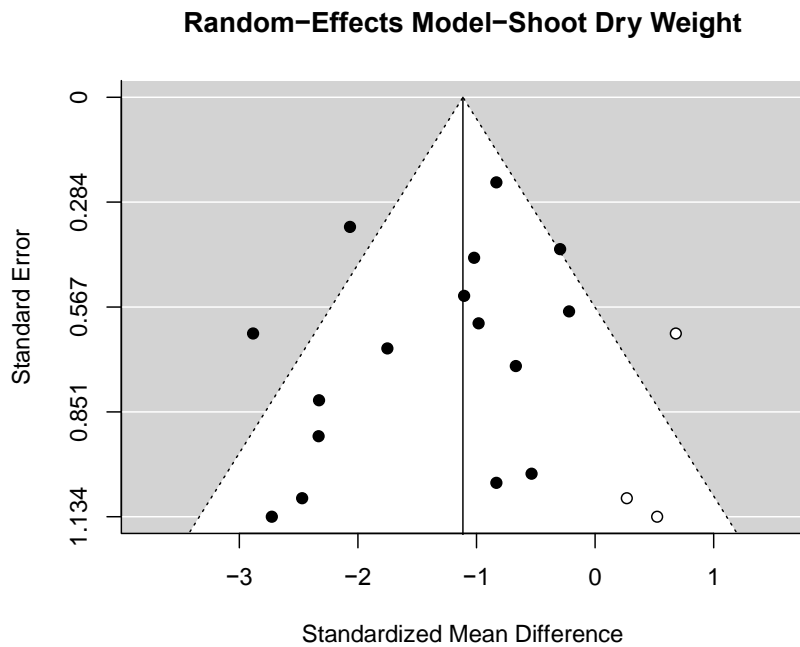**Figure 8.** A funnel plot for the 16 studies analyzed for shoot dry weight response to inoculation.
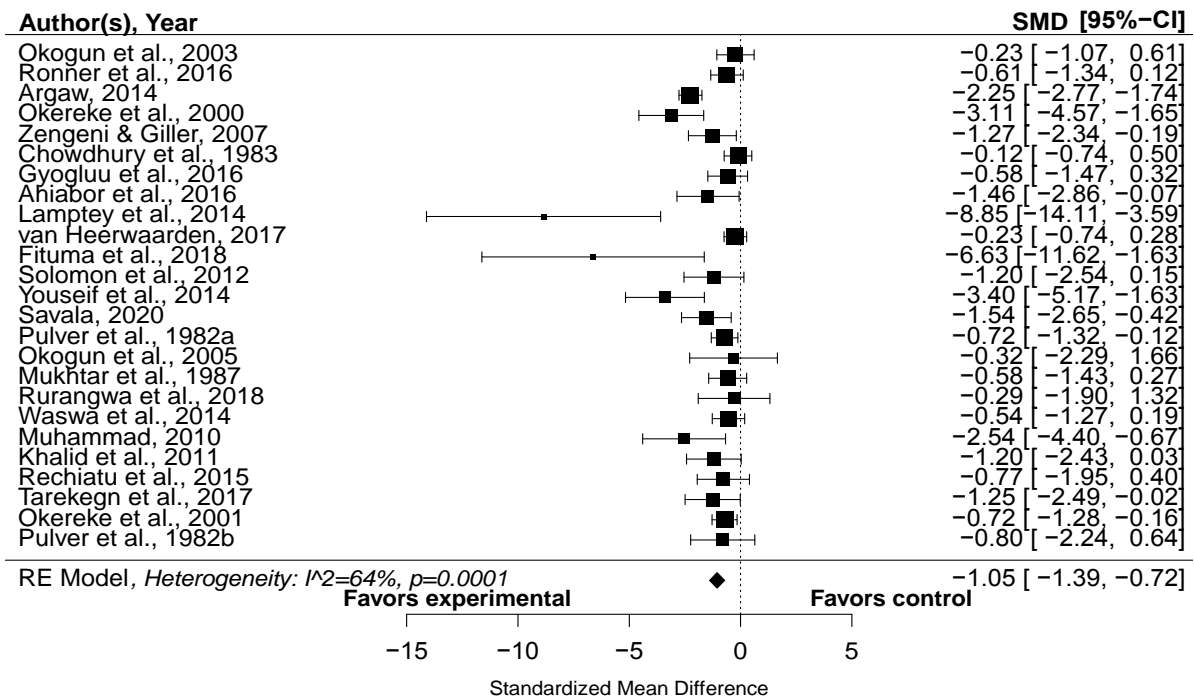

Figure 9. Effect of *Rhizobium* inoculation on soybean grain yield after doing meta-analysis on 25 studies.

that the efficacy of inoculants (*Bradyrhizobium and Sinorhizobium*)for nodule number varied from -28 to +178

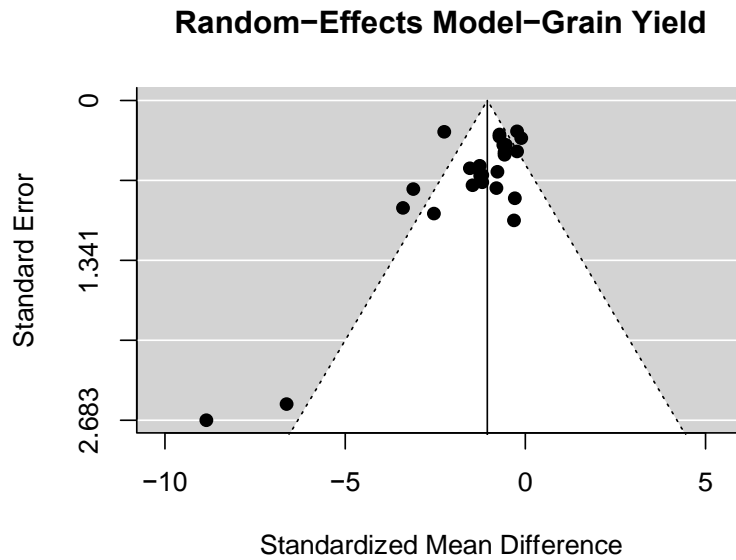nodules in contrast to the non-inoculated controls. According to the authors, the highest nodule number

Random−Effects Model−Grain Yield

Figure 10. A funnel plot on soybean grain yield response to inoculation.

Author(s), Year		SMD [95%−CI]
Dadson & Acquaah, 1984		−3.90 [−6.62, −1.17]
Akpalu, 2014		−0.90 [−2.20, 0.40]
Ahiabor et al., 2016		−1.92 [−4.30, 0.45]
Lamptey et al., 2014		−1.20 [−2.71, 0.30]
Fituma et al., 2018		−2.20 [−3.64, −0.77]
Savala, 2020		−0.26 [−1.39, 0.88]
Rurangwa et al., 2018		−1.07 [−2.78, 0.64]
Mulambula et al., 2019		−4.11 [−6.55, −1.66]
Tarekegn et al., 2017		−2.60 [−4.14, −1.06]
RE Model, *Heterogeneity: I^2=49%, p=0.0001*		−1.73 [−2.51, −0.94]

Favors experimental   Favors control
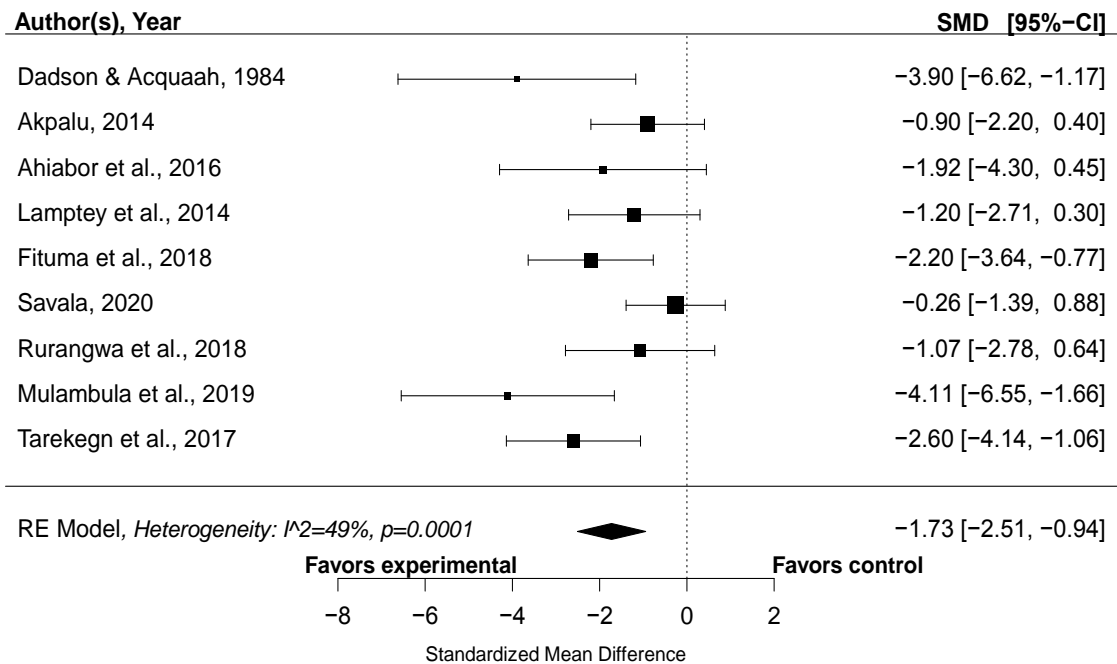
Standardized Mean Difference

Figure 11. Effect of phosphorus fertilizer to soybean on nodule number after doing meta-analysis on 9 studies.

occurs in soils where indigenous *Rhizobia* are absent or extremely low. This could be probably due to less competition between the commercial *Rhizobia* and indigenous strains. A recent field research conducted across three sites found that inoculation increased nodulation of different soybean genotypes ranging from 37-95% against the non-inoculated treatments (Savala and Kyei-Boahen, 2020). Okereke et al. (2000) also found that soybean inoculation with *Bradyrhizobia* strains significantly increased nodule number but with huge variability at 84 days after planting (DAP); and this was attributed to the variations in the ability to nodulate the
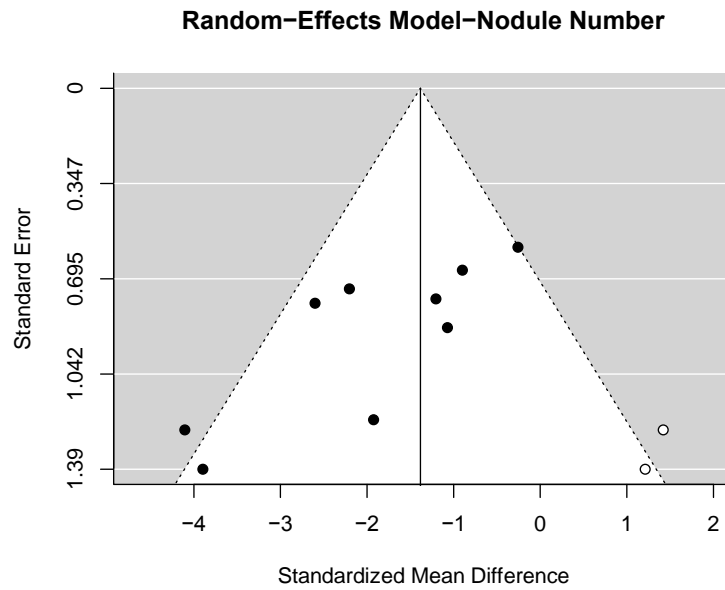
Random−Effects Model−Nodule Number

Figure 12. A funnel plot on nodule number response to P-fertilizer application for 9 studies.

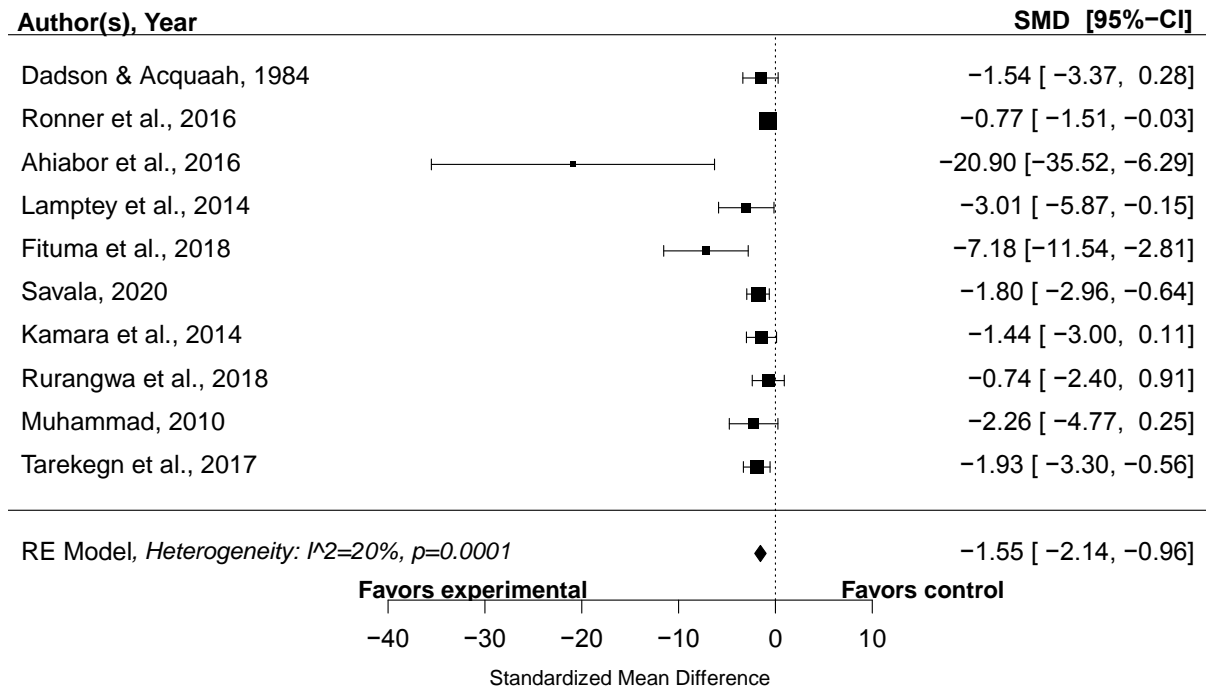| Author(s), Year		SMD [95%−CI]
Dadson & Acquaah, 1984		−1.54 [−3.37, 0.28]
Ronner et al., 2016		−0.77 [−1.51, −0.03]
Ahiabor et al., 2016		−20.90 [−35.52, −6.29]
Lamptey et al., 2014		−3.01 [−5.87, −0.15]
Fituma et al., 2018		−7.18 [−11.54, −2.81]
Savala, 2020		−1.80 [−2.96, −0.64]
Kamara et al., 2014		−1.44 [−3.00, 0.11]
Rurangwa et al., 2018		−0.74 [−2.40, 0.91]
Muhammad, 2010		−2.26 [−4.77, 0.25]
Tarekegn et al., 2017		−1.93 [−3.30, −0.56]
RE Model*, Heterogeneity: I^2=20%, p=0.0001*		−1.55 [−2.14, −0.96]

Figure 13. Effect of phosphorus application to soybean grain yield.

soybean variety used (TGX 536-02D). On the other hand, *Rhizobium* inoculation has failed to significantly increase nodule number as demonstrated by Ahiabor et al. 2016). These results implied that N might not be always the limiting factor to lack of nodulation but other nutrients like low phosphorus and molybdenum may impede the inoculation response; and also, indigenous *Rhizobia* could prevent the introduced *Rhizobia* from forming
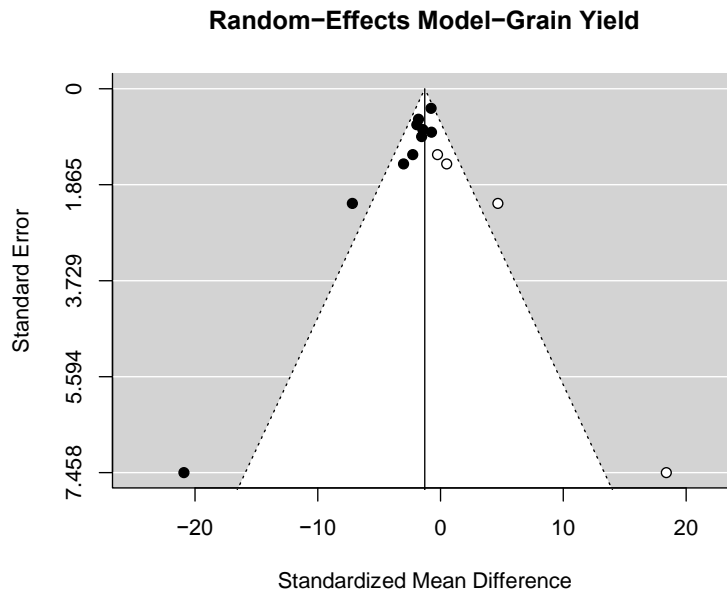
Random−Effects Model−Grain Yield

Figure 14. A funnel plot for the 10 studies analyzed for soybean grain yield response to P.
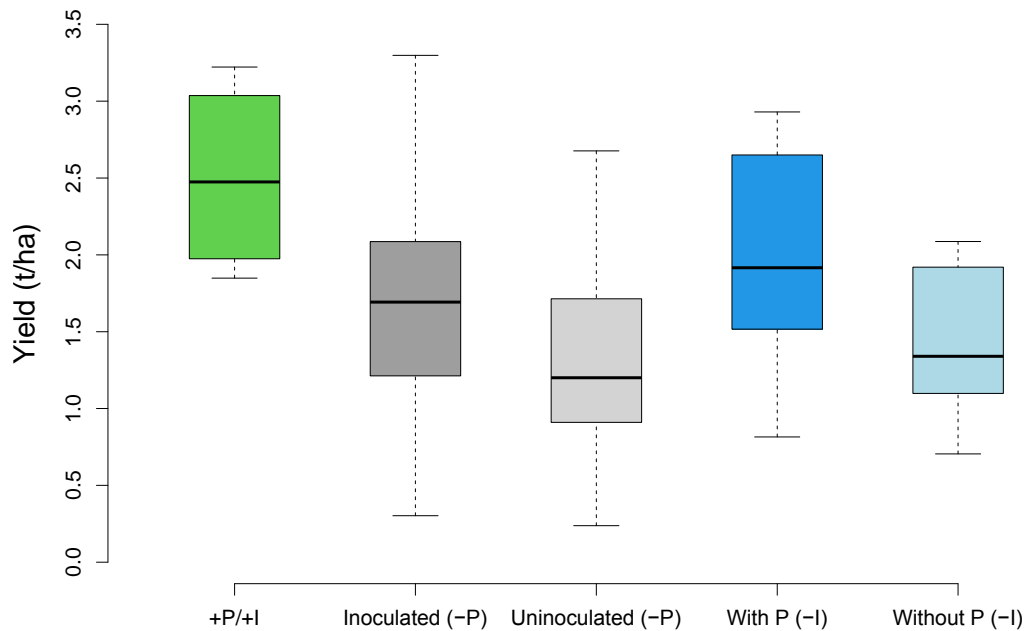

Figure 15. Effect of inoculation and phosphorus application on soybean grain yield. +P, phosphorus was applied; -P, no phosphorus applied; +I, inoculated; -I, non-inoculated.

nodules on soybean (Ahiabor et al., 2016).

The significant increase in the nodule dry weight was not surprising given the effective response of the nodule number to inoculation. This result concurs with the recent works conducted in Ethiopia and Kenya which demonstrated that rhizobial inoculation resulted in increased nodule dry weight per plant (Fituma et al., 2018; Mulambula et al., 2019). Different *Rhizobia* strains also showed significant effects on nodule dry weight, ranging from 0.33 to 0.44 g plant[-1] in contrast to the non-inoculated controls (Argaw, 2014). Similarly, a field research demonstrated a prolific nodule dry response to
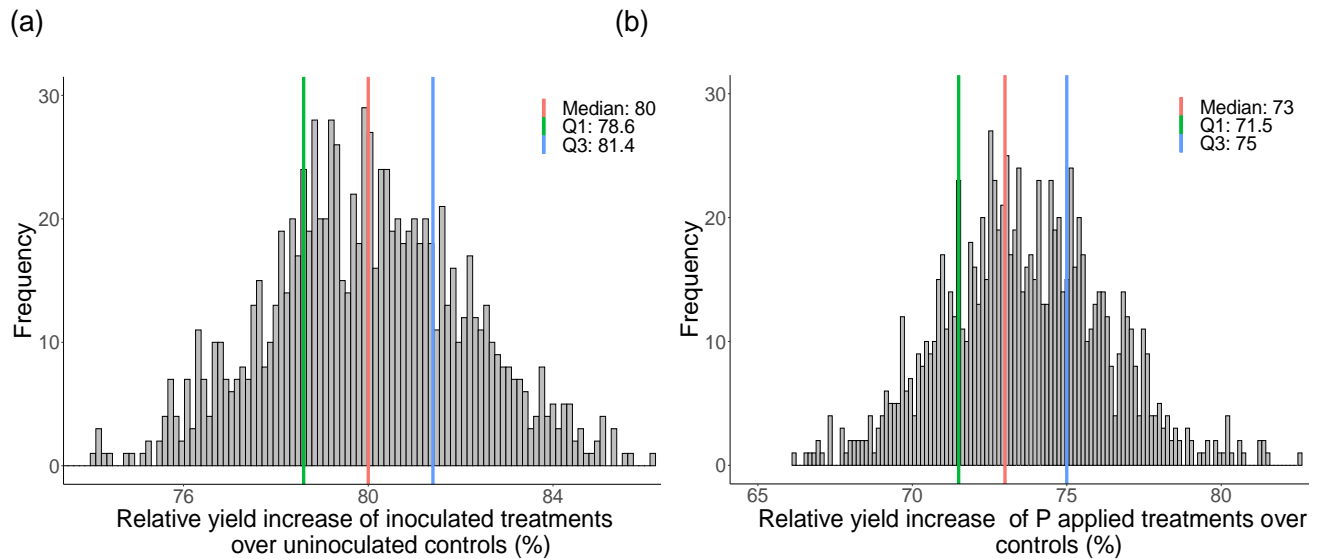
Figure 16. Relative yield increase of soybean in response to inoculation (a) and P fertilizer (b).

Rhizobium inoculation, which ranged from 0.27-0.36 g plant[-1] versus 0.02-0.03 g plant[-1] for non-inoculated treatments (Youseif et al., 2014). The results indicated the importance of soybean *Rhizobium* inoculation in African soils. Soybean shoot dry weight increase was highly significant in response to inoculation with *Rhizobia* strains as compared to the non-inoculated control. This result concurs with Ibrahim et al. (2011) who reported a significant increase of shoot dry weight in inoculated treatment over non-inoculated treatment. However, Lamptey et al. (2014) also reported the highest shoot fresh and dry weight following application of 30 kg P ha^{-1}. Inoculation of soybean with *Rhizobia* strains improves nodulation leading to higher nitrogen fixation which subsequently increases the vegetative growth as well as dry matter formation from the inoculated soybean (Lamptey et al., 2014). Interestingly, the results showed a highly significant grain yield of inoculated soybean increased by 25.5% over non-inoculated, confirming the benefits from *Rhizobium* inoculation to soybean in Africa. A global meta-analysis also reported the efficacy of various *Rhizobia* inoculants on soybean yield ranging from -34% to +109% over non-inoculated controls (Thilakarathna and Raizada, 2017). Another farmer-managed field research conducted across 10 sub-Sahara African countries estimated mean grain yield at 1.34 t ha^{-1} and 1.23 t ha^{-1} for inoculated and non-inoculated treatments, respectively, indicating a very narrow margin (van Heerwaarden et al., 2017). They mentioned huge varietal and spatial variations across the region as major contributing factors to their results. Ulzen et al. (2018)

also found that *Rhizobium* inoculation increases soybean yield, hence improving the livelihood of smallholders.

Effect of applied P on soybean productivity

Phosphorus is one of the irreplaceable nutrients (Giller and Cadisch, 1995), and its deficiency in many tropical regions is limiting legume performance (George et al., 1995). The BNF process in legumes is substantially driven by phosphorus, which functions as the energy storage and transfer component for the symbiotic bacteria (Dakora and Keya,1997), and increases tissue-%N as well as uptake of N derived from fertilizer (Thomas, 1995; cited by Giller and Cadisch, 1995). This study demonstrated that the supplementation of P-fertilizer on soybean at rates between 20 to 60 kg P ha^{-1} across African soils has a higher significant effect on nodule number. In their findings, Ahiabor et al. (2016) found that applying 22.5 kg and 45 kg P_2O_5 ha^{-1} also effectively increased the number of nodules in soybean by 12 and 22%, respectively, as compared to untreated control. Despite the benefits of this technology, about 24% of farmers were reportedly applying P-fertilizer on soybean in Western Kenya (Franke and Wolf, 2011), which remains true for the majority of smallholders especially, in the SSA (Sheahan and Barrett, 2017). The significant response of grain yield to applied P was not surprising because 11 out of 12 studies that reported on phosphorus fertilizer in this meta-analysis found concurring results. The yield was increased by 36.4% as

compared to the control treatment (Figure 15). A field experiment conducted in Nigeria demonstrated that P supplementation increased soybean yield by 452 kg ha^{-1} under smallholder farming (Ronner et al., 2016). Another recent study concluded that 23-46 kg P_2O_5 ha^{-1} of P-fertilizer applied together with a lower level of N (11.5 kg N ha^{-1}) as starter fertilizer potentially increases yield (Tarekegn and Kibret, 2017).

Rhizobium inoculation and P-fertilizer interaction effect on soybean grain yield

The combined application of *Rhizobia* inoculants and P-fertilizer on soybean has resulted in 50.3% and 28.7% yield increase, respective of the independent effects of the two technologies. Servani et al. (2014) reported that P plays a critical role in nodulation processes in legumes; hence its deficiency can limit the yield. Supplementing P-fertilizer will however enhance the BNF process in soybean through improved nodulation processes by the rhizobial bacteria. Ekeleme *et al*. (2009) mentioned that phosphorus is mostly deficient in many soils, and its optimum application improves the shoot weight and yield of legumes. Another field study also found that applying 30 kg P ha^{-1} of phosphorus together with *Rhizobium* inoculant significantly increased soybean grain yield (Lamptey et al., 2014). Relative yield increase from inoculated treatments over non-inoculated controls was 80%, on average, attesting the effectiveness of rhizobial inoculation in soybean grain yield's improvement. In this regard, van Heerwaarden et al. (2017) obtained an average yield response of 88 kg ha^{-1} from inoculated plants over non-inoculated controls. Phosphorus-treated plants had also a high relative yield increase over control plants, averaging 73.4%. Eleven studies reported that supplementation of P fertilizer resulted in improved BNF with direct impacts on grain yield. However, it was hard to conclude that rhizobial inoculation and P fertilizer result in higher relative yield increase given the huge variability in agronomic, climatic, and edaphic factors across African countries which could affect the biological nitrogen fixation process of soybean.

Although the meta-analysis ascertained the general effect of rhizobial inoculation on soybean, it should be noted that the efficacy of the commercial inoculants varies with the underlying soil factors like indigenous rhizobial level, soil available nutrients, soil pH, organic matter content, temperature, and precipitation. Unfortunately, the effects of the above-mentioned factors were not analyzed due to the huge variation of the data and scarcity of valid studies. It was also clear that P-fertilizer notably between 20 kg and above 60 kg P ha^{-1} had varying effects on soybean's nodulation and grain yield. Furthermore, it was not established that the rates between 20 or above 60 kg P ha^{-1} could not affect

soybean productivity.

Conclusion

Meta-analyses of the effects of *Rhizobia* inoculants and phosphorus fertilizer on soybean nodulation in Africa revealed rhizobial inoculation has, in absolute terms, highly significant effects on nodulation characteristics, shoot dry weight, and grain yield of soybean on African soils that may vary with the underlying soil characteristics, *Rhizobium* strain, climatic conditions, to name a few. Application of phosphate fertilizer at rates of 20-60 kg P ha^{-1} proved to increase the nodule number per plant and most importantly, soybean grain yield. Phosphorus showed a slightly higher effect on grain yield as compared to rhizobial inoculation, in absolute terms. Finally, the application of both inoculant and P-fertilizer on soybean greatly increased grain yield by 50.3% compared to a simple *Rhizobium* inoculation and 28.7% compared to P application alone. Therefore, it was recommended to African farmers to adopt this sustainable approach of combined application of both *Rhizobium* inoculants and phosphate fertilizer for reduced financial costs of production and increased yield.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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Classifying highland quality protein maize in bred lines into heterotic groups

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Efforts to boost maize productivity can be undertaken through systematic management of heterosis. Combining ability test is a critical step towards identifying a heterotic group of new parental lines. Different heterotic grouping methods has been used by different researchers. Among these, SCA and hybrid mean, hybrid index, combined use of amplified fragment length polymorphism (AFLP), SSR markers and heterotic group's specific and general combining ability (HSGCA) are the major grouping methods. This study aimed to (i) assign maize inbred lines into heterotic groups and (ii) compare efficiency of different grouping methods. An experiment with 21 maize inbred lines crossed to two testers with known heterotic groups was conducted in 2017. The hybrid and parent experiments were tested together and laid out side by side. This study identified good heterotic grouping procedure. The breeding efficiency of HSGCA was higher by 31.6, 11.0 and 9.6% over joint SCA and hybrid mean, SCA and hybrid index methods, respectively. While the hybrid index method was more efficient than joint SCA and hybrid mean and SCA by 20.1 and 1.3% respectively, the SCA grouping method was more efficient than joint SCA and hybrid mean method by 18.5%. The highest (37%) and lowest (28.1%) breeding efficiency value were scored by HSGCA and hybrid index heterotic grouping method, respectively. Based on the result, HSGCA grouping method was more efficient. The variable heterotic grouping of the 21 newly developed QPM lines in this study indicated that different heterotic grouping methods have different efficiency in grouping the germplasms.

Key words: General combing ability, heterotic-group, inter-group, specific combining ability, within-group, *Zea mays.*

INTRODUCTION

Maize (*Zea mays* L.) is one of the five major cereals (also including wheat, teff, barley and sorghum) in terms of production volume, area coverage and household consumption (Tsedeke et al., 2015; CSA and WB, 2015).

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It accounts for 27% of Ethiopia's total cereal production and is critical for food security for smallholder subsistence farmers. Roughly nine million smallholders account for 95% of the national maize production (Tsedeke et al., 2015; CSA, 2012). Over the last two decades, the maize sector in Ethiopia has experienced an unprecedented transformation. Maize yields have doubled from around 1.6 t/ha in 1990 to more than 3.7 t/ha in recent years, the highest level in sub-Saharan Africa after South Africa (FAOSTAT, 2019). Important causes for the increased productivity include increased availability and use of modern inputs (e.g., modern varieties and fertilizer), better extension services and increasing demand (Tsedeke et al., 2015). Despite the recent progress in productivity, yield levels in Ethiopia are still very low relative to what they could be. According to the Global Yield Gap Atlas (GYGA, 2019), the water-limited yield potential of maize in Ethiopia is on average 12.5 t/ha, implying that farmers realize only around 30% of that potential.

Hybrid maize is one of the prosperous technology that farmers adopted in Ethiopia due to its promising yield. Therefore, continuous yield increase should be guaranteed through exploitation of heterosis and hybrid vigor to meet future needs of the country. The classification of inbreeds into heterotic groups is a precondition to facilitate the exploitation of heterosis in maize (Bidhendi et al., 2012). Melchinger and Gumber (1998) defined a heterotic group "as a group of related or unrelated genotypes from the same or different populations, which display similar combining ability (CA) and heterotic response when crossed with genotypes from other genetically distinct germplasm groups. By comparison, the term heterotic pattern refers to a specific pair of two heterotic groups, which express high heterosis and consequently high hybrid performance in their cross.

The concept of heterotic groups and patterns is fundamental to hybrid breeding theory and practice (Reif et al., 2005). Grouping germplasm into divergent heterotic groups is advantageous due to (i) a higher mean heterosis and hybrid performance and (ii) a reduced specific combining ability (SCA) variance and a lower ratio of SCA to general combining ability (GCA) variance (Reif et al., 2005). Heterotic groups and patterns among inbred lines helps to identify the best hybrid combinations using information obtained from field crosses, mainly using diallel or topcrosses to testers (Han et al., 1991; Terron et al., 1997), pedigree information, morphological traits, and molecular markers (Smith and Smith, 1992). The magnitude of the combining ability effect plays a crucial role in heterotic grouping, since it indicates the types of gene action as a preliminary indicator of heterotic expression (Singode et al., 2017). Maximum heterosis can be exploited if the breeding program uses inbred lines having significant positive GCA effect for grain yield and classified in opposing heterotic group (Annor et al., 2020). Phenotypic descriptors and molecular markers have been widely used in crop diversity studies to measure genetic distances (Darvishzadeh, 2012). Molecular markers are a powerful tool to delimit heterotic groups and to assign inbred lines into existing heterotic groups (Abebe et al., 2004).

One of the major purposes of maize hybrid breeding is to develop hybrids with high grain yield (Fan et al., 2009). To develop a high yielding maize hybrid, a breeder usually makes hundreds of crosses among selected inbred lines. The better chance of obtaining superior hybrids can be achieved when the breeder decides to make crosses between lines from different maize heterotic groups. However, because of unlimited genetic combinations between any two inbred lines, no heterotic group classification method can be perfect. Thus, a good heterotic group classification method can be defined as one whose classified heterotic groups allow inter-heterotic group crosses to produce more superior hybrids than the within-group crosses (Fan et al., 2009). Heterotic patterns have a strong impact in crop improvement because they predetermine to a large extent the type of germplasm used in a hybrid breeding program over a long period of time (Melchinger and Gumber, 1998). The objectives of the study were to classify the tropical inbred lines into heterotic groups and to compare the efficiency of heterotic grouping methods.

MATERIALS AND METHODS

Study sites

The study was conducted at three locations in the highland agroecology of Ethiopia including; Ambo, Arsi-Negele (transition highland) and Kulumsa Agriculture Research Centers in the 2017 main cropping season (May to December).

Experimental materials

Twenty-one highland QPM inbred lines, named hereafter as lines (L1 to L21) and two elite QPM inbred lines (CML159 and CML144), named hereafter as testers (T1 and T2, respectively), constituted the basic genetic materials of this experiment (Table 1). From the 21 inbred lines and the two testers, 42 F1 hybrids were generated at Ambo Highland Maize Breeding Program (AHMBP).

Two separate but interrelated experiments were laid side by side during the main cropping season (May to December) of 2017 GC as described below. In the 1st experiment, the 42 F1 hybrids along with three standard checks: one QPM (AMH852Q) and two CM (Jibat and AMH853), designated as hybrid check, were tested. In the 2nd experiment, the 21 inbred lines (L1 to L21), the two testers (T1 and T2) and one elite CM highland inbred line (FS67), designated inbred parent check, were tested at two locations (Ambo and Arsi-Negele Agricultural Research Centers) presented in Table 2.

Heterotic group's specific and general combining ability effects and their use in classifying maize lines into known heterotic groups

The calculated HSGCA effects for grain yield of the 21 maize inbred

Table 1. Latitude, longitude, altitude (masl), long-term annual rainfall (mm), maximum temperature (MaxT) (^{o}C), minimum temperature (MinT) (^{o}C), soil type and soil pH of the study sites.

Site	Latitude	Longitude	Altitude	Annual rainfall	Max T	Min T	Soil type	pH
Ambo	8˚ 57ʹ N	38˚ 7ʹ E	2225	1115	25.5	11.7	Heavy clay (Vertisol)	7.8
Arsi-Negele	7˚19ʹ N	38˚ 39ʹ E	1960	886	26.0	9.1	Clay loam	6.5-7.5
Kulumsa	8˚ 02' N	39˚ 10' E	2200	830	23.2	10.0	Luvisol/Eutric nitosols	6.0

Table 2. List of parental inbred lines used to generate the single cross hybrids using line by tester mating design and standard checks use.

Code	Pedigree	Description type	Tryptophan (%)
L1	[CML144/[CML144/CML395] F2-8sx]-1-2-3-2-B*5-1-B-B-B-#	Line	0.056
L2	[CML144/[CML144/CML395] F2-8sx]-1-2-3-2-B*5-2-6-B-B-#	Line	0.062
L3	(CLQRCWQ50/CML312SR)-2-2-1-BB-1-B-B-B-#	Line	0.077
L4	[CML144/[CML144/CML395] F2-8sx]-1-2-3-2-B*5-1-B-B-B-#	Line	0.077
L5	([NAW5867/P49SR(S2#)]//NAW5867] F#-48-2-2-B*/CML511) F2)-B-B-39-1-B-#	Line	0.066
L6	(CML197/(CML197/[(CLQRCWQ50/CML312SR)-2-2-1-BB/CML197]-BB) F2)-B-B-9-1-B-#	Line	0.063
L7	(CML197/(CML197/[(CLQRCWQ50/CML312SR)-2-2-1-BB/CML197]-BB) F2)-B-B-35-2-B-#	Line	0.063
L8	(CML197/(CML197/[(CLQRCWQ50/CML312SR)-2-2-1-BB/CML197]-BB) F2)-B-B-44-2-B-#	Line	0.069
L9	(CML197/(CML197/(CLQRCWQ50/CML312SR)-2-2-1-BBB) F2)-B-B-18-2-B-#	Line	0.086
L10	(CML197/(CML197/(CLQRCWQ50/CML312SR)-2-2-1-BBB) F2)-B-B-30-1-B-#	Line	0.080
L11	(CML197/(CML197/(CLQRCWQ50/CML312SR)-2-2-1-BBB) F2)-B-B-35-2-B-#	Line	0.109
L12	(CML395/(CML395/[NAW5867/P49SR(S2#)]//NAW5867] F#-48-2-2-B*4) F2)-B-B-30-1-B-#	Line	0.076
L13	[CML144/[CML144/CML395] F2-8sx]-1-2-3-2-B*5-2-6-B-B-#	Line	0.060
L14	(CML395/(CML395/[CML144/[CML144/CML395] F2-8sx]-1-2-3-2-B*5) F2)-B-B-46-1-B-#	Line	0.063
L15	(CML395/(CML395/[CML144/[CML144/CML395] F2-8sx]-1-2-3-2-B*5) F2)-B-B-50-1-B-#	Line	0.062
L16	(CML395/(CML395/S99TLWQ-B-8-1-B*4-1-B) F2)-B-B-10-3-B-#	Line	0.061
L17	(CML395/(CML395/S99TLWQ-B-8-1-B*4-1-B) F2)-B-B-14-1-B-#	Line	0.073
L18	(CML395/(CML395/S99TLWQ-B-8-1-B*4-1-B) F2)-B-B-29-1-B-#	Line	0.060
L19	(CML395/(CML395/CML511) F2)-B-B-7-2-B-#	Line	0.060
L20	(CML395/(CML395/CML511) F2)-B-B-11-2-B-#	Line	0.066
L21	(CML395/(CML395/CML511) F2)-B-B-37-1-B-#	Line	0.061
T1	CML144	Tester	
T2	CML159	Tester	

lines and the two testers (CML144 and CML159) are shown in Figure 1. The following procedure was followed for classifying 21 maize lines into the known maize heterotic groups using the HSGCA method according to Fan et al. (2009).

Step 1: We placed all inbred lines with negative HSGCA effects into the same heterotic groups as their tester. The inbred lines were classified into the two known heterotic groups (Table 3). At this step, a line might be assigned to more than one heterotic group.

Step 2: If an inbred line was assigned to more than one heterotic group in Step 1, we kept the line in the heterotic group if its HSGCA had the smallest value (or largest negative value) and removed it from other heterotic groups.

Step 3: If a line had a positive HSGCA effect with all representative testers, we were cautious to assign that line to any heterotic group because the line might belong to a heterotic group different from the two testers.

Data analysis

The data obtained from field measurements was organized and analyzed using SAS statistical package (SAS, 2002). Analysis was conducted using the model of RCBD after confirming the non-significance of block effect, which implied there was uniformity among blocks. Accordingly, to test for the presence of variation among crosses and inbred lines for the trait in question; variance and CA analysis was carried out for individual locations and across locations. The details of data analysis are subsequently presented.

Analysis of variance

Individual and across locations data were subjected to analysis of variance using PROC GLM procedure in SAS software version 9.0 (SAS, 2002). In the analysis, treatments were used as a fixed factor, while replications and locations were considered as random factors. This was specified using RANDOM statement in the PROC
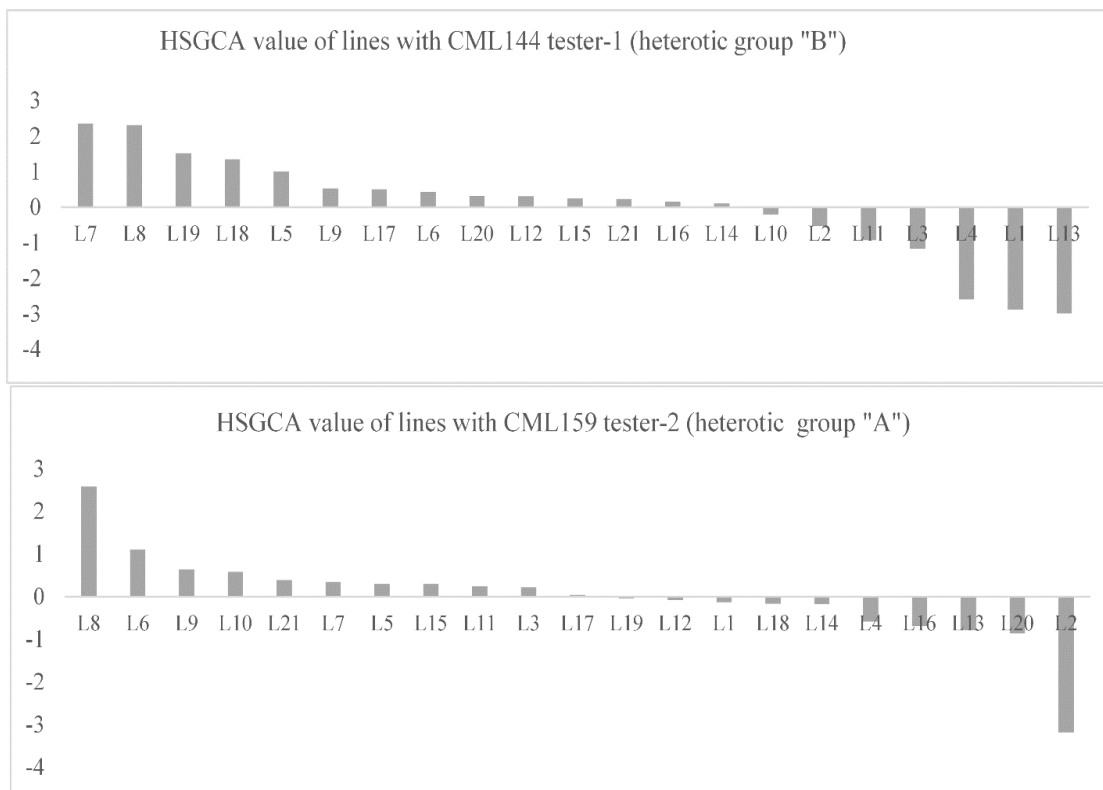
Figure 1. HSGCA value of lines with tester 1 (CML144) (heterotic group "B") and tester-2 (CML159) (heterotic group "A") for 21 maize lines.

Table 3. Mean square of 42 test crosses and three standard checks for grain yield, days to tasseling (DT) and days to silking (DS) at three locations (Ambo, Arsi-Negele and Kulumsa) in the 2017 cropping season.

Source of Variation	DF	GY	DT	DS
Rep (Location)	3	7.96***	16.2*	17.9*
Location	2	329.73**	2278.1**	1159.4**
Genotype	44	9.34***	87.9***	71.5***
Cross	41	9.67***	68.5***	50.8***
Line (Lgca)	20	13.59*	62.6*	50.7*
Tester (Tgca)	1	6.00	1102.1***	644.5***
Line x Tester (LxTsca)	20	5.94***	22.7***	21.2***
Check	2	0.38	5.1	11.6
Cross vs Check	1	13.59***	1050.8***	1038.2***
Genotype x Location	88	2.05***	6.0*	6.8
Cross x Location	82	1.81***	5.0	5.8
Lgca x Location	40	1.95***	4.0	5.0
Tgca x Location	2	2.76*	9.7	25.9**
LxTsca x Location	40	1.63**	5.8	5.6
Check x Location	4	4.85*	13.1	12.8
Pooled error crosses	123	0.87	4.3	5.1
Pooled error genotypes	132	0.93	4.2	5.1
Pooled error checks	6	0.67	4.4	3.6
GCA/SCA ratio		2.34	5.2	3.9

*, **, *** = significant at alpha 0.05,0.01 and 0.001 level respectively, DF=Degree of freedom, GCA= General combining ability, SCA= specific combining ability.

GLM model. Combined analysis was done for traits that showed significant difference at each location analysis after testing homogeneity of error variances. In combined analysis, the variation among genotypes, crosses and checks effects were tested against their respective interaction effect with location. Interaction effect of each source of variation by location was tested as per the expected mean square (MS) of error estimate.

Combining ability analysis

The LxT analysis was done for traits that showed statistically significant differences for L, T and LxT in each environment and across environment using the adjusted means based on the method described by Kempthorne (1957). GCA and SCA effects for grain yield and other traits were calculated using a LxT model.

The F-test of MS due to lines and testers was tested against LxT interaction but crosses were compared against MS due to error for individual locations (Singh and Chaudhry, 1985). In case of across locations analysis, the F-test for the main effects such as crosses, lines, testers and LxT interaction MS were tested against their respective interaction with the location. The MS attributable to all the interactions with the locations were tested against pooled error MS. The effect of location was tested by replication within environment as an error term. Significances of GCA and SCA effects of the lines and crosses were determined by t-test using standard errors of GCA and SCA effects. The main effects due to LxT were considered as GCA effects while, LxT interaction effects were represented as the SCA. The estimate of GCA effect considered for traits showed significant MS by both line GCA and tester GCA or only by line GCA. Similarly, SCA effect presented the traits which had significant SCA MS.

$$I = (MH-MT)/MT$$

where, I= hybrid index, MH= mean value of each hybrid, MT=mean value of each tester. Lines with hybrid index values less than 1.05 are classified under the same group.

Heterotic group's specific and general combining ability computation

SCA = Cross mean (X_{ij}) – Line mean ($X_{.j}$) – Tester mean ($X_{i.}$) + Overall mean ($X_{..}$)

GCA = Line mean ($X_{.j}$) – Overall mean ($X_{..}$).

HSGCA = Cross mean X_{ij} – Tester mean ($X_{i.}$) = GCA + SCA.

where X_{ij} is the mean yield of the cross between i^{th} tester and j^{th} line, $X_{i.}$ is the mean yield of the ith tester and $X.j$ is the mean yield of jth line.

The best classification method was identified based on the breeding efficiency proposed by Fan et al. (2009) and modified by Badu-Apraku et al. (2016). The equation for estimating the breeding efficiency is as shown below according to Annor et al. (2020):

$$\text{Breeding efficency} = \frac{\frac{\text{HYinterHG}}{\text{TNInterHG}} \times 100 + \frac{\text{LYwithinHG}}{\text{TNwithinHG}} \times 100}{2}$$

Where, HYinterHG = number of high yielding inter-heterotic group hybrids, TNinterHG = total number of inter-heterotic group hybrids, LYwithinHG = number of low yielding within-heterotic group hybrids, TNwithinHG = total number of within heterotic group hybrids.

To compare the breeding efficiency, first all hybrids were divided into three groups on the basis of their grain yields according to Fan et al. (2009).

RESULTS AND DISCUSSION

Analysis of variance for grain yield combined across three locations showed that mean square due to locations, crosses, and crosses × locations were significant at P = 0.001 (Table 3). SCA, SCA × location and GCA x location were also significant at the 0.01 level and GCA at the 0.05 level. The significant difference for cross by location interaction suggested that the crosses behaved differently at the three locations, presupposing selection of specific hybrids that performed best in each of the three locations. Regarding phenological traits, mean square due to genotype and crosses were highly significant (p < 0.01) for days to tasseling (DT) and days to silking (DS). All the mean squares of the partition of cross or both GCA and SCA had significant differences for DT and DS. The relative importance of GCA to SCA ratio was greater than one for all the three traits (Table 3). The higher value of GCA to SCA ratio, which is a greater unit for GY, DT and DS, indicated that additive type of a gene action gave a higher contribution to genetic variation than non-additive type of the gene action (Table 3). Similarly, Berhanu (2009), and Arifin et al. (2018) reported significant variation among genotypes, GCA, SCA and the higher contribution of additive gene action for the genetic variation for grain yield. In contrast, Abiy (2017) reported no significant variation among genotypes for GCA and SCA but this other reported the higher proportion of GCA over for the research conducted at Ambo.

Heterotic grouping of inbred lines

Heterotic grouping designates broad classes in maize with diverse genetic base that are complimentary and result in expression of heterosis after crossing (Melchinger and Gumber, 1998). A hybrid breeding program needs to organize its germplasm into heterotic groups (Hallauer et al., 1998; Reif et al., 2007) to ease its operation and increase genetic gain. In heterotic grouping, if lines expressed negative SCA effect when crossed to a certain tester, this implies that both the line and the tester belong to the same heterotic group, while the reverse is true when the SCA effect is positive (Vasal et al., 1992).

In this study, 21 newly generated QPM lines (with unknown heterotic group) were crossed to two testers of known heterotic group: tester 1 (CML144, HGB) and tester 2 (CML159, HGA). To group the 21 lines, four methods were followed: SCA, SCA and hybrid mean jointly and hybrid index. The heterotic grouping of 21 inbred lines using different methods is presented in Table 4.

a) Using SCA: 11 of the 21 inbred line, viz. L1, L3, L4, L6, L8, L9, L10, L11, L13, L15 and L21 that showed positive SCA effect when crossed with CML159 (HGA) and negative SCA effect with CML144 (HGB) were classified as group "HGB" (Table 4). The other 10 inbred lines viz. L2, L5, L7, L12, L14, L16, L17, L18, L19 and L20 that showed positive SCA effect when crossed with CML144 (HGB) and exhibited negative SCA effect when crossed with CML159 (HGB) were classified under "HGA" (Table 4).

b) Based on SCA and hybrid mean jointly, nine lines were grouped under "HGA", eight lines under group "HGB" and the other four lines (L1, L2, L4 and L13) grouped as unknown.

c) Using hybrid index method, 19 lines were grouped under "HGB" and one line (L2) was grouped under both heterotic groups ("HGA" and "HGB"). The hybrid index method, fail to indicate the HG of L8 because its hybrid index value was higher than 1.05%. But it was grouped under "HGB" using its SCA and hybrid mean.

Considering the intersection of the four different methods (SCA, SCA and hybrid mean jointly, hybrid index and HSGCA), three lines (L3, L10 and L11) were grouped under "HGB". Considering four methods intersections, no lines were grouped under "HGA". Under three methods intersections (SCA, SCA and hybrid mean jointly, and hybrid index), four lines (L6, L9, L15 and L21) were grouped under "HGB". Taking the intersection of three grouping methods: SCA, joint SCA and hybrid mean and HSGCA methods, L12, L14, L16, L18, L19 and L20 fall under "HGA". L1, L4, L13 fall under "HGB" using SCA and hybrid index. L8 was grouped under "HGB" using SCA and joint SCA and hybrid mean methods and unknown under hybrid index and HSGCA methods. Considering two methods SCA and SCA and hybrid mean, three lines (L5, L7 and L17) fall under "HGA". L2 was grouped under "HGAB) based on hybrid index and HSGCA grouping methods. Using HSGCA grouping method, six lines (L12, L14, L16, L18, L19 and L20) were grouped under "HGA", three lines (L3, L10 and L11) under HGB, four lines (l1, L2, L4 and L13) under "HGAB" and for eight lines (L5, L6, L7, L8, L9, L15, L17 and L21) the group was unknown. The three lines (L3, L10 and L11), which are grouped under "HGB" by HSGCA, also fall under the same heterotic group "HGB" across the four heterotic grouping methods, consistently. This can be settled by applying molecular marker based heterotic grouping. Previous studies by Legesse et al. (2009) using population, Gudeta et al. (2015) and Abiy (2017) using inbred line testers, separated inbred lines into different heterotic groups based on grain yield SCA values only. However, this study used different methods to reduce the chance of misclassification of lines into heterotic groups. The failure of the three heterotic grouping methods except SCA methods to classify some inbred lines into the heterotic groups of the two testers, suggested that those inbred lines belonged to heterotic groups other

than those of the two testers.

Comparison of heterotic grouping methods for combined analysis

Methodically, the heterotic grouping following SCA vs SCA and hybrid mean matches very well. These two methods deviated only in grouping four of the lines. The four heterotic grouping methods were matched only three times for 21 lines. However, divergence of grouping following the hybrid index-based method diverted much from the grouping done following SCA, joint SCA and hybrid mean and HSGCA methods. Hybrid index-based grouping matched with SCA based method only in ten of the 21 times, implying that the classification following these two methods agrees only by about 48%. The disagreement between the hybrid index-based and the joint SCA and hybrid mean method was wider; only in seven of the 21 times (33%) their grouping matched each other. The hybrid index method also showed the widest disagreement with HSGCA only in 3 of 21 times (14%) for their grouping with each other. The hybrid index-based method matched with the other two methods only in identifying lines grouped under HGB. The hybrid index-based method nearly grouped 19 of the new QPM lines under HGB. The four heterotic grouping methods were matched only with the grouping of three of the lines from 21 lines. The three grouping methods (SCA, joint SCA and hybrid mean and HSGCA) were matched in grouping nine of 21 lines (43%); whereas, the other three methods in combination (SCA, joint SCA and hybrid mean and hybrid index) were matched in 6 of 21 lines (27%). The four lines of which their grouping was unknown by the SCA and hybrid index methods was shifted to HGAB by the HSGCA method. In the reverse, the seven lines which were assigned into their heterotic grouping even if their grouping was varied across each method (SCA, SCA and hybrid method, hybrid index) were not grouped under any heterotic grouping by the HSGCA method. This implies that using a single method might not be enough to group the maize lines clearly into their heterotic groups. Generally, based on the heterotic grouping result, HSGCA method appears to be the more stringent than the other methods, and is followed by the hybrid index method. This implied that the task of heterotic grouping of materials should be supported by other supported methods like molecular methods to get a clear classification of the germplasms.

Comparison of heterotic grouping methods across sites using the breeding efficiency

Under four heterotic grouping methods, the number of crosses considered for grain yield comparison was varied. The crosses which were from lines heterotically

Table 4. Grouping of 21 QPM lines using different grouping methods.

Line code	GCA	Tester 1 (CML144) (Group "B") Ecuador		Tester 2 (CML159) (Group "A") Kitale		HSGCA (t/ha)		Hybrid Index						Grouping using different methods			
								Tester 1 (CML144)			Tester 2 (CML159)				SCA and hybrid	Hybrid	
		Grain Yield	SCA	Grain Yield	SCA	CML144	CML159	Location 1	Location 2	Mean	Location 1	Location 2	Mean	SCA	Mean	Index	HSGCA
1	-1.51	3.90	-1.37	6.96	1.37	-2.88	-0.13	-0.41	-0.20	-0.31	2.77	0.70	1.73	B	un	B	AB
2	-1.86	6.24	1.32	3.91	-1.32	-0.54	-3.18	0.36	0.04	0.20	0.51	0.01	0.26	A	un	AB	AB
3	-0.48	5.61	-0.69	7.31	0.69	-1.17	0.22	0.47	0.19	0.33	3.79	0.60	2.19	B	B	B	B
4	-1.59	4.18	-1.01	6.51	1.01	-2.6	-0.58	-0.22	-0.09	-0.15	1.94	0.71	1.33	B	un	B	AB
5	0.66	7.79	0.35	7.39	-0.35	1.01	0.30	0.72	0.68	0.70	2.86	0.99	1.93	A	A	B	un
6	0.76	7.21	-0.34	8.20	0.34	0.43	1.11	0.97	0.09	0.53	3.40	0.92	2.16	B	B	B	un
7	1.35	9.13	1.00	7.44	-1.00	2.35	0.35	1.06	0.79	0.93	3.37	0.91	2.14	A	A	B	un
8	2.43*	9.09	-0.13	9.67	0.13	2.31	2.58	1.09	1.02	1.05	4.24	1.76	3.00	B	B	un	un
9	0.58	7.31	-0.06	7.73	0.06	0.53	0.64	1.35	0.12	0.74	3.20	0.74	1.97	B	B	B	un
10	0.19	6.58	-0.39	7.67	0.39	-0.20	0.58	0.71	0.13	0.42	3.05	1.11	2.08	B	B	B	B
11	-0.34	5.85	-0.59	7.33	0.59	-0.93	0.24	0.63	-0.26	0.19	2.98	0.98	1.98	B	B	B	B
12	0.11	7.09	0.19	7.01	-0.19	0.31	-0.08	0.76	0.29	0.52	2.24	0.62	1.43	A	A	B	A
13	-1.89	3.79	-1.10	6.30	1.10	-2.99	-0.79	-0.37	-0.09	-0.23	1.76	0.56	1.16	B	un	B	AB
14	-0.04	6.88	0.14	6.91	-0.14	0.10	-0.18	0.48	0.36	0.42	2.88	0.44	1.66	A	A	B	A
15	0.27	7.03	-0.02	7.39	0.02	0.25	0.30	1.01	-0.01	0.50	2.17	0.86	1.51	B	B	B	un
16	-0.28	6.93	0.42	6.40	-0.42	0.15	-0.69	0.59	0.36	0.48	2.38	0.50	1.44	A	A	B	A
17	0.27	7.28	0.23	7.13	-0.23	0.50	0.04	0.98	0.20	0.59	4.01	0.61	2.31	A	A	B	un
18	0.59	8.13	0.75	6.93	-0.75	1.35	-0.16	1.31	0.53	0.92	1.82	0.85	1.34	A	A	B	A
19	0.74	8.30	0.78	7.05	-0.78	1.52	-0.04	0.53	0.69	0.61	2.47	0.81	1.64	A	A	B	A
20	-0.28	7.10	0.59	6.23	-0.59	0.32	-0.86	0.72	0.41	0.56	2.36	0.44	1.40	A	A	B	A
21	0.31	7.01	-0.08	7.48	0.08	0.23	0.39	0.78	0.09	0.44	2.83	0.60	1.72	B	B	B	un
Mean		6.78		7.09													

un = unknown, location1 = Ambo, location2 = Arsi-Negele.

undefined were not considered under each heterotic grouping methods. Based on this, the total of 42, 32, 38 and 18 crosses were divided into three grain yield variation ranges under each of the heterotic grouping methods, respectively. Under each of the heterotic grouping methods, five crosses for each SCA and joint SCA and hybrid mean methods, one cross for hybrid index and two crosses under HSGCA method had high mean grain yield > 8 t/ha, which are assigned to grain yield Group 1. Under lowest yielding (grain yield Group 3) with mean grain yield < 6.9 t/ha, 12, 4, 24, and 5 crosseshad lowest grain yield for each grouping method in respective order; and the rest of 24, 22, 10 and 11 hybrids were assigned to grain yield Group 2 (grain yield in between 6.9 and 8.0 t/ha) (Table 5). Crosses were later divided into inter-group and within-group crosses based on the heterotic groups of the lines used in a cross formation. Inter-group crosses are the crosses formed using lines from two different heterotic groups and the within-group crosses are the crosses between lines within the same heterotic group.

Based on the breeding efficiency definition, the SCA method identified five, SCA and hybrid mean gave five, hybrid index one and HSGCA two high

Table 5. The number of hybrids with mean grain yield greater than 8 t/ha (15% greater than crosses mean (6.9 t/ha) (yield Group 1), between 6.9 and 8.0 t/ha (yield Group 2), smaller than the mean of crosses 6.9 t/ha (yield Group 3) for the four different heterotic group classification methods and breeding efficiency of each grouping methods, their comparison.

Yield group	Cross type	SCA	SCA and hybrid mean	Hybrid index	HSGCA
1	Inter-group	5	5	1	2
1	within-group	1	1	3	0
2	Inter-group	13	11	4	5
2	within-group	11	11	6	6
3	Inter-group	3	0	10	0
3	within-group	9	4	14	5
	No of crosses	**42**	**32**	**38**	**18**
	No of lines classified into "A" or "B"	21	17	19	9
	No of lines grouped into "A"	10	9	0	6
	No of lines not grouped clearly into "A "or "B"	0	4	2	12
	Breeding efficiency of grouping method (%)	**33.3**	**28.1**	**33.8**	**37.0**
Heterotic grouping methods	HSGCA breeding efficiency over (%)	11.0	31.6	9.6	-
	Hybrid Index breeding efficiency over (%)	1.3	20.1	-	-
	SCA breeding efficiency over (%)	-	18.5	-	-

Lines with AB and un grouped once were not considered for the number of lines to be counted under the lines grouped into A and B, the crosses formed using these lines were not taken into account in the determination of the number of crosses determination for within and inter grouped success based on grain yield.

yielding hybrids from a total of 21, 16, 15, and 7 inter-group crosses under each grouping method. Under each of the methods, the high top yielders among the hybrids were: SCA 23.8, joint SCA and hybrid mean 31.3, hybrid index 6.7% and HSGCA 28.6% (Table 5). The breeding efficiency value of each method was 33.3, 28.1, 33.8 and 37.0% for SCA, joint SCA and hybrid mean, hybrid index and HSGCA, respectively (Table 5). HSGCA was more efficient than the other three heterotic grouping methods (Table 5). Joint SCA and hybrid mean method was less efficient than the HSGCA, Hybrid index and SCA methods by 31.6, 20.1 and 18.5% in breeding efficiency, respectively. The highest efficient method (HSGCA) showed higher breeding efficiency by 31.6, 11.0 and 9.6% over joint SCA and hybrid mean, SCA and hybrid index methods, respectively. This highest efficiency of HSGCA heterotic grouping method compared with the other methods indicated that the HSGCA method was more effective in classifying the inbred lines into heterotic groups. This result confirmed that the HSGCA method was the most reliable for grouping the parental lines into heterotic groups for the development of productive and stable hybrids as well as synthetic varieties. Hence, crossing inbred lines from opposite HSGCA heterotic groups could result in more productive hybrids. Furthermore, the inbred lines classified into the same heterotic group by the HSGCA method could be recombined to form heterotic populations that could be improved through recurrent selection for extraction of inbred lines and synthetics for use in breeding programs in the tropics. The result of this study was in line with the findings of Fan et al. (2009), Badu-Apraku et al. (2015),

Amegbor et al. (2017) and Annor et al. (2020) who reported that the HSGCA was the most efficient for classifying inbred lines under drought, low N and optimal environments. The classification of the inbred lines into four heterotic groups based on the most efficient method, HSGCA indicated that there was a broad genetic diversity among the set of inbred lines used in the present study.

In order to maximize heterosis during hybrid variety development using these inbred lines, one parent should come from the inbred lines belonging to heterotic group "HGB" while the other parent should be from the inbred lines belonging to heterotic group "HGA". In the case of the development of synthetic varieties, inbred lines belonging to the same heterotic group should be used.

The overall percent grain yield of crosses from inter-grouped parents exceeded the mean performance of crosses from within-grouped parents by 22.4%. The highest and lowest percent grain yield from inter-grouped parents was 78.5 and -0.4, respectively. These highest and lowest values were obtained through the advantage of crossing L1xT2 over L1xT1 and L14xT1 over L14xT2, respectively (Table 6). The theory of higher chance to obtain higher grain yield from crosses formed from parents under different heterotic groups may not always be true because under this study, we found crosses formed from parents of inter-grouped lines provided inferior performance compared with crosses formed from within-group parents. For example, L14xT1 from inter-grouped parents had lower mean grain yield performance (less by 0.4%) compared with the same line (L14) crossed with T2 (crosses from within-group parents). This line (L14) showed a negative GCA effect with the value of

Table 6. Mean grain yield of crosses formed between inter-group, within-grouped parent materials and the percent grain yield advantage of crosses formed from inter-group parents over crosses from within-grouped parent based on SCA classification method.

Cross	Grain Yield	SCA	SCA and hybrid mean	Hybrid index	HSGCA	PYA
L1xT1	3.90	Within (B)	unknown	Within (B)	unknown (AB)	-
L1xT2	6.96	Inter-group (B)	unknown	Inter-group (B)	unknown (AB)	78.5
L2xT1	6.24	Inter-group (A)	unknown	unknown (AB)	unknown (AB)	59.6
L2xT2	3.91	Within (A)	unknown	unknown (AB)	unknown (AB)	-
L3xT1	5.61	Within (B)	Within (B)	Within (B)	Within (B)	-
L3xT2	7.31	Inter-group (B)	Inter-group (B)	Inter-group (B)	Inter-group (B)	30.3
L4xT1	4.18	Within (B)	unknown	Within (B)	unknown (AB)	-
L4xT2	6.51	Inter-group (B)	unknown	Inter-group (B)	unknown (AB)	55.7
L5xT1	7.79	Inter-group (A)	Inter-group (A)	Within (B)	unknown	5.4
L5xT2	7.39	Within (A)	Within (A)	Inter-group (B)	unknown	-
L6xT1	7.21	Within (B)	Within (B)	Within (B)	unknown	-
L6xT2	8.20	Inter-group (B)	Inter-group (B)	Inter-group (B)	unknown	13.7
L7xT1	9.13	Inter-group (A)	Inter-group (A)	Within (B)	unknown	22.7
L7xT2	7.44	Within (A)	Within (A)	Inter-group (B)	unknown	-
L8xT1	9.09	Within (B)	Within (B)	unknown	unknown	-
L8xT2	9.67	Inter-group (B)	Inter-group (B)	unknown	unknown	6.4
L9xT1	7.31	Within (B)	Within (B)	Within (B)	unknown	-
L9xT2	7.73	Inter-group (B)	Inter-group (B)	Inter-group (B)	unknown	5.7
L10xT1	6.58	Within (B)	Within (B)	Within (B)	Within (B)	-
L10xT2	7.67	Inter-group (B)	Inter-group (B)	Inter-group (B)	Inter-group (B)	16.6
L11xT1	5.85	Within (B)	Within (B)	Within (B)	Within (B)	-
L11xT2	7.33	Inter-group (B)	Inter-group (B)	Inter-group (B)	Inter-group (B)	25.3
L12xT1	7.09	Inter-group (A)	Inter-group (A)	Within (B)	Inter-group (A)	1.1
L12xT2	7.01	Within (A)	Within (A)	Inter-group (B)	Within (A)	-
L13xT1	3.79	Within (B)	unknown	Within (B)	unknown (AB)	-
L13xT2	6.30	Inter-group (B)	unknown	Inter-group (B)	unknown (AB)	66.2
L14xT1	6.88	Inter-group (A)	Inter-group (A)	Within (B)	Inter-group (A)	-0.4
L14xT2	6.91	Within (A)	Within (A)	Inter-group (B)	Within (A)	-
L15xT1	7.03	Within (B)	Within (B)	Within (B)	unknown	-
L15xT2	7.39	Inter-group (B)	Inter-group (B)	Inter-group (B)	unknown	5.1
L16xT1	6.93	Inter-group (A)	Inter-group (A)	Within (B)	Inter-group (A)	8.3
L16xT2	6.40	Within (A)	Within (A)	Inter-group (B)	Within (A)	-
L17xT1	7.28	Inter-group (A)	Inter-group (A)	Within (B)	unknown	13.8
L17xT2	7.13	Within (A)	Within (A)	Inter-group (B)	unknown	-
L18xT1	8.13	Inter-group (A)	Inter-group (A)	Within (B)	Inter-group (A)	17.3
L18xT2	6.93	Within (A)	Within (A)	Inter-group (B)	Within (A)	-
L19xT1	8.30	Inter-group (A)	Inter-group (A)	Within (B)	Inter-group (A)	17.7
L19xT2	7.05	Within (A)	Within (A)	Inter-group (B)	Within (A)	-
L20xT1	7.10	Inter-group (A)	Inter-group (A)	Within (B)	Inter-group (A)	14
L20xT2	6.23	Within (A)	Within (A)	Inter-group (B)	Within (A)	-
L21xT1	7.01	Within (B)	Within (B)	Within (B)	unknown	-
L21xT2	7.48	Inter-group (B)	Inter-group (B)	Inter-group (B)	unknown	6.7
Mean						22.4
Minimum						-0.4
Maximum						78.5

Letters in bracket indicates the heterotic group of the new lines in different heterotic grouping methods under this study. T1 and T2 are heterotic group of B and A, respectively which are grouped before this study. PYA= Percent grain yield advantage of hybrids form inter group over within-group parents.

-0.04) (Table 4) and T1 which is grouped under the opposite side of the line (L14) based on the three grouping methods had negative GCA effect with value of 0.15. However, T2 had positive GCA effect with the value of 0.15. Hence, the inferior performance of the hybrid (L14 x T1) developed from the within-grouped parents might be due to the negative GCA effect, which showed both the line and tester found in different heterotic group. This result suggested that, to obtain high heterosis, combining germplasms from different heterotic groups might not guarantee that it will yield a high performance. Therefore, breeders should be careful while choosing germplasms and making crosses. Based on this, the breeders need to see the GCA effect of each germplasm in addition to their heterotic group while developing hybrids, OPV and other maize varieties. Annor et al. (2020) also suggested that to get the maximum heterosis, the breeding program should use inbred lines with significant positive GCA effects for grain yield; and should be classified into opposing heterotic groups by using the HSGCA method for hybrid or synthetic variety development. There was also a cross formed from within-group parents that had relative nearest performance with a cross formed from inter-group parents. For example, L8xT1 and L12xT2, which were the hybrids developed from the parents found under the within-group category. These two lines (L8 and L12) were grouped under the tester group (within) based on two heterotic grouping methods (SCA and joint SCA and hybrid mean) for both lines. L12 was grouped within the group of "HGA" by the HSGCA method; but line (L8) was unknown based on the other two methods (Hybrid index and HSGCA). The L12 group assignment was unknown by the hybrid index method. This hybrid (L8xT1) from within-grouped parents showed relative inferior yield less by 6.4% compared with grain yield obtained from the other hybrid (L8 x T2) developed from the same female parent, which is crossed with the other tester found in the other heterotic group (Table 6). The L12xT2 cross had lower mean grain yield by 1.1% developed from within-grouped parents compared with the cross (L12 x T1) developed using inter-grouped parents. The relative smaller difference of the cross performance from hybrids developed using inter-grouped parents (L12 x T1) compared to the hybrid (L12 x T2) formed using within grouped parents might be due to the negative GCA effect of tester (T1). It may be that the higher grain yield obtained from L8 crossed with both testers in different groups was due to the highest GCA effect (2.43) showed by the new line (L8) compared with other new lines and testers tested under this study (Table 4). This indicates that, there is the possibility to obtain higher grain yield from crosses formed using within grouped materials. Similarly, Fan et al. (2009) reported the existence of the chance to obtain crosses with high grain yield from within grouped maize in bred lines. On the other hand, this result tells us researchers should see the other way to use these materials for OPV varieties development because development of OPV is also the other good option to exploit germplasm in a different way. This is because OPVs are also helpful for the final users in reducing the seed cost that would incur if hybrid seed was purchased.

From 21 crosses developed from inter-grouped parents, 12 of them were obtained from 12 lines each crossed with T2. From these 12 crosses, seven of them were developed from lines and tester (T2) which had positive GCA effect; whereas, the remaining five crosses were from five lines with negative GCA effect with T2. From the other 21 crosses developed from inter-grouped parents, nine of them were developed from nine lines each crossed with tester 1 (T1). From nine crosses developed from lines with T1, four lines had negative GCA effect and the other five crosses were from lines with positive GCA effect crossed with T1, which had negative GCA.

The highest mean performance of hybrids (51.2%) was obtained from inter-grouped parents over the hybrids from within-grouped parents, which were developed from the female parents (lines) with negative GCA effect crossed with male (tester with positive GCA effect) (Table 7). The second higher value (20.4%) was obtained from the hybrids developed from female and male parents both with negative GCA effect. Even if the percent mean grain yield advantage obtained from lines which had positive, the GCA effect crossed with tester with positive GCA effect was the lowest (9%) (Table 7), the mean performance of the hybrids was higher while considering individual hybrids for grain yield (Table 6). This might be due to the relative higher GCA effect in magnitude from the female parent side in addition to its GCA effect to the positive side.

Regarding the direct grain yield comparison considering only the hybrids formed from the inter-grouped parents, or excluding the 21 hybrids developed using within-grouped parents, six hybrids formed from six lines which had positive GCA effect crossed with the tester (T2) with positive GCA having mean grain yield of 8.0 t/ha. The other set of six hybrids developed from six lines with positive GCA, each crossed with a tester (T1) with negative GCA, also had 8.0 t/ha performance. The other five hybrids generated by crossing five lines, which showed negative GCA effect and tester (T2) with positive GCA effect, had a mean value of 6.9 t/ha for GY. The other set of four hybrids formed from lines, which had a negative GCA effect crossed with tester (T1) with negative GCA, had the mean GY value of 6.8 t/ha. This direct mean GY performance comparison results of hybrids, formed from inter-grouped parents, showed that to get hybrids with good performance for grain yield, either both male and female parents of the hybrid should have positive significant GCA effect or at least the female parent should be with positive GCA effect. In addition, to realize this result, the basic criterion of both parents should come from different heterotic pool. Because as we

Table 7. The percent mean performance of hybrids calculated from the percent mean advantage of the hybrids developed from inter grouped parents over the hybrid developed from within-grouped parents considering the GCA effect and direct mean of the hybrid formed from inter-grouped parents without considering the hybrids of the within-grouped parents for grain yield.

Crosses formed from inter-grouped parents grouped based on GCA effect of parents involved in cross formation	Direct mean GY (t/ha)	Mean GY Performance (%)
Lines (6) x Tester (T2) both with positive GCA effect	8.0	9.0
Lines (5) x Tester (T2) with negative x positive GCA effect	6.9	51.2
Lines (6) x Tester (T1) with positive x negative GCA effect	8.0	13.0
Lines (4) x Tester (T1) both with negative GCA effect	6.8	20.4

The direction of GCA effect is mentioned in line and tester respective order. The number in bracket indicates the number of lines crossed with tester to form the single crosses.

see the hybrids formed from six lines with positive GCA and one tester with positive GCA showed equal GY (8.0t/ha), compared with the other set of six hybrids developed from lines which had positive GCA effect crossed with the other one tester with negative GCA effect (Table 7). Similarly, Annor et al. (2020) also suggested that, to get the hybrids or OPV with high heterosis for grain yield, the breeding program should use inbred lines, which had significant positive GCA effect; and the lines should be classified under an opposing heterotic group. This conclusion is supported by the lower mean GY performance obtained from hybrids developed from female parents, which had negative GCA effect when crossed with male parents with negative and positive GCA effects (Table 7).

Conclusion

For grouping new maize germplasms into a heterotic group, it is advisable to consider a range of different methods. The variable heterotic grouping of the 21 newly developed QPM lines in this study indicated that different heterotic methods have different efficiencies of grouping the germplasms. The HSGCA method was more efficient than the other heterotic grouping methods. The Joint SCA and hybrid mean method was less efficient, followed by the SCA method. The HSGCA method looks more seriously limited than the others, because a high number of the lines failed under unknown conditions for the heterotic grouping. To make the grouping of materials clearer, use of molecular methods might be more productive. Moreover, classifying new germplasms into their group based on the data collected from field experiments imply that integrated use of different methods can increase the chances of separating the germplasm into their heterotic group. The result of this study indicated the possibility of getting a high yielding hybrid by crossing parents from the same heterotic group. In so doing, the breeder should take care to make crosses and evaluate them. The breeder should also consider the parents, which are found within the same group, and had good GCA for OPV variety development.

The results indicate that, to get the high grain yield from hybrids or any other kinds of varieties, considering the GCA effect for each germplasm has a significant role in addition to their heterotic group. The direct mean GY performance comparison for hybrids developed from inter-grouped parents showed that, to get hybrids with good performance, either both male and female parents of the hybrid should have positive GCA; or at least the female parent should be with positive GCA effect in addition to fulfilling the basic criterion of both parents coming from a different heterotic pool, mostly.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Ethics approval and consent to participate

The researchers have obtained permission from funding

institution CIMMYT and EIAR. Accordingly, the information under this article had been developed in collaboration with CIMMYT, Ethiopian Institute of Agriculture Research investigators and university instructor.

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African Journal of Plant Science

Full Length Research Paper

Combining ability of quality protein maize inbred lines under low and optimum soil nitrogen environments in Ethiopia

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Maize (*Zea mays* L.) is the most important cereal crop on which many smallholder farmers in sub-Sahara Africa (SSA) depend on as a sole source of calories, proteins, and vitamins. However, conventional maize is deficient in two essential amino acids, lysine, and tryptophan. Thereafter, Quality Protein Maize (QPM) developed to combat protein malnutrition. In SSA low soil nitrogen is also the most limiting factor in maize production and productivity. To determine the combining ability of QPM inbred lines for grain yield and other agronomic traits under low and optimum N environments, 121 genotypes generated using 11 QPM inbred lines in a complete diallel mating design with 5 checks were evaluated under both low and optimum N environments at 3 locations in the 2018 cropping season. Significant differences were observed among the genotypes for major characters under both low and optimum N environments. Under low and optimum N environments, non-additive gene actions were more important than additive gene action for grain yield, number of ears per plant, plant and ear height, ear length and diameter. Under low N environments, more contributions of reciprocal effects than GCA effects were observed for number of ears per plant, plant aspect, ear diameter. Parental lines TL156579, TL156583, and TL148288 were considered as a good general combiner for grain yield under low N environments. Parental line TL156612 showed higher positive GCA effects when used as female while parental line TL156612 showed higher negative GCA effects when used as a male parent for grain yield under low N environments.

Key words: Complete diallel, general combining ability, low soil nitrogen, lysine, optimum soil nitrogen, quality Protein maize, reciprocal effect, tryptophan.

INTRODUCTION

Maize is one of the most important food crops and is widely grown all over the world on which the majority of developing countries depend on as a sole source of calories, proteins, vitamins and minerals (Babu and Prasanna, 2014; Ranum et al., 2014; Badu-Apraku et al., 2015b; Farfan et al., 2015). However, in conventional maize (normal maize), the concentration of lysine and tryptophan are low, which are more important for both

humans and monogastric animals. In countries where maize is consumed as the primary or sole source of protein, malnutrition is common due to the lack of essential amino acids in maize kernel endosperm lysine and tryptophan.

Nitrogen (N) is an important nutrient for maize yield. It is a constituent of amino acids, proteins, hormones, and chlorophyll. Reduced seed production, leaf chlorosis, and reduced plant root branching occur due to a low soil N supply. Maize yield is more dependent on the availability of soil N, which is one of the major limiting factors in sub-Saharan Africa (SSA). Even if better yield of maize is dependent on the amount of N fertilizer supplied to the crop, many developing countries' farmers apply N fertilizer at sub-optimal levels due to the high cost of fertilizer, which leads to low yield. In addition to the high cost of N fertilizer, maize can only uptake 30% of applied N; and then, the rest 70% of the N applied to maize is lost due to different mechanisms like leaching, de-nitrification and surface runoff from the soil, which causes soil acidification and affect both plant and animal biodiversity (Erisman et al., 2013). To address both economic and environmental problems related to N fertilizer, development of genotypes that yield better under low N supply by improving N-use efficiency (NUE) of maize genotypes is the most important strategy for sustainable agriculture (Gelli et al., 2014). NUE is the capacity of the crop to produce biomass and grain yield per unit of available N in the soil (Moll et al., 1982); and it is the product of N-uptake efficiency and N-utilization efficiency (Banzinger et al., 2000). Improving NUE of a crop through breeding methods can improve crop yield.

In maize breeding strategy, information on combining ability, heterosis, and heterotic groups are the most important factors for the development of high-yielding maize hybrids. A heterotic group is a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups. Analysis of combining ability for inbred lines indicates the type of gene action controlling quantitative characters, which are used for genetic diversity analysis, inbred selection, heterotic grouping, estimation of heterosis, and hybrid development (Fan et al., 2009; Hallauer et al., 2010). The advantage of grouping germplasms into divergent heterotic groups is advantageous because of higher mean heterosis and hybrid performance and a reduced specific combining ability (SCA) variance and a lower atio of SCA to GCA variance. Thus, early testing becomes more effective and superior hybrids can be identified and selected mainly based on their prediction from GCA effects (Reif et al., 2005). The most effective and direct way to solve the maize yield gap is to breed varieties with high yielding potential and wide adaptability. About 52.9% of maize yield increment was attributed to development of new varieties, and the rate of improvement was 89.1 kg

ha[-1] per year (Wang et al., 2009), which is attributed to heterosis.

Breeding strategies for maize under stress environments have been influenced by the breeding strategy for maize under non-stress environments, in which selection under high yielding environments may not be effective selection to increases yield under low yielding environments. This is due to the finding that plant traits that are less important under non-stressed environments, become more important for yield under stressed environments (Banzinger et al., 2000). Thus, the selection of germplasm under both low and optimum N environments can increase the chance to develop low N tolerant maize hybrids. Some organizations have developed QPM inbred lines with tolerances to low N; however, their adoptions in Sub-Saharan Africa countries were very low (Badaway, 2013).

According to the report of agricultural sample survey of Ethiopian Central Statistical Agency (CSA, 2021) for 2020/2021 main cropping season, out of 10.5 million hectares (about 81.19%) of land covered by cereal crops, maize covered 2.5 million hectares (about 19.46%) after teff 2.9 million hectares (about 22.56%). About 88% of maize produced in Ethiopia is consumed as food, both as green and dry grain (Tsedeke et al., 2015). However, the productivity of maize in Ethiopia during this growing season was about 4.2 t ha-1, which is far below the world average yield per hectare (FAOSTAT, 2019). Low productivity of maize in Ethiopia is associated with several limiting factors including, lack of high yielding and stable improved genotypes, drought, reduction in soil N fertility, inappropriate agronomic practices, low adoption of improved agricultural technologies including varieties by farming communities, foliar diseases, weeds, and high cost of inorganic fertilizer. Therefore, the objective of this study was to estimate the combining ability effects of QPM inbred lines under both low and optimum N environments for grain yield and other agronomic traits.

MATERIALS AND METHODS

Plant materials

Eleven inbred lines were selected based on their tolerance to a low N environment and high tryptophan content from a panel of 74 QPM inbred lines obtained from the maize breeding program at CIMMYT-Zimbabwe; and these were used in this study (Table 1). A complete diallel mating design was made among these 11 QPM inbred lines at Haramaya University research site (Raare), to generate 121 genotypes. The 121 genotypes with two non-QPM hybrid maize (BH547 and BH546) and three QPM hybrid (BHQPY548, BHQPY545 and MHQ138) checks were used in this study.

Experimental sites and design

The field experiments were conducted at three research sites (Raare, Fedis and Dire-Dawa) of Haramaya University, Ethiopia.

Table 1. List of quality protein maize parental lines used for complete diallel cross.

Code	Name	Pedigree	Source	Characteristics	Tryptophan in protein (%)
L1	TL156579	([NIP25-100-1-1-B-1-B*5/[GQL5/[GQL5/CML202]F2- 3sx]-11-1-3-2-B*4]-3/CML395IR)-BBB(IR)-1-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.11
L2	TL156583	(CML312IR/[[[CLQRCWQ83/CML312SR//CML312SR]/ CML312SR]-26-B/(CLQRCWQ50/CML312SR)-2-2-1-BB]- BB)F2-B-7-2-2-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.74
L3	TL148288	ObatanpaSR-278-B*4-2-B-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.89
L4	TL156591	([GQL5/[GQL5/CML202]F2-3sx]-11-4-1-1-BBB/([NIP25- 230-1-3-B-2-BBB/CZL066]-1/CML444IR)-B)F2-B-12-2-3-2-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.10
L5	TL148287	[VP047/CML511]-25-B-2-BBB-6-B-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.82
L6	TL116960	[Syn01E2/CML511]-16-B-2-BBB-1-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.79
L7	TL155932	([NAW5867/P49SR(S2#)//NAW5867]F#-48-2-2-B*4/(CLQRCWQ50/CML312SR)-2-2-1-BBB)F2-B-9-5-1-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.73
L8	TL147078	(CML197/(CML197/(CLQRCWQ50/CML312SR)-2-2-1- BBB)F2)-B-B-36-2-B	CIMMYT-ZIM	Tolerant to low N	0.11
L9	TL156612	[[CML506/[CML205/CML176]-B-2-1-1-B]F2- 1/[CML144/[CML144/CML395]F2-8sx]-1-2-3-2-B*4]-24-B-2-BBB-3-B-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.68
L10	TL155976	([CML144/[CML144/CML391]F2-8sx]-1-2-3-2-B*4-1-B/[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BB)F2-B-16-6-3-B-B-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.78
L11	VL05128	WWO1408-1-1-2-B*4-#-B-B-B	CIMMYT-ZIM	Tolerant to low N	0.68

The university is located in the Eastern part of the country, which represents the mid-altitude maize growing ecology of the country. The Raare research site lies between 9º40' N latitude and 42º03' E longitude at an altitude of 2020 m.a.s.l with an annual rainfall of 820 mm and average maximum and minimum temperature of 24.4ºC and 9.8ºC, respectively. Fedis lies between 8º49' N latitude and 42º00' E longitude at an altitude of 2,118 m.a.s.l with an annual rainfall of 750 mm and average maximum and minimum temperature of 30.4ºC and 13.6ºC, respectively. Dire-Dawa research site lies between 9º97' N latitude and 42º53' E longitude at an altitude of 1180 m.a.s.l with an annual rainfall of 637 mm and average maximum and minimum temperature of 33.0ºC and 18.9ºC, respectively. One hundred and twenty one hybrids generated from the complete diallel cross with 5 hybrid checks (126 genotypes) were arranged in 18 x 7 alpha lattice (0, 1) design with 2 replications in each location under both low N (30 kg ha^{-1}) and optimum N (100 kg N ha^{-1}) environments.

Depletion of N in experimental sites and soil analysis

Depletion of nitrogen in the soil was done by planting high population densities of maize plants without applying nitrogen fertilizer for two years and removing all biomass after harvest from the fields. The soil chemical properties of the sites were determined at two soil sample depths (0-30 cm and 30-60 cm) before planting at Haramaya university soil laboratory. From soil laboratory analysis results, the total N content at Raare was 0.070% and 0.056%, 0.098% and 0.084% at Fedis and 0.112% and 0.098% at Dire-Dawa for 0-30 cm and 30-60 cm soil depth, respectively; which were considered as low soil N at all locations.

Low soil N management

The field experiment was divided into two blocks (Low and optimum N blocks). Two seeds per hill on a one-row length of 4 m at 30 cm distance between plants and 75 cm between rows were planted. The plants were thinned to one plant per hill to give 44,444 plant populations per hectare. The recommended rate of N fertilizer for maize (100 kg N ha^{-1}) was applied in the form of Urea for optimum N environment and 30 kg N ha^{-1} for low N environment. Low N environment of 30 kg N ha^{-1} was used by considering sub-Saharan Africa countries' farmers apply 20-30% fertilizer below recommended rate because of the high cost of fertilizer and low access to fertilizer for their crops. In addition to N fertilizer, both experiments were supplied with 100 kg P$_2$O$_5$ ha^{-1} in the form of tricalcium phosphate. Split N fertilizer application was done on each plot for both low and optimum N environments. Under a low N environment, a mixture of 18 kg N ha^{-1} and 46 kg P$_2$O$_5$ ha^{-1} were applied at planting and 12 kg N ha^{-1}, 45 days after planting. Under optimum N environment, a mixture of 50 kg N ha^{-1} in the form of Urea and 46 P$_2$O$_5$ ha^{-1} were applied at planting, and 50 kg N ha^{-1} was applied in the form of Urea 45 days after planting. Weeds were removed by hand weeding at each location.

Data collection

Based on the International Board for Plant Genetic Resources (IBPGR) descriptor list (IBPGR, 1991) phenotypic characters were measured from five randomly selected plants except for days to silking and anthesis, anthesis-silking interval, plant aspect, ear aspect, stay green characteristics which were recorded on a pilot basis (Table 2).

Data analysis

Analysis of variance per environment was performed on plot means for grain yield and other agronomic traits using PROC GLM procedure of SAS software, version 9.4 (SAS Institute, 2012).

Combining ability analysis

Analysis of variance for a complete diallel cross excluding

checks was done using AGD-R (Analysis of Genetic Designs with R) version 5.0 (Rodríguez et al., 2018). Griffing's method I (parents, F_1s, and F_1's reciprocals were included) and model I (fixed effect) of diallel analysis was used for computing GCA effects of the parents, SCA and reciprocal effects of the crosses. Analysis of variance was done for the individual environment, and combined analyses of variance over low N, Optimum N and across all N environments were done for those traits showed significant genotypes mean squares in an individual environment. The mean squares for ENV and crosses were tested against the mean squares for ENV x crosses as mean square of error, while ENV x crosses interactions mean squares were tested against pooled error. The significance of GCA, SCA and reciprocal source of variations were tested against F-tests while the significance of GCA, SCA and reciprocal effects were tested against t-test, with the standard error of GCA, SCA and reciprocal effects (Dabholkar, 1999; Griffing, 1956). The linear model for combining ability analysis for Griffing's method-I of the complete diallel cross for a single environment was computed as (Griffing, 1956):

$$Y_{ij} = \mu + g_i + g_j + r_{ij} + s_{ij} + \frac{1}{bc} \sum_k \sum_l e_{ijkl}$$

The linear model for combining ability analysis for the method I of the complete diallel cross for across environments were computed as (Yao et al., 2013):

$$Y_{ijkl} = \mu + \alpha_l + b_{kl} + v_{ij} + (\alpha v)_{ijl} + e_{ijkl}$$
$$v_{ij} = g_i + g_j + s_{ij} + r_{ij}$$
$$r_{ij} = m_i + m_j + n_{ij}$$

Where, Y_{ijkl} is observed trait value from each environmental unit, μ is the population mean, α_l is location (environment) effects, b_{kl} is block or replication effect within each location, v_{ij} is the F_1 hybrid effect $= g_i + g_j + s_{ij}$ (where $g_i = $ GCA effect for the ith parent with

$$\sum_i g_i = 0$$

, $g_j = $ GCA effect of jth parent with

$$\sum_j g_j = 0$$

S_{ij} is the SCA for the ij^{th} F_1 hybrid with $S_{ij} = S_{ji}$ and

$$\sum_{i=j} S_{ij} = 0$$

, $(\alpha v)_{ijl}$ is interaction effect between ij^{th} F_1 hybrid and location, e_{ijkl} is a random residual effect, r_{ij} is Reciprocal of ij^{th} cross with $r_{ij} = -r_{ji}$, m_i is maternal effect (MAT) of ith parental line with

$$\sum_i m_i = 0$$

, m_j is MAT of jth parental line with

$$\sum_j m_j = 0$$

, n_{ij} is a nonmaternal effect (NMAT) of the cross between the ith and j^{th} parental lines with

$$\sum_{i=j} n_{ij} = 0$$

'$n_{ij} = n_{ji}$ and e_{ijkl} is an environmental effect associated with ijklth individual observation.

The relative importance of combining ability

The ratio of combining ability variance components determines the type of gene action involved in the expression of traits and allows inferences about the optimum allocation of resources in hybrid breeding. The closer this ratio is to one, the greater the prediction of GCA alone, whereas a ratio with a value less than one shows SCA action. However, because in many cases only a few parents are used in crosses, the magnitude of GCA, SCA and reciprocal effects evaluated using the ratio of their sum of squares to the total sum of squares for crosses where they were involved were used to determine the importance of combining ability.

RESULTS

Combined analysis of variances and importance of combining ability effects of inbred lines under low, optimum, and across N environments

Under combined low N environments, environmental effects were significant for all traits except the plant aspect (Table 3). Mean squares for GCA and SCA were highly significant for all traits and E x crosses and E x GCA interactions were highly significant for all traits; while reciprocal and non-maternal effects were significant for all traits except the ear aspect. Mean squares for maternal effects were highly significant for grain yield, days to anthesis and silking, plant height, ear height, ear length and ear diameter. E x SCA and E x reciprocal interactions were highly significant for all traits except plant aspect and ear aspect. E x maternal interactions were significant for grain yield, days to anthesis, number of ears per plant, plant height, ear height and stay green characteristics. The mean square of E x non-maternal interaction was significant for all traits except days to anthesis and plant aspects.

Under combined low N environments, the percent of sum of squares for GCA ranged from 8.69% for ear length to 46.95% for days to silking; while percent of sum of squares for SCA ranged from 39.55% for days to silking to 73.54% for ear length. Percent of sum of squares for reciprocal effects ranged from 5.11% for ear height to 34.34% for number of ears per plant (Table 4). High GCA sum of squares accounted for days to anthesis (45.95%), days to silking (46.95%) and stay green characteristics (41.61%); while low GCA sum of squares were detected for number of ears per plant (13.02%), plant aspect (12.5%), ear length (8.65%) and ear diameter (10.88%) of a total variation among crosses. A higher SCA sum of squares than for the GCA sum of squares were recorded for all traits; while a lower reciprocal sum of squares than for the SCA sum of

Table 2. List of characters.

Trait name	Acronym	Trait description
Grain yield (12.5% moisture) (t ha-1)	GY	Shelled grain weight at harvest adjusted to 12.5% moisture content for low N, and the weight of all ears per plot at harvest adjusted to 12.5% for optimum N
Days to 50% anthesis (days)	DTA	Number of days from planting to when pollens have shed 50% of the plants
Days to 50% silking (days)	DTS	Number of days from planting to when silks have emerged 50% of the plants
Anthesis- silking interval (days)	ASI	The difference between days to 50% silking and 50% anthesis
Plant aspect (1 - 5)	PASP	Plant aspect was recorded on a scale of 1 - 5 based on general plant type (plant and ear height), uniformity of plants, disease, and insect damage, and lodging was 1 good plant type and 5 poor plant type
Ear aspect (1 - 5)	EASP	Ear aspect could be recorded based on a scale of 1 - 5, where 1 means good ears (consider ear size, husk cover, ear rot, total numbers of ear harvested per plot, ear damage caused by insects or/and diseases and other acceptable characters), while 5 means poor ear with undesirable characters.
Number of ears per plant (count)	NEPP	It counted as several ears with at least one fully developed grain divided by the number of harvested plants
Plant height (cm)	PLH	From the ground level to the base of the first tassel branch. After the milk stage
Ear height (cm)	ERH	From ground level to the node bearing the uppermost ear. After the milk stage
Ear length (cm)	ERL	the length of the uppermost ears of sampled plants in cm after de-husking.
Ear diameter (cm)	ERD	The diameter of the sample plant in cm after de-husking in the middle of the cob
Stay-green Characteristics (1 - 9)	SG	Stay green scored four weeks after silking on a scale of 1-9 based on the % of dead leaf area below the ear

Stay green score: 1 = 0-10% dead leaf area; 2 = 10-20% dead leaf area; 3 = 20-30% dead leaf area; 4 = 30-40% dead leaf area; 5 = 40-50% dead leaf area; 6 = 50-60% dead leaf area; 7 = 60-70% dead leaf area; 8 = 70-80% dead leaf area; 9 = 80-90% dead leaf area (Banzinger et al., 2000).

squares were recorded for all traits under low N environments.

Under optimum N environments, mean squares for environments were highly significant for all traits (Table 4). Variances of GCA, SCA, E x GCA, and E x SCA interactions were significant for all traits; but, E x maternal interactions were only significant for grain yield, plant height, and ear height. Ex non-maternal interaction was only significant for grain yield, number of ears per plant, plant height, and ear height. Mean squares for reciprocal effects were significant for all traits, except days to anthesis and silking; while E x reciprocal interactions were highly significant for grain yield, number of ears per plant, plant height and ear height. Maternal and Non-Maternal mean squares were highly significant for all traits, except days to anthesis and silking and ear aspect.

GCA sum of squares under optimum N environments ranged from 11.87% for plant aspect to 54.92% for days to silking; while sum of squares for SCA ranged from 36.86% for days to silking to 75.60% for plant aspect. Percent of sum of squares for reciprocal effects ranged from 8.22% for days to silking to 24.79% for number of ears per plant (Table 4). High GCA sum of squares accounted for days to anthesis (53.99%), days to silking (54.92%) and ear height (40.68%) of a total variation among crosses. Small SCA sum of squares than for the GCA sum of squares were accounted for days to anthesis and silking of the total variation among crosses. A greater reciprocal sum of squares than for the GCA sum of squares were accounted for number of ears per plant and plant aspect of the total variation among the crosses under optimum N environments.

Combined mean squares for environmental effects were significant for all traits across all environments. Highly significant differences were also found among crosses for all measured traits

(Table 5). Mean squares of GCA, SCA, reciprocal, non-maternal, and E x Crosses, E x GCA, and E x SCA interactions were highly significant for all traits. E x reciprocal and E x maternal interaction mean squares were significant for all traits except for days to anthesis and silking and ear aspect; while E x non-maternal interaction was highly significant for all traits except days to anthesis, days to silking and plant aspect.

Across all N environments, the GCA sum of squares ranged from 12.78% for plant aspect to 56.22% for days to silking; while SCA sum of squares ranged from 34.19% for days to silking to 75.97% for plant aspect. The reciprocal sum of squares ranged from 5.15% for grain yield to 24.60% for number of ears per plant (Table 5). A higher contribution of GCA effects was found for days to silking (56.22%), and lower contribution for plant aspect (12.78%); while there was a high

Table 3. Combined mean squares of traits for 11 QPM inbred lines evaluated under low N environments at Raare, Fedis, and Dire-Dawa in 2018 cropping season.

Sources of variation	df	Mean squares										
		GY	DTA	DTS	NEPP	PASP	EASP	PLH	ERH	ERL	ERD	SG
Environment (E)	2	19.36**	27512.60**	27548.91**	0.89**	0.24	9.49**	41120.37**	40869.22**	9.64**	0.98**	59.31**
Crosses	120	5.28**	30.84**	31.02**	0.03**	1.09**	1.58**	1793.02**	773.33**	11.34**	0.90**	1.84**
GCA	10	13.55**	170.04**	174.75**	0.05**	1.63**	5.33**	6038.36**	3141.81**	11.83**	1.17**	9.19**
SCA	55	8.38**	27.23**	26.76**	0.03**	1.67**	2.11**	2641.63**	1029.74**	18.20**	1.28**	1.66**
Reciprocal	55	0.68**	9.14**	9.14**	0.02**	0.40**	0.37	172.53**	86.29**	4.40**	0.47**	0.69**
Maternal	10	1.13**	16.61**	16.31**	0.02	0.33	0.35	193.74**	95.00**	2.96**	0.26**	0.64
Nonmaternal	45	0.58**	7.48**	7.55**	0.02*	0.42**	0.38	167.82**	84.35**	4.72**	0.52**	0.70**
E x Crosses	240	1.20**	5.67**	5.79**	0.02**	0.26**	0.40*	140.59**	110.12**	1.23**	0.22**	1.13**
E x GCA	20	3.58**	21.37**	20.29**	0.03**	0.67**	0.96**	242.74**	215.92**	1.30**	0.34**	3.98**
E x SCA	110	1.47**	5.11**	5.31**	0.02**	0.23	0.35	183.58**	150.27**	1.23**	0.27**	1.19**
E x Reciprocal	110	0.48**	3.38*	3.63*	0.02**	0.22	0.35	79.02**	50.74**	1.22**	0.14**	0.56**
E x Maternal	20	0.60**	2.31*	2.14	0.03**	0.22	0.19	116.00**	78.46**	0.74	0.09	0.63**
E x Nonmaternal	90	0.46**	3.62	3.97**	0.02**	0.21	0.39*	70.80**	44.58**	1.33**	0.15**	0.55**
Pooled error	255	0.13	2.62	2.68	0.01	0.19	0.30	17.65	16.27	0.52	0.08	0.39
% SS GCA		21.3	45.95	46.95	13.02	12.51	28.04	28.06	33.86	8.69	10.88	41.61
% SS SCA		72.70	40.47	39.55	52.63	70.43	61.12	67.53	61.03	73.54	65.17	41.28
% SS Reciprocal		5.93	13.58	13.50	34.34	17.06	10.84	4.41	5.11	17.77	23.95	17.10

GY= grain yield (t ha⁻¹); DTA= days to anthesis; DTS= days to silking (days); NEPP = number of ear per plant; PASP = plant aspect (1–5); EASP = ear aspect(1–5); PLH = plant height (cm); ERH = ear height (cm); ERL= ear length (cm); ERD = ear diameter; SG = stay green characteristics (1–9) ; %SS GCA=.relative contribution of GCA to the total crosses sum of squares; %SS SCA = relative contribution of SCA to the total crosses sum of squares; %SS Reciprocal = relative contribution of reciprocal to the total crosses sum of squares.

contribution of SCA effects for ear length (73.90%), with a low contribution for days to anthesis (35.48%). A greater reciprocal sum of squares than GCA sum of squares was accounted for number of ears per plant across all N environments.

Combining ability effects

General combining ability and maternal effects

From combined analysis under a low N environment, inbred lines TL156579, TL156583, TL148288, and TL156612 had significant positive GCA effects for grain yield; while inbred lines TL156612 and VL05128 had significant positive MAT effects for grain yield (Table 6). TL148288, TL148287, and TL116960 inbred lines had positive and significant GCA effects for plant aspects. Positive and significant GCA effects were found in TL156579, TL147078, and VL05128 inbred lines for the ear aspect. Inbred lines TL156583, TL147078, and TL156612 had significant positive GCA effects for plant height, ear length, and ear diameter. Significant positive GCA and MAT effects were found for ear height in the VL05128 inbred line. GCA effects were found to be significant and positive in TL156579, TL156583, TL147078 and TL156612

inbred lines for ear length and diameter. Significant positive GCA effects with significant negative MAT effects were found in the VL05128 inbred line for ear length. Inbred lines TL148288, TL148287, TL155976, and TL116960 had significant positive GCA effects for stay green (Table 6).

Under optimum N environments for combined analysis, inbred lines TL156579, TL148288, TL147078, and TL156612 had positive significant GCA effects for grain yield. Inbred lines TL156583 and TL155976 had both positive and significant GCA and MAT effects for grain yield; while inbred lines TL156591 and VL05128 had negative significant GCA and MAT effects for grain yield.

Table 4. Combined mean squares of traits for 11 QPM inbreds evaluated under optimum N environments at Raare, Fedis, and Dire-Dawa in 2018 cropping season.

Source of variation	df	GY	DTA	DTS	NEPP	PASP	EASP	PLH	ERH	ERL	ERD
Environment (E)	2	94.17**	22445.96**	22389.52**	0.16**	15.84**	7.04**	16221.52**	21857.67**	9.51**	28.27**
Crosses	120	11.61**	42.43**	43.69**	0.04**	1.39**	1.77**	1915.26**	777.97**	13.86**	0.96**
GCA	10	33.29**	274.89**	287.92**	0.08**	1.97**	5.70**	7333.87**	3797.44**	31.44**	2.60**
SCA	55	17.05**	34.63**	35.13**	0.05**	2.29**	2.25**	2489.41**	864.46**	20.74**	1.33**
Reciprocal	55	2.22**	7.97	7.83	0.02**	0.38**	0.58*	355.91**	142.47**	3.78**	0.30**
Maternal	10	3.70**	10.56	10.11	0.03**	0.50**	0.53	491.47**	179.93**	4.10**	0.39**
Nonmaternal	45	1.90**	7.39	7.33	0.02**	0.35**	0.59*	325.79**	134.15**	3.71**	0.28**
E x Crosses	240	2.28**	16.74**	17.42**	0.03**	0.30**	0.58**	149.63**	75.93**	0.99**	0.13*
E x GCA	20	5.24**	55.53**	55.94**	0.05**	0.94**	0.78**	477.31**	167.70**	3.01**	0.24
E x SCA	110	2.78**	21.10**	22.45**	0.03*8	0.25*	0.60**	170.89**	80.60**	0.96**	0.13*
E x Reciprocal	110	1.23**	5.33	5.38	0.02**	0.23*	0.52	68.79**	54.57**	0.66	0.10
E x Maternal	20	1.08**	4.11	4.20	0.01	0.29*	0.62	84.01**	55.51**	0.70	0.09
E x Nonmaternal	90	1.27**	5.61	5.64	0.02**	0.22	0.50	65.41**	54.36**	0.65	0.10
Pooled error	255	0.18	12.12	12.89	0.01	0.17	0.41	11.42	12.45	0.56	0.10
% SS GCA		23.90	53.99	54.92	16.87	11.87	26.81	31.91	40.68	18.90	22.49
% SS SCA		67.32	37.40	36.86	58.35	75.60	58.18	59.57	50.93	68.59	63.39
% SS Reciprocal		8.78	8.61	8.22	24.79	12.53	15.00	8.52	8.39	12.51	14.12

GY= grain yield (t ha⁻¹); DTA= days to anthesis; DTS= days to silking (days); NEPP = number of ear per plant; PASP = plant aspect (1–5); EASP = ear aspect(1–5); PLH = plant height (cm); ERH = ear height (cm); ERL= ear length (cm); ERD = ear diameter; %SS GCA = relative contribution of GCA to the total crosses sum of squares; %SS SCA = relative contribution of SCA to the total crosses sum of squares; %SS Reciprocal = relative contribution of reciprocal to the total crosses sum of squares.

Positive and significant GCA effects, with negative significant MAT effects, were observed in TL147078 inbred lines for grain yield. The TL156583 inbred line had a positive and significant GCA effect for number of ears per plant. Two inbred lines (TL148287 and VL05128) had significant and positive GCA effects for plant and ear aspects. Inbred lines TL148287, TL155976 and VL05128 had significant positive MAT effects for plant height. GCA and MAT effects were found to have significant positive otucomes in the VL05128 inbred line for ear height. Significant positive GCA and MAT effects were observed in the TL156579 inbred line for ear length. Inbred lines TL156579, TL155976, and VL05128 had significant positive GCA effects for ear diameter (Table 7).

Significant and positive GCA effects were found for grain yield in TL156579, TL156583, TL148288, TL147078, and TL156612 inbred lines across all N environments (Table 8). A significant positive GCA effect with a significant negative MAT effect was found in TL147078 for grain yield. A significant negative GCA effect with a significant positive MAT effect was observed in TL155976 inbred line for grain yield. Inbred lines TL156583 and TL156612 had significant positive GCA effects. For plant and ear aspects, inbred lines TL148287 and VL05128 had significant positive GCA effects.

Significant negative GCA effects with significant positive MAT effects were observed in TL148287 and VL05128 inbred lines, but positive and significant GCA effects with negative and significant MAT effects were observed in TL156612 inbred lines for plant height. Positive and significant GCA and MAT effects were found in the TL156579 inbred line for ear length. Lines TL156579 and TL147078 had significant positive

Table 5. Combined mean squares of traits for 11 QPM inbreds evaluated across all N environments at Raare, Fedis, and Dire-Dawa in 2018 cropping season.

Source of variation	df	Mean squares									
		GY	DTA	DTS	NEPP	PASP	EASP	PLH	ERH	ERL	ERD
Environment (E)	5	191.08**	20033.18**	20023.81**	0.60**	6.46**	8.23**	23057.60**	25203.49**	71.10**	35.75**
Crosses	120	14.97**	66.03**	66.59**	0.05**	2.23**	2.99**	3576.93**	1462.00**	22.37**	1.48**
GCA	10	43.46**	433.15**	449.20**	0.10**	3.42**	10.83**	13144.33**	6775.88**	38.92**	3.09**
SCA	55	23.07**	51.12**	49.67**	0.06**	3.69**	3.97**	4968.16**	1779.92**	36.08**	2.23**
Reciprocal	55	1.68**	14.19**	13.93**	0.03**	0.55**	0.58**	446.17**	177.92**	5.66**	0.45**
Maternal	10	2.29**	25.23**	24.22**	0.03*	0.35*	0.57	603.94**	248.72**	5.18**	0.41**
Nonmaternal	45	1.55**	11.74**	11.65*	0.03**	0.59**	0.59**	411.11**	162.19**	5.77**	0.46**
E x Crosses	600	1.77**	10.41**	10.90**	0.02**	0.27**	0.47**	142.36**	92.28**	1.46**	0.21**
E x GCA	50	4.20**	33.11**	33.18**	0.04**	0.68**	0.74**	333.60**	186.12**	2.59**	0.37**
E x SCA	275	2.17**	12.63**	13.55**	0.03**	0.24**	0.46**	174.36**	115.21**	1.45**	0.24**
E x REC	275	0.93**	4.07	4.21	0.02**	0.23*	0.42*	75.58**	52.29**	1.26**	0.16**
E x Maternal	50	1.18**	2.95	2.97	0.02**	0.30**	0.39	96.26**	58.83**	0.95**	0.12**
E x Nonmaternal	225	0.88**	4.32	4.49	0.02**	0.21	0.43**	70.98**	50.84**	1.32**	0.17**
Pooled error	510	0.15	7.37	7.78	0.01	0.18	0.35	14.52	14.36	0.54	0.09
% SS GCA		24.20	54.67	56.22	17.95	12.78	30.16	30.62	38.62	14.50	17.37
% SS SCA		70.65	35.48	34.19	57.46	75.97	60.91	63.66	55.80	73.90	68.75
% SS Reciprocal		5.15	9.85	9.59	24.60	11.26	8.94	5.72	5.58	11.60	13.88

GY= grain yield (t ha^{-1}); DTA= days to anthesis; DTS= days to silking (days); NEPP = number of ear per plant; PASP = plant aspect (1–5); EASP = ear aspect(1–5); PLH = plant height (cm); ERH = ear height (cm); ERL= ear length (cm); ERD = ear diameter; %SS GCA = relative contribution of GCA to the total crosses sum of squares; %SS SCA = relative contribution of SCA to the total crosses sum of squares; %SS Reciprocal = relative contribution of Reciprocal to the total crosses sum of squares.

GCA effects for ear diameter (Table 8).

DISCUSSION

Under both low and optimum N environments, significant differences were found among genotypes for grain yield, days to anthesis, days to silking, plant aspect, ear aspect, plant height, ear height and length, and ear diameter. This indicates genetic variability among genotypes, which creates a great opportunity for a maize breeder to improve genotypes for yield and other agronomic traits under both soil environments.

Different authors also reported similar results for QPM hybrids both under low and optimum N environments (Badu-Apraku et al., 2015b; Wegary et al., 2014).

Significant differences among genotypes for traits of interest at a specific location under specific N environments indicate that each environment was unique for each genotype; and each genotype responded differentially under low and optimum N environments. Similar findings were reported by Badu-Apraku et al. (2010) who reported the effects of stress environments on the performance of tropical early-maturing maize cultivars in multiple stress environments.

Single cross hybrids VL05128 x TL156612 (4.89 t ha^{-1}) and TL156612 x TL155932 (4.74 t ha^{-1}) were selected as high yielding hybrids which out-yielded by 16.76% and 14.14%, respectively, than the best check (4.07 t ha^{-1}) under low N environments due to better tolerance effects of the genotypes to stress environments than the checks. Under low N environments, a 20% grain yield increment over local commercial check was reported by Worku et al. (2001) for normal maize varieties. Overall, mean percentage of relative grain yield reduction for hybrids was 32% under low N environments as compared to optimum environments, which are within a range of 25% to35% yield reduction as

Table 6. Combined general combining ability (GCA) and maternal (MAT) effects of 11 QPM inbred lines for traits evaluated under low N environments at Raare, Fedis, and Dire-Dawa in 2018.

Line	GY GCA	GY MAT	DTA GCA	DTA MAT	DTS GCA	DTS MAT	PASP GCA	EASP GCA	PLH GCA	PLH MAT	ERH GCA	ERH MAT	ERL GCA	ERL MAT	ERD GCA	ERD MAT	SG GCA
TL156579	0.31**	0.08	-0.06	0.14	-0.07	0.12	-0.06	0.12*	-0.51	0.92	-0.54	0.20	0.28**	0.21*	0.14**	0.06	0.07
TL156583	0.39**	-0.12*	-0.44**	-0.24	-0.48**	-0.26	-0.02	-0.08	2.09**	-0.47	0.18	-0.04	0.20**	-0.04	0.05*	-0.01	0.05
TL148288	0.07*	-0.10	-1.64**	0.03	-1.62**	0.01	0.11**	-0.06	-6.93**	-0.01	-2.74**	-0.61	-0.06	0.15	0.07**	0.06	0.26**
TL156591	-0.18**	-0.05	0.94**	0.31	0.91**	0.31	0.07	0.08	-0.64	-1.20	-3.16**	-1.02	-0.13*	-0.25*	-0.11**	-0.09*	-0.36**
TL148287	-0.19**	-0.05	-1.06**	0.23	-1.08**	0.22	0.14**	0.14**	-8.86**	1.67**	-3.98**	0.38	-0.52**	0.03	-0.07**	-0.01	0.22**
TL116960	-0.63**	0.02	-0.16	0.36	-0.19	0.36	0.11**	-0.04	-0.16	-0.57	-2.93**	-0.59	-0.52**	0.04	-0.14**	-0.03	0.25**
TL155932	-0.21**	-0.02	-0.90**	-0.61*	-0.90**	-0.62*	0.02	-0.16**	-6.70**	-1.43*	-4.02**	-0.47	0.00	-0.02	-0.03	0.01	0.05
TL147078	0.06	-0.07	2.32**	0.30	2.40**	0.26	-0.19**	0.12*	12.97**	0.06	11.56**	-0.29	0.28**	-0.02	0.12**	0.02	-0.57**
TL156612	0.50**	0.12*	-0.19	-0.28	-0.19	-0.24	-0.08*	-0.33**	3.45**	-1.30*	-2.25**	-0.26	0.35**	0.07	0.06**	0.03	-0.03
TL155976	-0.09**	0.02	1.31**	0.30	1.31**	0.34	-0.15**	-0.19**	9.23**	0.01	6.16**	0.57	0.01	0.10	-0.02	-0.02	0.19**
VL05128	-0.02	0.17**	-0.12	-0.53*	-0.07	-0.50*	0.06	0.41**	-3.95**	2.31**	1.72**	2.13**	0.12*	-0.27*	-0.06**	-0.03	-0.13*
SE (gi)	0.03		0.13		0.14		0.04	0.05	0.35		0.33		0.06		0.02		0.05
SE (*MAT*)		0.05		0.23		0.24				0.60		0.58		0.10		0.04	

GY= grain yield (t ha^{-1}); DTA= days to anthesis; DTS= days to silking; ASI = anthesis silking interval; PASP= plant aspect (1–5); EASP = ear aspect (1–5); PLH = plant height (cm); ERH = ear height (cm); ERL= ear length (cm); ERD = ear diameter (cm); SG = stay green characteristic (1-9).

recommended by Banzinger et al. (2000) to be selected as resistant/ tolerant cultivars under low N environments. This level of yield reduction is closer to that of Below (1997) and Presterl et al. (2003), who reported yield reduction under low N stress of 35% and 37%, respectively, for normal maize hybrids. Yield reduction of 42% for tropical early maturing cultivars was reported by Badu-Apraku et al. (2010) and 40% of grain yield reduction for QPM hybrids by Badu-Apraku et al. (2015b) under low N environments. Different authors have reported different percentages of relative yield reduction under low N environments because of differences in the severity of low N environments and the germplasm used for the study.

From the analysis of genetic designs, GCA effects provide an estimation of additive gene action, while SCA effect provides non-additive gene action (Baker, 1978); and reciprocal effects provide an estimation of additive gene action through maternal (MAT) effects and non-additive gene action through non-maternal (NMAT) effects (Fan et al., 2013; Mahgoub, 2011). Significant differences were found among QPM hybrids for almost all traits both under low and optimum N environments, indicating the available genetic variability among the hybrids under low and optimum N environments, which would allow good progress to the selection of the hybrids under the target environments. Similar results were reported by different authors for QPM and non-QPM hybrids for grain yield, days to anthesis and silking, plant and ear height, plant and ear aspect under low and optimum N environments (Badu-Apraku et al., 2015a; Njeri et al., 2017; Wegary et al., 2014).

Under combined low, optimum, and across all N environments, significant mean squares of GCA, SCA and reciprocal effects were found for grain yield and other agronomic traits indicating the importance of additive, non-additive gene action and maternal cytoplasmic genes under these target environments. This implies that in addition to additive and non-additive gene actions, the inheritance of the traits was controlled by maternal effects that created differences in the performance of grain yield and other traits between F_1 hybrids and their F_1 reciprocals, which allow selection of superior hybrid under the target environments. Fan et al. (2013) and Mahgoub (2011) reported the effects of reciprocal crosses for maize grain yield under non-stress environments.

Highly significant E x crosses interactions were observed for all traits under low, optimum, and across all environments indicates that crosses

Table 7. Combined general combining ability (GCA) and maternal (MAT) effects of 11 QPM inbred lines for traits evaluated under optimum N environments at Raare, Fedis and Dire-Dawa in 2018.

Lines	GY		DTA	DTS	NEPP	PASP	EASP	PLH		ERH		ERL		ERD	
	GCA	MAT	GCA	GCA	GCA	GCA	GCA	GCA	MAT	GCA	MAT	GCA	MAT	GCA	MAT
TL156579	0.43**	0.09	0.14	0.16	0.00	0.00	0.18**	-2.08**	0.77	-2.42**	0.10	0.61**	0.25*	0.30**	0.07
TL156583	0.73**	0.16*	-0.26	-0.26	0.04**	-0.08*	-0.09	3.78**	-0.09	2.48**	0.10	0.30**	-0.03	-0.01	-0.02
TL148288	0.30**	-0.08	-2.63**	-2.71**	0.01	0.06	-0.08	-7.14**	-0.13	-3.66**	-0.43	-0.27**	0.10	-0.01	-0.01
TL156591	-0.16**	-0.22**	1.30**	1.33**	0.00	0.06	-0.03	-1.69**	-3.79**	-2.89**	-1.86**	-0.42**	-0.34**	-0.12**	-0.06
TL148287	-0.05	0.12	-1.21**	-1.20**	0.02*	0.13**	0.13*	-9.10**	2.16**	-3.48**	1.34	-0.49**	-0.10	-0.13**	-0.03
TL116960	-0.98**	-0.06	-0.42	-0.45	-0.04**	0.19**	0.02	-4.20**	0.95	-6.42**	0.21	-0.94**	0.03	-0.20**	0.00
TL155932	-0.48**	0.14*	-1.21**	-1.22**	-0.01	-0.07	-0.13*	-6.05**	-2.02**	-2.99**	-1.63**	-0.07	-0.19	-0.08**	-0.10*
TL147078	0.20**	-0.25**	2.75**	2.83**	0.02	-0.21**	0.05	15.03**	-0.63	11.86**	-0.19	0.64**	0.09	0.08**	0.03
TL156612	0.55**	-0.03	0.17	0.18	0.01	-0.04	-0.36**	2.68**	-1.44**	-1.56**	-0.63	0.15*	-0.12	-0.03	0.00
TL155976	-0.31**	0.27**	1.19**	1.18**	-0.02	-0.14**	-0.14**	10.49**	1.31**	6.44**	0.93	0.25**	0.17	0.07**	0.09
VL05128	-0.23**	-0.14*	0.18	0.16	-0.04**	0.10**	0.44**	-1.74**	2.90**	2.65**	2.05**	0.24**	0.14	0.13**	0.04
SE (gi)	0.03		0.29	0.30	0.01	0.04	0.05	0.28		0.29		0.06		0.03	
SE (MAT)		0.06							0.49		0.51		0.11		0.05

GY= grain yield (t ha^{-1}); DTA= days to anthesis; DTS= days to silking; NEPP = number of ears per plant; PASP= plant aspect (1–5); EASP = ear aspect (1–5); PLH = plant height (cm); ERH = ear height (cm); ERL= ear length (cm); ERD = ear diameter (cm).

reacted differently to different testing environments; thus, the breeder should test the hybrids in a wide range of environments for the selection of best performing and stable crosses. This finding is consistent with the result reported by Badu-Apraku et al. (2015a) and Wegary et al. (2014) for QPM hybrids under multiple stress environments.

Significant mean squares for E x GCA interaction effects were observed for grain yield and all other agronomic traits under combined low, optimum, and across all N environments; which indicated that GCA effects of the parental lines exhibited variations under the considered environments of this study. Similar results were reported by different authors (Badu-Apraku et al., 2015; Badu-Apraku et al., 2010, 2017; Njeri et al., 2017; Wegary et al., 2014). This finding also

points out that, for the development of high yielding and stable hybrids across a range of environments for commercial purposes, there is the need to test parental lines for their GCA and SCA effects across a range of environments, and/or different parental lines need to be selected for each environment for hybrid development. Significant mean squares of E x SCA interaction effects were observed for grain yield and other measured traits under combined low, optimum and across all N environments, except for plant and ear aspects under combined low N environments. This indicates that except for plant and ear aspects under combined low N environments, all traits were significantly different across environments of this study. This finding is in disagreement with the finding of Wegary et al. (2014), who reported non-significant E x SCA

interaction effects of QPM hybrids for grain yield and most of the other agronomic traits evaluated under drought, low and optimum N environments. However, this finding is in agreement with the finding of Badu-Apraku et al. (2015a), who reported significant E x SCA interaction effects of QPM hybrids for grain yield and most of the other measured traits evaluated under drought, low and optimum N environments.

Under combined low, optimum, and across all N environments, greater contributions of GCA sum of squares than SCA sum of squares were observed for days to anthesis and silking, indicating the greater importance of additive gene action than non-additive gene actions in the inheritance of the traits. A greater reciprocal sum of squares than GCA sum of squares were observed for number of ears per plant, plant

Table 8. Combined general combining ability (GCA) and maternal (MAT) effects of 11 QPM inbred lines for traits evaluated across all environments at Raare, Fedis, and Dire-Dawa in 2018.

Lines	GY		DTA	DTS	NEPP	PASP	EASP	PLH		ERH		ERL		ERD
	GCA	MAT	GCA	GCA	GCA	GCA	GCA	GCA	MAT	GCA	MAT	GCA	MAT	GCA
TL156579	0.37**	0.08	0.04	0.05	0.00	-0.03	0.15**	-1.29**	0.85	-1.48**	0.15	0.44**	0.23*	0.22**
TL156583	0.56**	0.02	-0.35*	-0.37*	0.04**	-0.05*	-0.09**	2.94**	-0.28	1.33**	0.03	0.25**	-0.04	0.02
TL148288	0.18**	-0.09	-2.13**	-2.17**	0.01	0.09**	-0.07*	-7.03**	-0.07	-3.20**	-0.52	-0.16**	0.13	0.03
TL156591	-0.17**	-0.14*	1.12**	1.12**	0.01	0.07**	0.03	-1.16**	-2.49**	-3.02**	-1.44**	-0.28**	-0.29**	-0.12**
TL148287	-0.12**	0.03	-1.13**	-1.14**	0.00	0.13**	0.14**	-8.98**	1.91**	-3.73**	0.86	-0.51**	-0.03	-0.10**
TL116960	-0.81**	-0.02	-0.29	-0.32*	-0.03**	0.15**	-0.01	-2.18**	0.19	-4.68**	-0.19	-0.73**	0.03	-0.17**
TL155932	-0.35**	0.06	-1.05**	-1.06**	-0.01	-0.03	-0.15**	-6.38**	-1.72**	-3.50**	-1.05	-0.04	-0.11	-0.05**
TL147078	0.13**	-0.16**	2.54**	2.61**	0.01	-0.20**	0.08*	14.00**	-0.28	11.71**	-0.24	0.46**	0.04	0.10**
TL156612	0.52**	0.04	-0.01	0.00	0.02**	-0.06*	-0.35**	3.07**	-1.37*	-1.90**	-0.45	0.25**	-0.03	0.01
TL155976	-0.20**	0.14**	1.25**	1.25**	-0.01	-0.14**	-0.16**	9.86**	0.66	6.30**	0.75	0.13**	0.14	0.02
VL05128	-0.12**	0.02	0.03	0.05	-0.02**	0.08**	0.42**	-2.84**	2.61**	2.18**	2.09**	0.18**	-0.06	0.03
SE (gi)	0.02		0.16	0.16	0.01	0.03	0.03	0.22		0.22		0.04		0.02
SE (*MAT*)		0.06							0.55		0.54		0.11	

GY= grain yield (t ha-1); DTA= days to anthesis; DTS= days to silking; NEPP = number of ears per plant; PASP= plant aspect (1–5); EASP = ear aspect (1–5); PLH = plant height (cm); ERH = ear height (cm); ERL= ear length (cm); ERD = ear diameter (cm).

aspect, ear length and diameter under combined low N environments. This indicates that the inheritances of the traits were more affected by the additive gene actions of the maternal cytoplasm genes than the additive gene actions of the crosses nuclear genes for ears per plant, plant aspect, ear length, and diameter under combined low N environments. Different authors have also reported different results for contributions of the GCA and SCA sum of squares for QPM and non-QPM maize hybrids under multiple stress environments (Badu-Apraku et al., 2015a and Njeri et al., 2017) who reported greater magnitude of SCA sum of squares than GCA sum of squares of QPM germplasm for grain yield under drought stress. Wegary et al. (2014) also reported a high contribution of non-additive gene actions for the inheritance of grain yield, ears per plant and plant height; while there was a greater importance of

additive gene actions for the inheritance of days to anthesis and silking under low N environment for QPM hybrids. More importance of non-additive gene actions than additive gene action for grain yield under low N environments for non-QPM hybrids was reported by Worku et al. (2008).

Significant and positive GCA effects were observed in TL156579, TL156583, TL148288, and TL156612 parental lines, for grain yield across N environments, indicating that the inbred lines are good general combiners for grain yield across N environments; and the inbred lines can be used as donor parents for the target trait under the target environments. Different effects of SCA were observed in hybrids under low and optimum N environments, indicating that selection of hybrids based on only SCA effects is not a guarantee for the selection of the best grain yielding hybrids under target environments. Menkir et al. (2004)

recommended that for better hybrid development, parental lines should have to be grouped into heterotic groups based on the grain yield performance and SCA effects of the inbred lines.

For effective maize hybrid development, the classification of maize inbred lines into appropriate groups could be essential in a maize breeding program that maximizes the importance of inbred lines (Badu-Apraku et al., 2015b). In this study, classification of inbred lines into heterotic groups was done based on SCA effects for grain yield and grain yield performance of the crosses as suggested by Fan et al. (2013). Because, asper se performance of inbred lines is not a good indicator of the hybrids grain yield performance for maize due to the strong dominance effects in maize (Badu-Apraku and Akinwale, 2011). Inbred lines were classified into 3 heterotic groups under low, optimum and across all N environments,

which maximize the development of superior maize hybrids under stress and non-stress soil environments. Different researchers have used different methods for inbred lines classification into heterotic groups to maximize the potential importance of inbred lines for the development of productive hybrids. Badu-Apraku et al. (2015a) used heterotic grouping based on GCA of multiple traits (HGCAMT) methods for QPM inbred lines using a classification into 3 heterotic groups under a low N environment. Fan et al. (2009) classified 23 normal maize inbred lines into 4 known maize heterotic groups by using the heterotic group's specific and general combining ability (HSGCA) method. Non-QPM inbred lines were classified into 4 heterotic groups based on SCA effects for yield and yield performances of the crosses (Fan et al., 2013).

Conclusion

Mean squares for GCA, SCA and reciprocal effects showed significant for grain yield and other major phenotypic traits both under low and optimum N environments. Under low and optimum N environments, additive gene actions were more important than non-additive gene action for days to anthesis and silking; whereas, non-additive gene actions were more important than additive gene action for grain yield, the number of ears per plant, plant and ear aspects, plant and ear heights, ear length and diameter.

Single cross hybrids VL05128 x TL156612 and TL156612 x TL155932 were selected as high yielding hybrids, which out-yielded 16.76% and 14.14%, respectively, more than the best check (4.07 t ha^{-1}) under low N environments; while hybrids TL156583 x TL156612, TL156612 x TL148288, TL148287 x TL156583, TL156612 x TL156583, and TL156583 x TL148287 were selected as single cross hybrids; and these out-yielded the best check under optimum N environments.

Parental lines TL156579, TL156583, and TL148288 were considered as a good general combiner for grain yield under low N environments. Parental line TL156612 showed higher positive GCA effects when used as female; while parental line TL156612 showed higher negative GCA effects when used as the male parent for grain yield under low N environments. Under low N environments, hybrids TL156591 x TL155976, TL116960 x TL147078 and TL156583 x TL155932 were recorded as showing high SCA effects for grain yield; while hybrids TL156583 x TL148287, TL148288 x TL155976 and TL148287 x TL155932 were recoded as showing high SCA effects for grain yield under optimum N environments.

ABBREVIATIONS

CSA, Central Statistical Agency; GCA, general Combining Ability; IBPGR, International Board for Plant Genetic Resources; Low N, Low N environment; MAT, Maternal effect; Optimum N, optimum N environment; QPM, Quality protein Maize; SCA, Specific Combining ability.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

ACKNOWLEDGEMENTS

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Correlation and path coefficient analysis studies in Ethiopian Mustard (*Brassica carinata* A. Braun)

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Ethiopia is the center of origin for Ethiopian mustard (*Brassica carinata*). The crop is one of the oldest oil crops and farmers in the highlands of the country grow as a leafy vegetable in their gardens. However, no attempt has been made to assess the association of characters and path analysis in Ethiopian mustard leaf. This study was conducted to assess association of traits among leafy vegetable yield and yield related traits and to determine the direct and indirect effects of the traits. A total of 36 Ethiopian mustard genotypes were evaluated at Holleta in 2017/2018. The results from correlation study showed that the genotypic correlation coefficient among edible vegetable leaf yield as well as all of agro-morphological qualities was positive and significant apart from leaves per plant and leaf width ratio to length. Length of leaf petiole, leaf length, leaf width, petiole width and plant height had positive and highly significant correlation with edible vegetable leaf yield both at levels of genotypic and phenotypic. These traits also had indirect positive effect on yield either though each other or via other traits at genotypic level. The strong association of these traits with leaf yield, the high to low effects of direct and indirect through other traits at level of genotypic for these traits is an indication of the importance of the traits to use in Ethiopian mustard genotypes for high edible vegetable leaf yield selection.

Key words: Correlation, direct, indirect and edible vegetable leaf yield.

INTRODUCTION

The genus *Brassica* is one of the fifty-one genera, and the foremost economically important genus, in the Brassicaceae family, or previously known as Cruciferae (an older name for the family). It means "cross-bearing," because the 4 petals of their flowers are reminiscent of a cross). The genus *Brassica* contains 37 distinctive species (Gomez-Campo and Prakash, 1999). Several species and of Brassicas are significant oilseed crops, vegetables, forage crops, and are utilized in the production of condiments, such as mustard (Nagaharu, 1935). Ethiopian mustard is believed to have originated from the Ethiopian highlands, and its cultivation is thought to have begun about 4000 years B.C. (Schippers, 2000; Nigussie and Becker, 2002).

In plant genetic and breeding sciences, correlated traits are of top significance due to genetic causes of correlations through pleiotropic action, or gene developmental interactions; as well as changes brought

about by natural or artificial selection (Singh, 1993; Falconer and Mackay, 1996; Sharma, 1998). A clear delineation of direct and indirect effects of interaction among traits upon each other in some conditions was not realized using correlation alone; therefore, use of coefficient of path evaluation is better, particularly because it can observe the direct and indirect associations causes, and provide a measure of the relative importance of all (Sharma, 1998). Given the absence of sufficient information on the association of characters and path analysis in Ethiopian mustard leaf, a correlation and path coefficient study has critical importance in generating information that could help in designing breeding methods for the purpose of developing new varieties. Hence, this study was conducted with the objectives of (i) to estimate association among leafy vegetable yield and yield related traits, and (ii) to determine the direct and indirect effects of yield related traits on leafy vegetable yield of Ethiopian mustard genotypes.

MATERIALS AND METHODS

This research was at Holetta Agricultural Research Center throughout the main cropping season of 2017/2018 under rain fed conditions. The location of Holetta Agricultural Research Center is at 9° 00'N, 38° 30'E at an altitude of 2400 m.a.s.l. The main rainy season is from June to September, which accounts for 70% of the rainfall; while the remaining 30% is from February to April (EIAR, 2005).

For this study, 36 genotypes of Ethiopian mustard were used. Among the tested genotypes, five check varieties were included; which were released for seed production purposes but not for leaf purposes. The experiment was conducted using 6×6 simple lattice design. Each genotype was planted in a plot size of 1.2 by 3 m length in each block of replication. The recorded observations were for ten quantitative characters; viz: Days to 50% maturity, Leaf petiole length (cm), Leaf length (cm), Leaf width (cm), Ratio of leaf blade width to leaf length (cm), Petiole width (cm), Leaves per plant (No), Plant height(cm), Canopy diameter (cm), and Edible vegetable leaf yield (ton ha^{-1}).

Phenotypic and genotypic correlation coefficient

Phenotypic (r_p) and genotypic (r_g) correlations among two traits were assessed using the suggested formula by Johnson et al. (1955) and Singh and Chaudhury (1985).

$$r_p = \frac{P\,cov_{xy}}{\sqrt{(V_p x . V_p y)}}$$

Where, r_p = Phenotypic correlation coefficient;

$$r_g = \frac{G\,cov_{xy}}{\sqrt{(V_g x . V_g y)}}$$

r_g = Genotypic correlation coefficient;

$Pcov_{xy}$ = Phenotypic covariance among variables x and y; $Gcov_{xy}$ = Genotypic covariance among variables x and y; $V_p x$ = Phenotypic variance of variable x; $V_g x$ = Genotypic variance of variable x; $V_p y$ =

Phenotypic variance of variable y; $V_g y$ = Genotypic variance of variable y.

The calculated phenotypic correlation value was tested for its significance using t-test:

$t = r_{ph}/SE\,(r_p)$

Where, r_p = Phenotypic correlation; SE (r_p) = Standard error of phenotypic correlation obtained using the following formula (Sharma, 1998).

$$SE\,(r_p) = \sqrt{\frac{1 - r^2\,ph}{n - 2}}$$

Where, n is the number of genotypes tested; r^2_p is phenotypic correlation coefficient.
Levels of genotypic coefficients of correlations were tested for their significance by the formula described by Robertson (1959); namely:
$t = r_{gxy}/SEr_{gxy}$.

The calculated "t" value was compared with the tabulated "t" value at (n-2) degrees of freedom at 5% level of significance. Where, n is number of genotypes.

$$SEr_{gxy} = \sqrt{\frac{1 - r^2 gxy}{h^2 x} . h^2 y}$$

Where, $h^2 x$ = Heritability of trait x; $h^2 y$ = Heritability of trait y.

Path coefficient analysis

Based on genotypic and phenotypic correlations; path coefficient analysis, that refers to the direct and indirect estimation effects of leaf yield identified characters (independent character) on leaf yield (dependent character), was calculated based on the methods of Dewey and Lu (1959) as follows:

$rij = Pij + Σrik\,pkj$

where, rij = mutual association among the independent character (i) and dependent character (j) as measured by the genotypic and phenotypic correlation coefficients; Pij = direct effects of the character of independent (i) on the dependent variable (j) as measured by the genotypic path coefficients; and Σrikpkj = components summation of indirect effects of a given character of independent (i) on a given character of dependent (j) through all other characters of independent (k).
The remaining effect, which controls how best the causal factors account for the variability of the dependent yield factor, was calculated using the formula:

$1 = p^2 R + Σ\,p\,ij\,rij$

Where, $p^2 R$ is the residual effect; p ij rij = the product of direct effect of any variable and its correlation coefficient with yield.

RESULTS AND DISCUSSION

Phenotypic and genotypic correlation coefficient of leaf yield with other characters

Most of crop phenology and growth traits (viz. days to

Table 1. Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients among 10 traits of 36 Ethiopian mustard genotypes.

Parameter	DTM	LPL	LL	LW	RLWLL	PTW	LPP	PH	CD	YLD
DTM		0.63**	0.59**	0.53**	-0.57**	0.64**	-0.04	0.59**	0.67**	0.50**
LPL	0.56**		0.95**	0.90**	-0.54**	0.91**	0.50**	0.84**	0.94**	0.78**
LL	0.52**	0.93**		0.97**	-0.48**	0.96**	0.49**	0.78**	0.96**	0.84**
LW	0.45**	0.86**	0.96**		-0.27	0.94**	0.51**	0.77**	0.93**	0.81**
RLWLL	-0.47**	-0.39**	-0.35**	-0.09		-0.43**	-0.15	-0.38*	-0.50**	-0.43**
PTW	0.59**	0.87**	0.93**	0.90**	-0.30**		0.39*	0.75**	0.95**	0.83**
LPP	-0.09	0.48**	0.49**	0.54**	-0.02	0.38**		0.56**	0.42*	0.33
PH	0.53**	0.82**	0.78**	0.75**	-0.29*	0.73**	0.51**		0.84**	0.55**
CD	0.62**	0.89**	0.94**	0.90**	-0.36**	0.93**	0.40**	0.82**		0.76**
YLD	0.47**	0.75**	0.82**	0.76**	-0.35**	0.79**	0.28*	0.55**	0.73**	

DTM = Days to 50% maturity, LPL (cm) = Leaf petiole length in centimeter, LL (cm) = Leaf length in centimeter , LW (cm) = Leaf width in centimeter, RLWLL (cm) = Ratio of leaf blade width to leaf length in centimeter , PTW (cm) = Petiole width in centimeter, LPP (no) = Number of leaves per plant, PH (cm) = Plant height in centimeter, CD (cm) = Canopy diameter in centimeter, YLD (ton ha^{-1}) = Edible vegetable leaf yield in tons per hectare, respectively.

50% maturity, leaf petiole length, leaf length, leaf width, petiole width, plant height and canopy diameter) had positive and significant correlations with edible vegetable leaf yield in ton ha^{-1} at both levels of genotypic and phenotypic. In addition, leaf number per plant had positive as well as significant correlations at phenotypic level. Among the growth parameters, leaf blade width ratio to length of leaf showed negative and significant correlations with leaf yield both at genotypic and phenotypic levels (Table 1). The magnitude of the genotypic correlation coefficient was higher than the traits of phenotypic correlation coefficient for all parameters. Phenotypic correlation (rp) measures the extent to which the two detected characters are linearly connected; although genotypic correlation (rg) measures the extent to which degree of the same genes, or closely related genes, cause covariation (simultaneous variations) in two characters that are different (Singh and Chaudhary, 1977; Falconer and Mackay, 1996; Sharma, 1998). The more significant genotypic association between the different pairs of characters than the phenotypic correlation indicates the presence of strong association between those characters genetically, but the phenotypic value is lessened by the significant interaction of environment (Singh and Chaudhary, 1977; Falconer and Mackay, 1996; Sharma, 1998). Thus, the presence of significant correlation of phenology and most of the growth traits with edible vegetable leaf yield per hectare (both at genotypic and phenotypic levels) suggested the major significance of the traits in selecting program to identify Ethiopian mustard genotypes with high leaf yield.

Edible vegetable leaf yield per hectare showed negative correlation with ratio of leaf width and length at both genotypic and phenotypic levels (Table 1). The existence of negative correlation indicated the associated traits are in opposite direction; and thus, genotype selection for high performance of one trait leads to the reduction of performance in the other traits. Therefore, it is vital to give attention to the two crop traits in the selection process of genotypes for high yield. Association of negative traits is difficult or virtually impossible to improve through concurrent selection of those traits (Akinyele and Osekita, 2006; Nwangburuka et al., 2012; Ahiakpa et al., 2013). Genetic correlation signs amid two characters can either facilitate or obstruct progress of selection (Singh and Chaudhary, 1977; Falconer and Mackay, 1996; Sharma, 1998).

A similar result reported by Buhroy et al. (2017) showed that total yield of the amaranth leaves had significantly and positive correlation with plant height, petiole length, leaf width and leaf length. Kumar et al. (2017) reported most of growth traits (leaf petiole length, leaf length, leaf width, petiole width, plant height and plant spread) had positive and significant correlations with curd yield quintal ha^{-1} of *Brassica oleracea* L. var. *botrytis* both at genotypic and phenotypic levels.

Estimate of correlation coefficients among other characters

The phenology and growth traits (days to 50% maturity, leaf petiole length, leaf length, leaf width, petiole width, plant height and canopy diameter) showed positive and significant associations among them; both at genotypic and phenotypic levels. There were positive as well as significant associations of leaf number per plant with the variables of leaf petiole length, leaf length, leaf width and petiole width, at both genotypic and phenotypic levels. There were positive and significant associations of plant height and canopy diameter with number of leaves per plant at both genotypic and phenotypic levels. Among

Table 2. Estimates of direct (bold and diagonal) and indirect (off diagonal) effects of traits on edible vegetable leaf yield per hectare at the genotypic level.

Parameter	DTM	LPL	LL	LW	RLWLL	PTW	LPP	PH	CD	rg
DTM	**-0.1248**	0.1349	0.0285	0.5872	0.2251	0.3538	0.0094	0.0108	-0.7223	0.5025
LPL	-0.0790	**0.2132**	0.0457	0.9843	0.2117	0.5021	-0.1020	0.0152	-1.0084	0.7829
LL	-0.0740	0.2031	**0.0480**	1.0615	0.1904	0.5281	-0.0996	0.0141	-1.0317	0.8398
LW	-0.0667	0.1908	0.0463	**1.0998**	0.1068	0.5170	-0.0456	0.0139	-0.9936	0.8099
RLBLL	0.0715	-0.1149	-0.0233	-0.2991	**-0.3929**	-0.2370	0.0314	-0.0070	0.5365	-0.4346
PTW	-0.0802	0.1944	0.0460	1.0326	0.1691	**0.5506**	-0.0793	0.0135	-1.0130	0.8339
LPP	0.0057	0.1060	0.0233	0.5603	0.0600	0.2128	**-0.2053**	0.0101	-0.4466	0.3263
PH	-0.0743	0.1792	0.0375	0.8469	0.1509	0.4116	-0.1147	**0.0181**	-0.9005	0.5547
CD	-0.0842	0.2007	0.0462	1.0202	0.1968	0.5208	-0.0856	0.0152	**-1.0711**	0.7591

Residual factor = 0.204. rg = Correlation coefficient at genotypic level, DTM = Days to 50% maturity, LPL = Leaf petiole length , LL = Leaf length , LW = Leaf width , RLWLL= Ratio of leaf blade width to leaf length, PTW= Petiole width , LPP = Number of leaves per plant, PH = Plant height and CD = Canopy diameter.

growth parameters, leaf blade ratio width to leaf length had negative and significant association with days to 50% maturity, and all growth traits, except leaf width and leaves per plant (Table 1).

Similarly, Jangde et al. (2017) studied amaranth genotypes, and observed positive and significant association among leaf petiole length, leaf length, leaf width, petiole width, and plant height at both genotypic and phenotypic levels. In addition, leaf number per plant had a positive significant association with petiole length and leaf yield. Anyaoha et al. (2015) reported a strong positive correlation among leaf length, leaf width, petiole length and days to maturity of *Brassica juncea* genotypes. Kumar et al. (2017) also found strong and positive correlations among leaf number, leaf length and leaf width; similarly, plant height was positively and significantly correlated with leaf length, leaf width, petiole length in his study on analysis of midseason *Brassica oleracea* L. var. *botrytis.*

Path analysis

A total of 8 traits showed significant correlations with edible vegetable leaf yield in tons ha^{-1} at genotypic and phenotypic levels, respectively. Therefore, path coefficient analysis was conducted for these traits, taking edible vegetable leaf yield in tons ha^{-1} as the dependent variable and other traits as causal variables to understand the direct and indirect effects of the traits. The results of genotypic and phenotypic path coefficient analyses are presented in Tables 2 and 3, respectively. Information obtained from correlation coefficients can be enhanced by partitioning them into direct and indirect effects for a set of a priori cause-effect interrelationships; thus, providing a convenient method in selecting the characters that have direct and indirect effects, as has

been demonstrated in various crops (Kang et al., 1983; Gravois and Helms, 1992; Gravois and McNew, 1993; Board et al., 1997; Murtadha et al., 2004).

Genotypic path analysis of edible leaf yield with other traits

Leaf petiole length, leaf length, leaf width, petiole width and plant height had positive and highly significant genotypic correlation with edible vegetable leaf yield, and also exerted a confident direct effect on crop yield. Lenka and Mishra (1973) set the direct and indirect effects into five categories: negligible (0.00-0.09), low (0.10-0.19), moderate (0.20 -0.29), high (0.30-1.00) and very high (>1.00). Accordingly, using this categorization scheme, leaf width had very high (1.099) effects on edible leaf yield; whereas, petiole width (0.550) and leaf petiole length (0.213) exerted high and moderate positive direct effects on edible leaf yield, respectively; while leaf length and plant height had positive but negligible direct effects (0.048 to 0.018) on edible leaf yield (Table 2).

Leaf width had high positive indirect effects (>0.3) via days to 50% maturity, leaf petiole length, leaf length, petiole width, leaves per plant, plant height, and canopy diameter. Petiole width via days to maturity, leaf petiole length, leaf length, leaf width, plant height and canopy diameter exerted high indirect effect on leaf yield; and, via leaves per plant, exerted a moderate indirect effect on leaf yield. Leaf petiole length and leaf length exerted positive and high-to-low indirect effect via each other along with low-to-negligible effect through days to maturity, petiole width, number of leaves per plant, plant height and canopy diameter.

Plant height had positive but insignificant indirect effects on leaf yield through maturity days, leaf petiole length, length of leaf, leaf width, petiole width, leaf

number per plant as well as canopy diameter. This suggested that selection of genotypes for leaf width,petiole width, leaf petiole length, leaf length and plant height themselves (and through other traits) could be regarded as a reliable source of getting high leaf yield in Ethiopian mustard.

Similarly, Hasan et al. (2013) found a positive direct effect of leaf width on marketable yield of amaranths, and a low positive indirect effect of leaf width via plant height and number of leaves per plant on marketable yield. Sabaghina et al. (2013) reported a positive direct effect on leaf yield of spinach, that was categorized as high for leaf length, medium for leaf width and low for petiole length. Similarly, Kumar et al. (2017) reported negligible positive direct effect of plant height and days to maturity on marketable yield of *B. oleracea* L. var. *botrytis*; and also observed a moderate indirect effect on marketable yield by leaf width via petiole length and number of leaves per plant. Days to 50% maturity and leaves per plant had positive significant genotypic correlations with edible leaf yield per hectare, but had negative and low direct effect on the trait. Canopy diameter and ratio of leaf blade width to leaf length showed highly significant positive and negative genotypic correlation with edible leaf yield per hectare and exerted negative and negligible to moderate direct effect on the trait, respectively. The negative direct effect of leaves per plant and canopy diameter on leaf yield was due to these traits negligible to high negative indirect effects via each other. Moreover, the negative effect of days to maturity is due to negligible to high indirect effect of canopy diameter via each other. Whereas, the positive and significant genotypic correlations of days to 50% maturity, leaves per plant and canopy diameter with leaf yield was due to the traits negligible to high positive indirect effects on leaf yield via leaf width, petiole width, leaf length, ratio of leaf blade width to leaf length, leaf petiole length and plant height.

Therefore, days to 50% maturity, ratio of leaf blade width to leaf length, number of leaves per plant and canopy diameter should not be considered for selection of genotypes for high leaf yield; but it is necessary to consider the indirect traits that caused the positive and significant correlations of these traits with leaf yield. If the variable has correlation that is positive, and the variable direct effect is negative or negligible; the positive correlation of the trait is due to the indirect effects through other traits. The indirect causal factors/traits are to be considered simultaneously for selection in such a situation (Singh and Chaudhary, 1977).

Outstanding effect in the current study was the residual factor of 0.204 (Table 2) showing that 79.57% of the edible vegetable leaf variability yield per hectare was accounted for by the factors of component. The remaining is explained by other traits in the study that are not considered. The determination of the residual effect inidicates to what extent the causal factors or dependent variables account for the variability of the dependent variable (Dabholkar, 1992; Singh and Chaudhary, 1977).

Phenotypic path analysis of edible leaf yield with other traits

Leaf petiole length, leaf length, and leaf and petiole width had highly positive significant phenotypic correlation with edible vegetable leaf yield and also exerted a positive direct effect on yield. Leaf length (0.7076), leaf width (0.3748) and petiole width (0.3161) had positive direct effects on edible leaf yield, whereas leaf petiole length (0.0643) had negligible positive direct effects.

Leaf length had high and positive indirect effects (>0.3) via days to 50% maturity, leaf petiole length, leaf width, petiole width, number of leaves per plant, plant height and canopy diameter. Leaf width via leaf petiole length, leaf length, petiole width and canopy diameter exerted high indirect effect on leaf yield, and leaves per plant and days to maturity, exerted moderate and negligible indirect effect on leaf yield, respectively. Petiole width via leaf petiole length, leaf length, leaf width, plant height and canopy diameter exerted high and via days to 50% maturity and leaves per plant exerted low indirect effect on leaf yield.

Petiole width exerted positive and moderate indirect effect via leaf petiole length, leaf length, leaf width, plant height and canopy diameter and low through days to 50% maturity and number of leaves per plant. Leaf petiole length had positive but negligible indirect effects on leaf yield and through days to maturity, leaf length, leaf width, petiole width, leaves per plant, plant height and canopy diameter. This suggested that selection of genotypes for leaf length, petiole width, leaf width and leaf petiole length of leaf themselves, and through other traits, could be regarded as a reliable source of getting high leaf yield in Ethiopian mustard. Similarly, Jangde et al. (2017) reported positive and significant phenotypic correlations among leaf length and leaf width, and had direct effect on leaf yield of vegetable amaranths. Kumar et al. (2017) reported leaf width and petiole length had positive low direct effect on marketable yield. In addition, leaf width had positive indirect effect via leaf length, plant height and number of leaves per plant; and plant height had negligible indirect effect via leaf length, leaf width and number of leaves per plant.

Days to 50% maturity, plant height, canopy diameter and leaves per plant had positive significant phenotypic correlations with edible leaf yield per hectare; but had negative and negligible to high direct effect on the trait. Ratio of leaf blade width to leaf length showed highly significant negative phenotypic correlation with edible leaf yield per hectare, and exerted negative and low direct effect on the trait. This trait also had negative direct effects on leaf yield via other traits. The negative direct effect of several variables (days to 50% maturity, number of leaves per plant, plant height and canopy diameter on.

Table 3. Estimates of direct (bold and diagonal) and indirect (off diagonal) effect of traits on edible vegetable yield per hectare at the phenotypic level.

Parameter	DTM	LPL	LL	LW	RLBLL	PT	LPP	PH	CD	rp
DTM	**-0.0009**	0.0359	0.3690	0.1668	0.0855	0.1863	0.0152	-0.0361	-0.3505	0.47
LPL	-0.0005	**0.0643**	0.6582	0.3233	0.0708	0.2759	-0.0766	-0.0556	-0.5036	0.75
LL	-0.0005	0.0598	**0.7076**	0.3585	0.0624	0.2953	-0.0787	-0.0529	-0.5323	0.82
LW	-0.0004	0.0555	0.6769	**0.3748**	0.0172	0.2860	-0.0865	-0.0511	-0.5087	0.76
RLBLL	0.0004	-0.0252	-0.2445	-0.0357	**-0.1805**	-0.0956	0.0030	0.0199	0.2062	-0.35
PT	-0.0005	0.0561	0.6610	0.3391	0.0546	**0.3161**	-0.0609	-0.0498	-0.5248	0.79
LPP	0.0001	0.0307	0.3468	0.2020	0.0034	0.1198	**-0.1606**	-0.0351	-0.2268	0.28
PH	-0.0005	0.0524	0.5487	0.2808	0.0528	0.2309	-0.0827	**-0.0682**	-0.4656	0.55
CD	-0.0006	0.0571	0.6645	0.3364	0.0657	0.2928	-0.0643	-0.0560	**-0.5667**	0.73

Residual factor = 0.27. rp = Correlation coefficient at phenotypic level, DTM = Days to 50% maturity, LPL = Leaf petiole length , LL = Leaf length , LW = Leaf width , RLWLL= Ratio of leaf blade width leaf length, PTW = Petiole width , LPP = Number of leaves per plant, PH = Plant height and CD = Canopy diameter.

leaf yield) was due to these traits negligible to high negative indirect effects via each other on leaf yield Whereas, the positive and significant phenotypic correlations of days to 50% maturity, leaves per plant, plant height and canopy diameter with leaf yield was due to the traits negligible to high positive indirect effects on leaf yield via leaf petiole length, leaf length, leaf width, ratio of leaf blade width to leaf length and petiole width.

In the current study, the residual effect was 0.27 (Table 3), showing that 72.9% of the edible vegetable leaf variability yield per hectare was explained by the factors of the component. The remaining is explained by other traits in the study that are not considered.

Conclusion

The genotypic correlation coefficient among edible vegetable leaf yield and all of agro-morphological traits was positive and significant, except leaves per plant and ratio of leaf width to length. Leaf petiole length, leaf length, leaf width, petiole width and plant height had a positive and highly significant genotypic correlation with edible vegetable leaf yield, both at genotypic and phenotypic levels. These traits also had a positive indirect effect on yield, either though each other or via other traits at the genotypic level. The strong association of these traits with leaf yield, and the high to low direct and indirect effects through other traits at the genotypic level, are an indication of the importance of the traits to use in selection of Ethiopian mustard accessions for high edible vegetable leaf yield. Days to maturity, leaves per plant and canopy diameter had positive and significant correlations with edible leaf yield per hectare, both at genotypic and phenotypic levels. However, the traits had negative and low to high direct effects on edible leaf yield at the genotypic level. In addition, ratio of leaf blade width to leaf length showed a significant and negative correlation with edible leaf yield per hectare; and exerted

a negative high direct effect on the trait. This suggested that it is not necessary to consider these traits for selection of genotypes for high yield, rather it is necessary to consider the traits such as leaf petiole length, leaf length, leaf width and petiole width.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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