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African Journal of Agricultural Research

Full Length Research Paper

Herbicidal effects of *Datura stramonium* (L.) leaf extracts on *Amaranthus hybridus* (L.) and *Tagetes minuta* (L.)

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Evolution of weeds resistant to herbicides demands new solutions to cope with the problem since economic losses generated by weeds can be higher than those caused by insect pests. Bioactive compounds known as allelochemicals have the potential to act as natural herbicides in weed management in agro-ecosystems. Laboratory, pot and greenhouse experiments were carried out to investigate the herbicidal effects of *Datura stramonium* aqueous leaf extracts on the germination and early growth of *Tagetes minuta* and *Amaranthus hybridus*. The laboratory and greenhouse experiments were arranged as completely randomised design, and the open field pot experiment was arranged as a randomised complete block design. Four concentrations of 2, 4, 6 and 8%, respectively of *D. stramonium* aqueous leaf extracts were used. Distilled water was the control. Data for germination, radicle and plumule length was collected within the first 10 days for the laboratory experiment. Root length, shoot length and biomass yield was collected 30 days after germination for both the greenhouse and field experiments. Results indicated that germination percentage, radicle length, plumule length and dry matter significantly decreased (P<0.001) as concentration of *D. stramonium* leaf extracts increased in all the experiments. This implies that *D. stramonium* has pre-emergence and early post emergence herbicidal effects on the two weeds. This study revealed that allelopathic sprays of *D. stramonium* can be used by resource poor small scale farmers or organic farmers for the control of *Amaranthus hybridus* and *T. minuta* in Zimbabwe.

Key words: Allelopathy, aqueous leaf extract, *D. stramonium*, *Amaranthus hybridus, Tagetes ereca*, herbicidal effects, germination, early growth.

INTRODUCTION

Allelopathy is defined as any process involving secondary metabolites produced by plants, algae, bacteria and fungi

that influence the growth and development of agricultural or biological systems (Macías et al., 2007; Thi et al., 2015). Allelopathy involves synthesis of bioactive compounds known as allelochemicals which are capable of acting as natural pesticides. Plants produce these compounds as a mechanism to defend themselves in the course of co-evolution. The fact that allelopathy is a crucial defense and attack weapon of the plant to gain a foothold on the community can not be ignored (Marcias et al., 2007).

According to Asaduzzaman et al. (2014), Casimiro et al. (2017) and Farooq et al. (2011), the wise exploitation of allelopathy in the cropping systems may be an effective, economical and natural method of weed management. These compounds are usually degraded easily in the environment due to their short half life as they contain fewer halogen constituents in their structures. Due to their impure nature, they usually contain a number of active compounds which can act on more than one site like a mixture of herbicides and may control a wider spectrum of weeds (Solts et al., 2013). This discourages the development of resistance. Abandoning of chemical control with current agriculture is rather impossible, it is therefore necessary to create new classes of herbicides with new mechanism of action and target site not previously exploited. Natural compounds pose as a potential source for the discovery of eco-friendly herbicides, so called bio herbicides (Solts et al., 2013).

The herb, *D. stramonium* is an annual upland weed that is widely distributed throughout the world. In Mexico, the plant inhabits open, cultivated and disturbed sites where they attain an average height of 1 m (Valverde et al., 2002). According to Fatoba et al. (2001), the plant is characterised by solitary white trumpet shaped flowers. Weed surveys done in Zimbabwe by Thomas (1971) and Chivinge (1983, 1988) classified the weed as aggressive and difficult to control. The plant has been increasing in the cropping systems and farmers cut it and use the leaves as mulch.

Currently, the weed has turned invasive, thereby making available its leaves for mulch placement in gardens and agronomic fields. Other farmers have reported that it reduces weed germination. It has been said that several chemicals have been identified and phytochemical investigators believe that there are still many other chemicals in *D. stramonium* which have not been identified to be exploited as bioherbicides (Elisante et al., 2014). Allelochemicals found in *D. stramonium* have allelopathic effects on survival of native plants. *D. stramonium* contains a series of allelochemical in form of alkaloids, atropine, hiosciamine and scopolamine (Butnariu, 2012), which inhibits the growth and development of root and shoots of *Trigonella* and *Lepidium* in a concentration dependent manner (EL-Shora and Abd EL-Gawad, 2014; 2015a; An et al., 1996). Currently, there is no basic information of the allelopathic effects of *D. stramonium* on *A. hybridus* and *T. minuta* which are seriously problematic arable weeds in Zimbabwe. The objective of this current study was to determine the multi-herbicidal effects or mode of actions of *D. stramonium* leaf extracts on the germination and early establishment of the *A. hybridus* and *T. minuta.*

MATERIALS AND METHODS

Experiment 1: Effect of *D. stramonium* concentration on the germination and early establishment of two weeds in the laboratory experiment.

Study site

The laboratory experiment was carried out at Midlands State University, located in Midlands province of Zimbabwe. The geographical location is 19˚45' S (line of latitude) and 29˚85' E (line of longitude). It experiences mean annual temperature of 18°C. The site is in agro-ecological region III, at an altitude of 1428 m (Vincent and Thomas, 1960; Mugandani et al., 2012).

Experimental design

The experiment was arranged as a complete randomised design with five treatments replicated three times. Treatments were 20 ml of distilled water (control) and aqueous *D. stramonium* leaf extracts applied at 2, 4, 6 and 8% concentration as a ratio of plant extract powder to 100 ml distilled water. 2 g of extract powder was added to 100 ml of distilled water to give 2% concentration of aqueous and the same was done for 4, 6 and 8% concentrations.

Preparation of *D. stramonium* aqueous leaf concentrations for the three experiments

Leaves of fully grown plants collected from the wild were washed to remove soil particles. The material was then cut into pieces and shed dried for one month. After drying, the material was crushed into powder form manually using a traditional mortar and pistil. Further grinding was done by using an electric mortar. The material (powder and distilled water) was mixed and poured into a conical flask with its mouth closed and kept for 24 h in the dark at room temperature according to the method used by Dhawan and Narwal (1994). The four flasks were marked with stickers according to the *D. stramonium* concentrations (2,4 ,6 and 8%, respectively). This was followed by filtration process in two steps. In the first step, muslin cloth was used, and later the filtrate was allowed to pass

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through Whatman filter paper no.1. The prepared aqueous concentrations were kept in a refrigerator for the duration of the experiment to prevent conversions of some of the compounds upon exposure to light and high temperature.

Experimental procedure

Two hundred and twenty five seeds of the selected weeds were surface sterilized with 0.1 % mercuric chloride solution for two minutes and washed twice with distilled water. The petri dishes were labelled with a permanent marker in relation to concentration level. Fifteen seeds of each weed were placed in petri dishes on Whatman filter paper no.1. Twenty millilitres of each *D. stramonium* aqueous concentration (2, 4, 6 and 8%, respectively) was added to each petri dish. The same amount of distilled water was used as a control. Watering was done after every three days, and the petri dishes were kept in an incubator at 24°C room temperature for 10 days.

Experiment 2: Pot experiment: Effects of different *D. stramonium* aqueous concentrations on germination and early seedling growth of weeds in the field.

Study site

The field experiment was carried out at Midlands State University, located in Midlands province of Zimbabwe. The geographical location is 19˚45' S (line of latitude) and 29˚85' E (line of longitude). It experiences mean annual temperature of 18°C. The site is in agro-ecological region III, at an altitude of 1428 m.

Experimental design

The experiment was arranged as a complete randomised design with five treatments replicated three times and two weeds were tested.

Experimental procedure for field and green house experiments

Two hundred and twenty five seeds of the selected crops were surface sterilized with 0.1% mercuric chloride solution for two minutes, and washed twice with distilled water. Five litre pots were used and they were filled with mixtures of soil (loamy sand). Fifteen seeds of each of the tested weeds were sown in each pot at 0.5 cm, and then irrigated with various solutions to field capacity every three days.

Data collection for field and green house experiments

Data on seed emergence, shoot, and root length; seedling fresh and dry weight was recorded. Seed emergence was determined by physically counting the number of seedlings on the 8th day after planting. During the experiment period (after 30 days after planting), shoot and root length was also measured using a 30 cm ruler. The dry weight was determined by placing the tested samples in the oven to a temperature of 110°C for 48 h until a constant weight was realised.

Experiment 3: Effects of different *D. stramonium* aqueous concentrations on germination and early seedling growth of weeds in the greenhouse.

Study site

The greenhouse experiment was carried out during the 15/16 summer season at Morningside suburb in Masvingo Province of Zimbabwe at a geographical location of latitude 20˚ 7' 17S and longitude 30˚ 49' 58 E. The site is in agro-ecological zone 4, at an altitude of 1034 m above the sea level. It receives an average of 600 mm of rain annually with a mean annual temperature of 28°C.

Experimental design

The experiment was arranged as a complete randomised design with five treatments replicated three times.

Data analysis

Collected data was subjected to Analysis of Variance at 5% significance level using Genstat 4.0 version 2013. Fishers protected least significance test at 5% was used to separate the means where significant differences were noted.

RESULTS

Germination and emergence

The results showed that the germination percentage as affected by *D. stramonium* aqueous leaf extracts was significantly ($P<0.001$) lower than the control at all levels in the laboratory percentage compared to the rest of the treatment (Figure 1) in the laboratory. As concentrations increased, germination percentage decreased. The highest germination (100%) was recorded where distilled water was applied in all tested species whilst 8% concentration significantly ($p<0.001$) decreased germination. The same trend was observed in the field (Figure 2) and in the greenhouse (Figure 3) where the emergence percentage decreased with increase in the concentration.

Radicle and root length

Results indicated that as the concentration decreased from 8 to 0%, the radicle and root length increased with a decrease in the concentration of *D. stramonium*. Results showed highly significant effects ($p<0.001$) of *D. stramonium* on *A. hybridus* and *T. minuta* as shown on Table 1 across all the environments.

Plumule and shoot length

Results indicated that aqueous concentrations of thorn

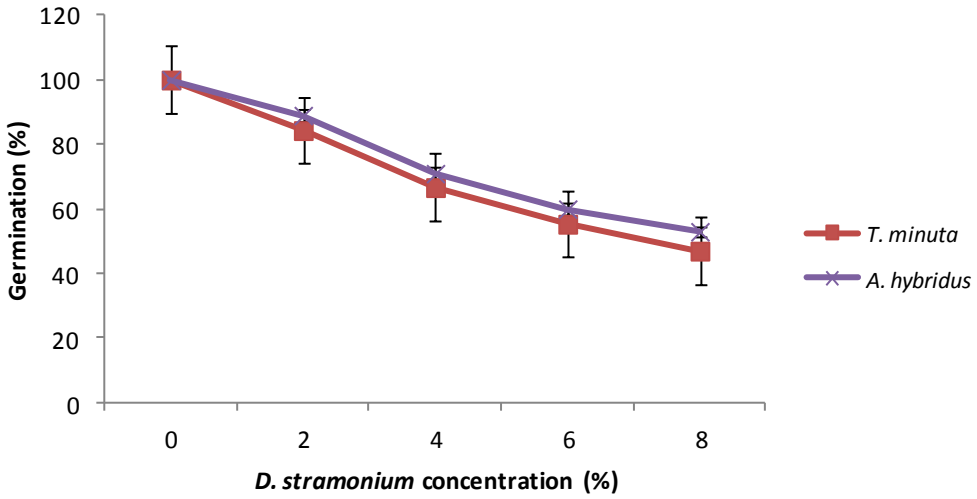

Figure 1. Effect of *Datura stramonium* concentration on the germination of the two weeds in the laboratory.

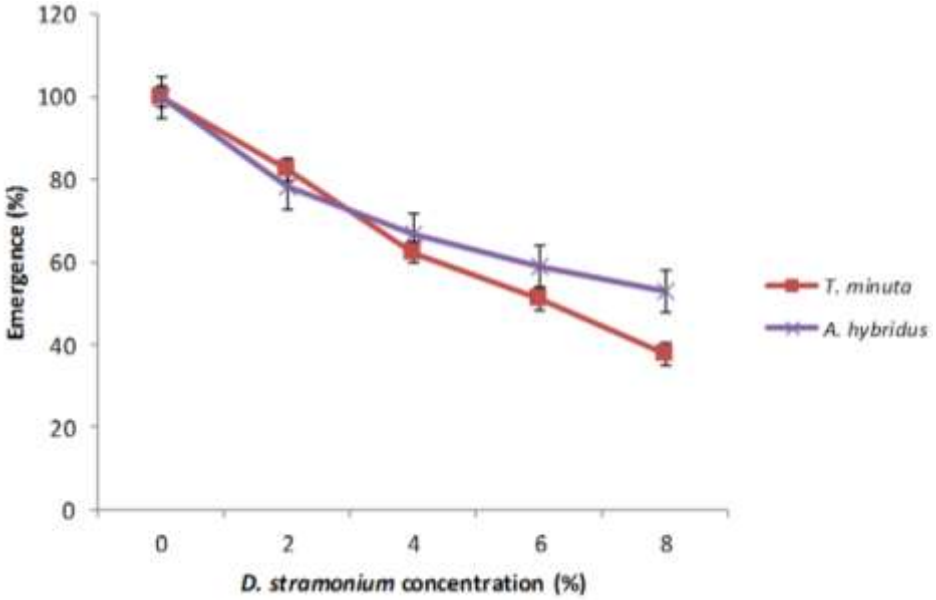

Figure 2. Effect of *D. stramonium* concentrations on the emergence percentage of the two weeds in the pot experiment in the field.

apple on plumule and shoot length was highly significant (P<0.001). Distilled water recorded the highest plumule length and shoot length when all treatments were compared on all tested species. It was observed that the rate of percentage decrease in plumule and shoot length was concentration dependent across all the tested species. Shoot length decreased as the concentration of *D stramonium* increased from 0 to 8% as presented on Table 2.

Dry matter traits

Results indicated that the effects of aqueous concentrations of thorn apple on seedling dry weight was significant (P<0.001). There was a general percentage

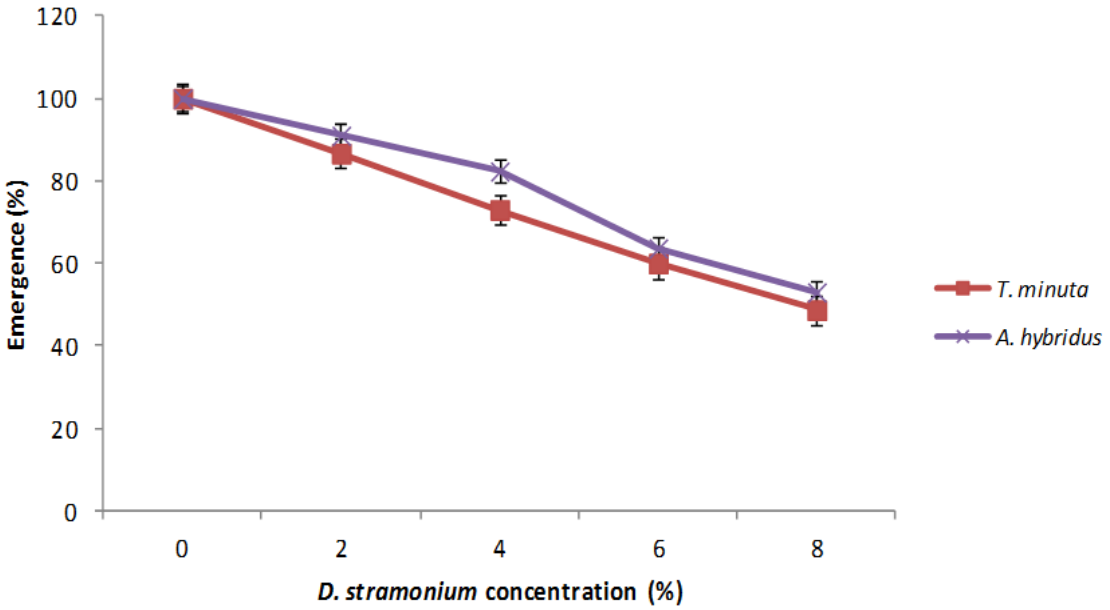

Figure 3. Effect of *D. stramonium* concentrations on the emergence of the two weeds in the greenhouse.

Table 1. Effect of the *D.stramonium* concentration on radicle and root length of *T. minuta.* and *A. hybridus* in laboratory, field and greenhouse conditions.

Concentrations	Laboratory		Field		Greenhouse	
	T. minuta	*A. hybridus*	*T. minuta*	*A. hybridus*	*A. hybridus*	*T. minuta*
0	26.97±0.14^{a}	28.67±0.491^{a}	44.17±2.09^{a}	80.4±1.21^{a}	80.13±1.07^{a}	44.17±2.09^{a}
2	25.1±0.14^{b}	24.97±0.491^{b}	38.37±2.09^{b}	75.2±1.21^{b}	74.50±1.07^{b}	38.37±2.09^{b}
4	22.1±0.14^{c}	22.83±0.491^{c}	34.3±2.09^{c}	64.9±1.21^{c}	64.43±1.07^{c}	34.30±2.09^{bc}
6	20.93±0.14^{d}	21.35±0.491^{d}	31.7±2.09^{d}	54.47±1.21^{d}	54.27±1.07^{d}	31.70±2.09^{c}
8	19.37±0.14^{e}	19.67±0.491^{e}	26.8±2.09^{e}	44.6±1.21^{e}	44.27±1.07^{e}	26.80±2.09^{d}
P-value	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
CV (%)	0.7	2.3	6.6	2.1	1.8	6.6

*Means followed by the same letter in the same column are not significantly different.

Table 2. Effect of the *D. stramonium* concentration on plumule and shoot length of *T. minuta* and *A. hybridus* in laboratory, field and greenhouse conditions.

Concentration (%)	Laboratory		Field		Green house	
	T. minuta	*A. hybridus*	*T. minuta*	*A. hybridus*	*T. minuta*	*A. hybridus*
0	16.5±0.09^{a}	20.07±0.705^{a}	65.47 ±2.85^{a}	80.13±1.07^{a}	65.03±0.27^{a}	80.40±1.22^{a}
2	14.83±0.09^{b}	18.40±0.705^{b}	63.17±2.85^{b}	74.50±1.07^{b}	62.37±0.27^{b}	75.20±1.22^{b}
4	12.90±0.09^{c}	16.30±0.705^{c}	60.13±2.85^{ab}	64.43±1.07^{c}	55.43±0.27^{c}	64.90±1.22^{c}
6	11.53±0.09^{d}	15.40±0.705^{cd}	55.50±2.85^{bc}	54.27±1.07^{d}	50.93±0.27^{d}	54.47±1.22^{d}
8	10.17±0.09^{e}	14.60±0.705^{d}	50.27±2.85^{c}	44.27±1.07^{e}	49.90±0.27^{e}	44.60±1.22^{e}
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	0.8	4.6	5.3	1.8	0.5	2.1

*Means followed by the same letter in the same column are not significantly different.

Table 3. Effects of the various *Datura stramoniium* concentrations on the dry matter properties of the *Targetis minuta* and *Amaranthus hybridus* in the greenhouse and the field experiments.

Concentration (%)	Field		Greenhouse	
	T. minuta	***A. hybridus***	***T. minuta***	***A. hybridus***
0	0.786±0.06[a]	2.013±0.069[a]	0.803±0.051[a]	2.037±0.076[a]
2	0.736±0.06[a]	1.86±0.069[b]	0.723±0.051[a]	1.887±0.076[a]
4	0.413±0.06[b]	1.673±0.069[c]	0.406±0.051[b]	1.697±0.076[b]
6	0.266±0.06[c]	1.49±0.069[d]	0.273±0.051[c]	1.513±0.076[c]
8	0.116±0.06[d]	1.26±0.069[e]	0.123±0.051[d]	1.28±0.076[d]
P value	<0.001	<0.001	<0.001	<0.001
CV (%)	14.3	4.6	12.4	5

*Means followed by the same letter in the same column are not significantly different.

decrease in seedling dry weight as aqueous concentration increased from 0 to 8% on all tested species. *Tagetes minuta* recorded the highest decrease of 54.8% whilst wheat and *A. hybridus* recorded seedling dry weight decreases of 22 and 15.3% respectively as concentration increased from 6 to 8%. The concentration of 2 % was not significantly different from the treatment watered by distilled water except for *A. hybridus* in the field (Table 3).

DISCUSSION

The results showed a reduced germination percentage with increasing concentration of allelochemicals from *D.stramonium* across all the measured weeds. These results concur with the findings of many authors (Hassannejad and Ghafarbi, 2013; Yu et al., 2003; Elisante et al., 2013; Levitt et al., 1984; Oyun, 2006; Alam and Islam, 2002). *D. stramonium* allelochemicals contains chemicals that retard the metabolism of food reserves in the seed (Levit et al., 1984) and the secondary effects of these processes include reduced germination and early growth of radicles (Levitt and Lovetti, 1984).

Altikat et al. (2013), Ullah et al. (2015) and Alam and Islam (2002) concur with these findings and reported that allelochemicals disturb the activities of the peroxidase alpha amylase enzyme and acid phosphatases which aid the breaking down of starch for successful germination to occur. Another assertion by EL-Shora *et al*. (2015a) and Oyun (2006) posits that allelochemicals inhibit water absorption which is a precursor for physiological processes that should occur before germination is triggered. All this help to support the assertion that *D. stramonium* has pre-emergence herbicidal effects.

Both shoot and root lengths of the two weeds were reduced by leaf extracts and the level of decrease depended on the concentration of the allelochemicals. Hussain and Reigosa (2011) found similar results on *D. glomerata, L. perenne* and *R. acetosa*. Gholami et al. (2011) concluded that *D. stramonium* alkaloids (hiosciamine and scopolamine) can reduce cell division or interferes with the auxin that induces growth of shoots and roots. Findings by EL-shora et al. (2015a) found that *D. stramonium* inhibit cell division. This can serve as a confirmation of the existence of the early post emergence effects of the allelochemicals. This further confirms the existence of more than one mode of action of herbicide which is critical in developing herbicides that are not prone to resistance development.

Total dry matter for all the weeds was reduced as concentration increased. Total dry matter is the function of the ability of the whole plant to obtain edaphic resources (minerals and water). Whilst all parameters were analysed individually, the cumulative contributions of the small differences has bigger effects on the metabolism of the whole plant (Robeiro, 2011). Any inhibition at each stage in the growth of the plant contributes towards reduced ability of the plant to capture resources for its survival. The various concentrations are therefore able to reduce dry mass of both weeds which indicated the presence of herbicidal effects.

Conclusion

We conclude that *D.stramonium* leaf extracts have both pre-emergence and early post emergence herbicidal effects towards the weeds studied. This study therefore recommends the use of *D. stramonium* leaf extracts at high concentrations as cheap bio herbicides to control *T. minuta* and *A. hybridus* in Zimbabwe. However, there is need for further research on the efficacy of other plant parts like the roots and fruits and solvent extraction method (ethanol and aqueous) of *D. stramonium*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Implications of climate change and variability on food security in Kenya

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This study assessed the impact of climate change and climate variability on food security in Kenya based on a sample size of 270 farmers randomly selected from semi-arid agro-ecological zones in Kenya. Both primary and secondary data were used in the present study. Data analysis was conducted using descriptive statistics and regression models. The results revealed that increase in temperature and reduction in rainfall had significant effect ($p<0.01$) on food security in Kenya. Increase in the amount of rainfall by 1 mm in the semi-arid areas enhanced food security by 0.01 kg while increase in maximum temperatures by 1°C reduced food security among households by 0.03 kg. Further, food security of Kenya is affected by increase in temperature and decline in rainfall. One unit increase in temperature would cause USD 3.9 million worth of value to become at risk as compared to USD 118,942 worth when rainfall decreased by 1 mm. Overall, the value at risk in the Kenyan economy is USD 112,351,112.46.

Key words: Food security, climate change, temperature, rainfall, risk.

INTRODUCTION

In Africa, Climate Change manifests itself as increasing trends in drought, temperature and reducing amounts and distribution of rainfall (Butt et al 2005). Drought, high temperatures, shortage of rainfall and floods exert heavy pressure on food resource availability, thereby, causing food insecurity (Dinar et al 2008; Mendelsohn and Tiwari, 2000). IPCC (2007) includes variability in the definition of climate change and state that climate change refers to a change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and that it is in addition to natural climate variability observed over comparable time periods. In order to ensure food security, it is necessary to minimize the impact of climate change and climate variability. This can be achieved by undertaking mitigation and adaptation measures. Food security is a situation when

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all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life. Household food security is the application of this concept to the family level, with individuals within households as the focus of concern (FAO, 2002). Conversely, food insecurity is a situation when people at any specified level, household, community, village, or country do not have adequate physical, social or economic access to food. Climate Change has negative affect on food security in sub-Saharan African (Downing, 1992). There have been limited studies in Kenya to establish quantitatively the impact of climate change and variability on crop and livestock production systems (KPSA 2014; IPCC, 2007). The food crops used in this study included maize, potatoes and beans.

The climatic conditions required for maize, potatoes and beans production are: (i) maize is grown under divergent physical conditions but best climatic conditions; a) temperatures between 18 and 27°C during the day and around 14°C during the night, b) annual rainfall between 60 and 110 cm but it is also grown in areas having rainfall as low as 40 cm and moderate altitude; ii) sweet potato requires warm weather of at least four to five months. It requires temperature range of 21 to 27°C and a well distributed rainfall of 75 to 150 cm. It cannot tolerate heavy rainfall which induces excessive vegetative tuber development. The optimum temperature for Irish potato production is between 15 and 20°C but can tolerate higher temperatures at 30°C that gives very low yield. Soil pH of 5 to 5.5 ($Cacl_2$) are favourable. Irish potatoes require 70 to 120 cm of rainfall but can be produced at higher rainfall beyond 120 cm so long as water logged conditions is avoided. In areas with heavy rainfall the crop becomes more susceptible to late blight. Irrigation requirements: provide days 25 to 30 mm of water for 3 to 4 days a week in light soils and 30 to 35 mm of water for 5 to 7 days a week in areas with heavy soil; and iii) common beans grow over a wide range of climate conditions. Beans grow in temperatures ranging from 14 to 32°C but the optimum growing temperature range is from 20 to 25°C. Extreme temperatures result into poor flower development and poor pod setting and therefore poor yields. Beans grow at an optimum altitude range of between 1,000 and 2,100 m above sea level. French beans mature faster in warmer areas. In rain fed cultivation production systems, beans require a well distributed medium to high rainfall of 90 to 120 cm per annum. It also requires well drained loams to heavy clay soils, with high organic matter contents and soil pH of between 6.5 and 7.5, but plants can tolerate up to pH 4.5. In the wake of Climate Change it is becoming rather hard to experience such ideal prodution conditions (Kurukulasuriya et al., 2006). This study assessed the impact of climate change and variability on food security in Kenya.

A number of studies undertaken to support this study include the risk to climate change, climate change waraness among farmers, adaptation options and some recommendations to improve resilience to climate change impacts.

Stefanovic et al. (2017) used a binary logistic regression to identify factors that influence individual's adoption of adaptation measures among two distinctive farmer groups; food crop producers and horticultural crops farmers. Adaptation measures employed by food crop farmers were mainly risk-reducing, such as mixed- and inter-cropping, planting early-maturing crop varieties and early planting. In contrast, horticultural farmers tended to focus more on intensifying crop production and applied crop rotation, irrigation and application of agro-chemicals, artificial fertilizer and manure. Factors positively influencing adaptation included access to extension services and risk perception among horticultural farmers, and access to workforce and farmers groups among food crop farmers. Furthermore, food crop farmers with access to less risk-prone income sources than agriculture seemed to have less motivation to adapt. The study showed that as climate change progresses, social differences between horticultural and food crop farmers are likely to increase, hence leading to inequalities in adaptation at local levels. Adaptation planners need to address these differences if sustainable adaptation is to be achieved.

Sennhenn et al. (2017) stated that climate variability is the major risk to agricultural production in semi-arid agroecosystems and the key challenge to sustain farm livelihoods for the 500 million people who inhabit these areas worldwide. Short-season grain legumes have great potential to address this challenge and help to design more resilient and productive farming systems. Results highlight that, based on specific morphological, phonological, and physiological characteristics, the three short-season grain legumes common bean, cowpeas and lablab follow different strategies to cope with climate variability. The climate-smart site-specific utilization of the three legumes offers promising options to design more resilient and productive farming systems in semi-arid Eastern Kenya.

Njeru et al. (2016) stated that the drier parts of Central Kenya are characterised by poor crop harvest due to unpredictable, unreliable and poor rainfall distribution patterns. The field experiment was laid out in Partially Balanced Incomplete Block Design (PBIBD) with 36 treatments replicated three times. The treatments of tied ridges and contour furrows under sorghum alone and intercrop plus external soil amendment of 40 kg P/ha + 20 kg N/ha + manure 2.5 t/ha had the highest grain yield of 3.1 t/ha. The soil fertility levels differed significantly from one another ($p = 0.0001$) in terms of sorghum grain yield. Generally, all experiment controls had the lowest grain yields as low as 0.3 to 0.5 t/ha. Therefore, integration of minimal organic and inorganic inputs under

various water harvesting technologies could be considered as an alternative food security initiative towards climate smart agriculture for climate change mitigation in drought-prone areas of Central Kenya.

Omoyo et al. (2015) carried out a study on the effects of climate variability on maize yield in the arid and semi arid lands of lower Eastern Kenya. The study confirmed that the arid and semi arid counties suffer from significant climate variability which has huge implications on maize yields and food security of lower Eastern Kenya. Thus, to counter the adverse effects of climate change, it is necessary to climate-proof agricultural crops through adaptation strategies such as developing maize varieties that tolerate water stress and mature early, practice early planting, increase the awareness of climate change and its impacts on agriculture, and develop appropriate mitigation measures. These findings are crucial in planning appropriate adaptation mechanisms in support of enhancing resilience of maize production and food security.

Gichangi et al. (2015) carried out a study on assessment of climate variability and change in semi-arid Eastern Kenya that revealed that a better understanding of farmers' perceptions of climate change, ongoing adaptation measures, and the decision-making process was important to inform policies aimed at promoting sustainable adaptation of the agricultural sector.

Thorlakson and Neufeldt (2012) revealed that subsistence farmers are among the people most vulnerable to current climate variability. They explored ways and techniques such as agroforestry that could help subsistence farmers reduce their vulnerability to climate change. Data from farmers in Western Kenya revealed that households though aware of climate change are not currently coping with climate-related hazards in a sustainable way. Evaluation of agroforestry as one possible means of improving farmers' well-being enabled a comparison of farmers engaged in an agroforestry project with a control group of neighboring farmers. Their findings show that involvement in agroforestry improves household's general standard of living via improvements in farm productivity, off-farm incomes, wealth and the environmental conditions of their farm. Thus, it was concluded that agroforestry techniques was an effective development strategy to help subsistence farmers reduce their vulnerability to climate-related hazards.

MATERIALS AND METHODS

This study was conducted in the semi-arid zones of Kenya, which are transitional areas between arid and medium to high agricultural potential areas that receive 300 to 900 mm of rainfall per annum. Semi-arid zones were considered for the study based on the sole criterion of their agricultural potential (agro-ecological zone) to support different crops and livestock. The sampling frame consisted of farmers in nine counties in the semi-arid zones. The nine semi-arid counties were randomly selected from 28 semi-arid counties, which is part of the 47 counties of Kenya. The counties involved in the study were Narok, Kajiado, Embu, Machakos, Makueni, Kilifi, TaitaTaveta, Kitui and TharakaNithi.

Multi-stage random sampling procedure was used to select 270 farmers from the nine counties who were interviewed to provide primary data for the study. From each of the chosen counties, one sub-county (district) was randomly selected and subsequently one ward (formally division) and finally one village were randomly selected. Farmers were then randomly selected from each of the villages based on farmers' lists held by the agriculture extension officers. The total population of all farmers in the nine counties was more than 10,000. Hence, the sample size was determined using the Scott Smith (2013) formula as follows:

$$n = \frac{Z^2 pq}{e^2} = \frac{1.645^2 x 0.5^2}{0.05^2} = 270$$

where n=number of respondents; p=estimated proportion of the distribution of the attribute which for this study was 0.5; q=1-p; z=is the statistical confidence level (in this study was pegged at 90%) whose corresponding statistical t-values is 1.645; and e=the error term of the desired level of precision (in this study was pegged at 5%). Based on the formula, the sample size for the study was taken as 270 farmers.

This study used both primary and secondary data. Primary data was collected from 270 farmers and 10 key informants using questionnaires and interview guides, respectively. Secondary data covering the period 1961 to 2016 was collected from published materials, government and non-governmental organization reports and websites.

Assessment of food security was undertaken at two levels. Firstly, the on farm food supply was computed from the primary data collected on food security and the factors that contribute to a household's food insecurity vis a vis security. Then multiple linear regression was run on food security (FS) as a function of affecting factors (Xi); FS=f(Xi). Thus, the evaluation of the farm households food security using regression for food security on its factors to sustain food supply at farm level involved factors such as climatic conditions, namely, rainfall and temperatures to sustain on farm production, employment, education, extension services, mechanization, use of certified seed, interactive effects of other factors such as the health of the farmer, area of land being cultivated and socio-economic factors like exportation/excess sale of food products from the farm. The model that was run using cross-sectional primary data is specified as follows:

$$FS = \alpha 0 + \alpha 1R + \alpha 2T + \alpha 3E + \alpha 4D + \alpha 5M + \alpha 6P + \alpha 7S + \alpha 8L + \alpha 9I + \alpha 10V + \alpha 11G + \mu$$

where FS = food security at farm level (in number of meals per day and the number of months a household remains food self-sufficient in a year), R = current rainfall amounts received (mm), T= current maximum temperature (°C), E = extension services (number of visits), D = farmer's education level (number of years in school), M = mechanization level, P = employment (on-farm versus off-farm), S = use of certified seed (Kgs of seed used), L = area of land (acres), V = soil improvement (Soil additives; manure and fertilizer (Kgs of fertilizer/manure)), I = interactive effects of other factors like the health of farmers, farmers social capital (no units), G = market derived factors such as availability/non-availability of key crop commodities, distance from the market, ease of access for a farmer to sell his/her crop surplus (no units) maize, beans, potatoes derived from the net trade of crops on the local markets as market information about the net scarcity of the crops in the various villages.

The reason for using the food indicators as variables in the food security model is to determine the contribution of each variable to

the household food security in Kenya.

Secondly, evaluation of the risk of hunger among farm households in Kenya was computed by carrying out a vulnerability analysis that addresses the issues of future incidents of food insecurity. Rosenzweig and Hillel (1998) and Rosenzweig et a. (1995) stated that climate change may influence food security or insecurity. For this purpose, the FAO value-at-risk model (2006) was used to calculate the risk of hunger, stated as:

$$V(y^*,X) = \int W(y)\delta F(y \mid X).$$

where y* = critical benchmark value for food security indicator (A minimum of 90 kg of maize per year or its equivalent), y = food security indicators (number of meals per day, dietary diversity (number of food sources consumed per day) and number of months the farm produce lasts before access from the market), W(y) = individual household welfare function for each household as part contribution to the nationwide welfare (∫W(y)) that would be determined, ∫W(y)) = nationwide welfare summed from individual household welfare, ∫ = integration function; summation of the individual household welfare into nationwide welfare, $F(y \mid X)$ = conditional distribution function of the future food security associated with each food security indicator as Xi in the food security regression model. After deriving the food security situation as a summation of the food indicators, an evaluation was carried out against each of the conditional variable X (level of education, area of land level of mechanization, amount of rainfall, maximum temperature, level of employment, access to extension services, soil improvement factors, interactive effect and amount of certified seed used), X = vector of conditioning variables (Household coping strategies, risk management government policies and other factors outside the household and government control), V(y, X) = welfare loss associated with food insecurity also called value at risk.

This gave the severity of the current food insecurity position and by projection future indications of becoming more food insecure or secure and what needed to be done to improve the situation in the communities under study. This information was captured and summarized in the food insecurity transition matrix.

RESULTS AND DISCUSSION

The study established that 34.43% of the farm households were food insecure taking 1.3 out of the possible 3 weighted food security score as the critical benchmark (Figure 1). The 1.3 mark implied that a farm household had at-least one meal per day and harvested food on-farm that would support at least the family for 2 months. Further, this graph showed that only 10% of the respondents were more than 60% food secure. This implied that a farm household may afford at least 2 meals per day and may harvest on-farm food that may support the family for at least 5 months. The weighted food security was revealed by three parameters; number of months households are food self-sufficient, average number of meals households had per day and the percent off-farm food source of households interviewed per year in semi-arid areas.

Multiple linear regression revealed that correct levels of temperature and rainfall contributed positively to food security scenarios among the households and this was statistically significant at 1% (Table 1).

The results showed that increase in the amounts of rainfall received by 1 mm in the semi-arid areas increased food security by 0.01 kg while increase in maximum temperatures by 1°C reduced food security among households by 0.33 kg. Additionally, level of education, employment and soil improvement initiatives all had a significantly positive contribution to food security. These result implied that correct levels of temperature and rainfall increased food security among the farm households in Kenya. Additionally, level of education, employment and soil improvement initiatives, use of farm yard manure and fertilizer, had a statistically significant positive contribution to food security. This implied that when people get more education and better jobs, their food security component increases. Likewise, increasing soil fertility would lead to better yields which in turn would improve food security. However, the rest of the factors, level of mechanization, use of certified seed, area of land under cultivation and access to extension services, had a negative attribution to food security. This is attributable to the fact that firstly, level of mechanization in Kenya is mainly used by large scale farmers whose food security is not really an issue but income. This was also the reason for increased land area under cultivation that had a negative coefficient though statistically non-significant in the model. The use of certified seed and access to extension services had a very small negative impact on food security which indicated that even without these two factors Kenyan farmers may become food secure.

Assessment of the value at risk was carried out to evaluate the vulnerability and therefore the impact of climate change on food security in Kenya. The FAO's value at risk model developed in 2006 was used to measure the probability of a household and community to fall below the critical food security threshold. This model integrated the social welfare loss realized from the loss in revenue and the food security indicators to simulate the loss attributed to each of the food security indicators as used in the food security regression model in Table 1.

The FAO value-at-risk (VAR) model (2006) was used to calculate the risk of hunger caused by climate change. Use of this method is consistent with the study by Scaramozzino (2006). The VAR was stated as an indirect component of the welfare loss function evaluated as $V(y^*, X) = \int W(y)\delta F(y \mid X)$. In this case, y* = critical benchmark value for food security indicator, y = food security indicators, W(y) = household welfare function, $F(y \mid X)$ = conditional distribution function of the future food security indicator and X = vector of conditioning variables (household coping strategies, risk management government policies and other factors outside the household and government control). This gave an indication of the severity of the farmers' food insecurity position and by projection future indications of becoming more food insecure or secure and what needs to be done to improve the situation in the communities under study. Using the loss in the social welfare due to climate change

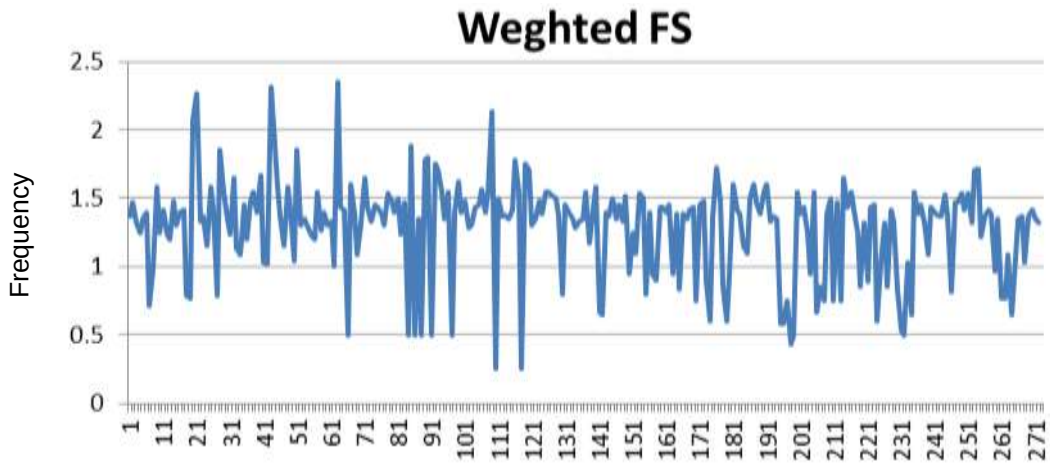

Figure 1. A graph of the frequency of food security occurrences among the farm households.

Table 1. Regression output of food security and climate change.

Parameter	Coefficient	Standard value	t-value	P-value	Beta-value
Rainfall	0.007	0.000	4.65	0.000	0.253
Max. temperature	-0.033	0.007	-4.96	0.000	-0.209
Level of education	0.056	0.023	2.45	0.015	0.146
Soil improvement factors	0.000	4.42×10^{-6}	3.16	0.002	0.180
Interactive	9.07×10^{-9}	4.33×10^{-9}	2.09	0.037	0.122
Interaction	-1.02×10^{-6}	8.6×10^{-7}	-1.19	0.236	-0.081
Area (acres)	-0.007	0.003	-2.80	0.006	-0.144
Level of mechanization	-0.026	0.038	-0.70	0.417	-0.043
Level of employment	0.058	0.038	1.52	0.129	0.111
Access to extension services	-0.034	0.037	-0.92	0.356	0.061
Use of certified seed (kg)	-0.001	0.001	-0.98	0.328	-0.060

$F_{(11, 254)} = 335.32$, $p<0.01$, Adjusted $R^2 = 0.9267$ and Root MSE = 0.4743

$$FS = 0.01R - 0.0328Tmax + 0.0565\ Educ + 1.0X\,10 - 5Soil\ improve + 9.07X10 - 9\ G(Interactive) + 0.058Employment - 1.026I\ (Interact) - 0.007Area - 0.0262\ Mechanization - 0.0344\ Extension - 0.001Seed$$

as:

$$S* - S0 = Wl = \int \{PS - CS\}\delta S = -115{,}948{,}398.5 - 2{,}993{,}776.79 = -118{,}942{,}175.29 = \int W(y)$$

The value at risk due to climate change is given as $V(y^*, X) = \int W(y)\delta F(y \mid X)$. Based on results from Table 1 and the coefficients in the earlier stated equation, the only parameter not estimated is $\delta F(y \mid X)$ which is derived from the function $Y=f(x_i)$. This was achieved through a linear regression of food self-sufficiency as captured by the number of meals per day a family was able to afford on level of education, area of cultivated land in acres, level of mechanization, amount of rainfall, maximum temperature, level of employment, access to extension services, soil improvement factors, interactive effect and amount of certified seed used in kilograms (Table 1).

The value at risk was deduced using the coefficients contained in the model as follows:

(a) Rainfall impact on value at risk if $V(y^*,X) = \int W(y) dF(y \mid X_i)$ = -118,942,175.29 $dF(y \mid X_{rainfall})$ = -118,942.175 × 0.001 = -118,942.175

This resulted into a negative value that signifies that excess rainfall beyond the critical values had a negative impact on food security. This is specifically true in cases of floods and flood prone areas.

(b) The value at risk due to maximum temperature fluctuations will be

$V(y^*, X) = \int W(y) dF(y \mid X_i)$ = -1,517,631.995 $dF(y \mid X_{max.temp})$ = -118,942,175.29 X0.033 = -3,925,091.785

This results show that increasing temperature had a

negative effect on food security in Kenya

(c) Likewise, the value at risk due to education

$V(y^*,X) = \int W(y) dF(y|X_i) = -1,517,631.995\ dF(y|X_{level\ education}) = -118,942,175.29 \times 0.056 = -6,660,761.816$

The results also indicated that increased education had a negative impact on food security since it withdraws farm labour from rural areas in search for white colar jobs.

(d) Soil improvement: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{soil\ improvement}) = -118,942,175.29 \times 0.000014 = -1665.19$

Soil improvement had a small negative impact on food security since it costed the family reources when it was most needed during the growing season.

(e) Interactive effect of climate change and farm inputs: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{interactive}) = -118,942, 175.29 \times 0.00000000907 = -1.07881$

This interactive effect has a negative impact on food security

(f) Employment: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{employment}) = -118,942,175.29 \times 0.058 = -6,898,646.167$

Employment has a negative impact to food security since it exposes families to cyclical food insecurity spells dictated by seasonal incomes, usually monthly intervals.

(g) Interaction of adaptation methods and soil improvement: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{interaction}) = -118,942,175.29 \times -1.026 = +122,034,671.8$

The use of adaptation methods improved the effects of soil fertility to give a positive impact on food security in Kenya

(h) Area: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{Area}) = -118,942,175.29 \times -0.007 = +832,595.227$

The amount of land a farm household owned has a positive effect on the family's food security.

(i) Mechanisation: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{Mechanization}) = -118,942,175.29 \times -0.0262 = +3,116,284.993$

The results support the theory that mecahnisation improved food security in rural areas and likewise;

(j) Extension: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{Extension}) = -118,942,175.29 \times -0.0344 = +3,972,668.655$

This implies that extension services improved food security and use of

(k) Certified seed: $V(y^*,X) = \int W(y) \delta F(y|X_i) = -1,517,631.995\ \delta F(y|X_{seed}) = -118,942,175.29 \times -0.001 = +118.942.175$

This means that the use of certified seed improved food security among the farm households in Kenya.

These computations are shown in Table 2. These results depict positive contributions of extension services, area under cultivation, mechanization and use of certified seed to food security. Reduction in rainfall and increases in temperature lower the food security positon of households in Kenya. These results suggest a need for mitigation and adaptation measures for climate change and variability to minimize food insecurity in Kenya.

Computation of transitional levels of food security and insecurity revealed that the benchmark levels of the various factors that signify food insecurity or security indicators are shown in Table 3. The results demonstrated that access to extension services and improvements in the levels of education have positive interaction with food security in Kenya. This can be explained by the fact that relatively educated farmers exerted more efforts and take measures to improve food production and become food secure. On the other hand information received from extension workers provided required skill to improve food production that may lead to improved farmer's access to food thereby, improving food security.

Comparison of optimal climatic conditions for production of the four crops with those experienced in Kenya is shown in Table 4.

The climatic conditions received in Kenya can marginally support maize and potato production. Thus, the perpetual crop failures and food insecurity experienced in Kenya.

This study tested the hypotheses that climate change and variability had no effect on food security in Kenya. The evidence for this hypothesis is anchored on the premise obtained from a regression of food security on climate change parameters and other food security factors whose output is shown in Table 5.

The results revealed that an increase in rainfall had a positive impact on food security and this was statistically significant at 1%. Maximum temperatures also had an impact which was significant at 1%. A compounding effect is realized from calculating the value at risk which revealed the extent of rainfall changes and temperature changes causing reduction in the net effect of food security. Given that:

$$V(y*,X) = \int W(y) dF(y|Xij) = -1,517,631.995\ dF(y|Xrainfall, temperature$$

For rainfall,

$$V(y*,X) = \int W(y) dF(y|Xij = -118,942,175.29X\ 0.001 = -118,942.175$$

Table 2. Summary of the value at risk.

Food security factorr	Corresponding effect ('000)
Rainfall	-118.942
Temperature	-3,925.092
Education	-6,660.762
Soil improvements	-1.665
Interactive effect of climate change and farm inputs	-0.001
Employment	-6,898.646
Interaction of adaptation methods and soil improvement	+122,034.672
Area under cultivation	+832.595
Mechanization	+3,116.285
Extension services	+3,972.669
Certified seed	+118.942
Net VAR in USD	112,351.112

Table 3. Food insecurity (FI)/Security (FS) transition matrix in Kenya.

Description of variable	Food secure	Moderately food secure	Benchmark	Slightly food insecure	Food insecure	Acute food insecure
Food consumption levels (kg maize/year)	>150	135 - 150	135	100 - 135	75 - 100	<75
Rainfall (mm)$^{\phi\phi}$	≥1200	1000	750	500	400	≤250
Temperature (°C)$^{\phi}$	20	25	28	30	33	≥33
Education level	Tertiary	Secondary	Completed primary	Incompleted primary	No education	No education
Employment	Both farmer and employed	Formal employment	Farmer	Small business	None	none
Extension services	Closer Extension	More frequent extension	Moderate	Lower extension	Lack of extension	Lack of extension
Access to purchased inputs seed	Adherence: recommend'	Moderately	Yes	Lower	0	0
Fertilizer	High use	Moderate use	Yes	Low use	0	0
Agroecological zone	I-II	III-IV	V	VI	VII	VIII
Access to land acres	≥10	10	5	2	1	0
Social capital (No. of social groups)	≥3	3	2	1	0	0
Amount of Wealth inherited	Good inheritance	Medium inheritance	Lower inheritance	None	0	0

The parameters marked $^{\phi}$ and $^{\phi\phi}$ are the critical determinatns of food crop production conditions.

While for temperature,

$$V(y*,X) = \int W(y)dF(y|Xj) = -1{,}517{,}631.995\, dF(y\,|\,\text{Xmax.temp}) = -1{,}517{,}631.995\text{X}\,0.033 = -3{,}925{,}091.175 \quad ,$$

respectively.

Based on the results, the null hypothesis was rejected, which means acceptance of the alternative hypothesis that lends credibility to the fact that climate change would have impact on food security. More specifically, increases in temperature and reduction in rainfall reduce food

Table 4. The optimal climatic conditions compared to those received in Kenya.

Condition	Maize		Potato		Beans	
	Optimal	Experienced in Kenya	Optimal	Experienced in Kenya	Optimal	Experienced in Kenya
Temperature$^{\phi}$ (°C)	22.5	27	17.5	27	22.5	27
Rainfall$^{\phi\phi}$ (%)	85	70	95	70	105	70
Soil	Loams	-	Non-logged	-	Well drained	-

Table 5. Hypothesis testing the impact of climate change on food security.

Parameter	Coefficients	Standard deviation	t-value	P-value	Beta-value	Significance
Rainfall (mm)	0.001	0.000	4.65	0.000	0.253	Sign. at 1%
Max. Temperature (°C)	0.033	0.007	4.96	0.000	0.209	Sign. at 1%
Level of education (No. of years in school)	0.056	0.023	2.45	0.015	0.146	Sign. at 5%
Soil improvement factors	0.000	4.42×10^{-6}	3.16	0.002	0.180	Sign. at 5%
Interactive	9.07×10^{-9}	4.33×10^{-9}	2.09	0.037	0.122	Sign. at 10%
Interaction	-1.02×10^{-6}	8.6×10^{-7}	-1.19	0.236	-0.081	Not Sign.
Area (acres)	-0.007	0.003	-2.80	0.006	-0.144	Sign. at 1%
Level of Mechanization	-0.026	0.038	-0.70	0.4168	-0.043	Not Sign.
Level of employment	0.058	0.038	1.52	0.129	0.111	Not Sign.
Access: extension services (No. of visits)	-0.034	0.037	-0.92	0.356	0.061	Not Sign.
Certified seed (kg)	-0.001	0.001	-0.98	0.328	-0.060	Not Sign.

security.

Assessing household's social welfare change caused by climate change was conducted using the FAO's Value at Risk model (2006) that allowed for assessment of the social welfare of households and communities. This was realized by integrating the loss in revenue and the risk to food insecurity to simulate the loss attributed to each food security indicators as used in the food security regression model in Table 1. The model used to calculate the risk of hunger in this study as discussed earlier is $V(y^*,X) = \int W(y)dF(y|X)$, which involved estimation of the conditional parameters to the main function F(y), that were pre-disposing factors X_i for a household to either become food secure or insecure such as level of education, access to farm inputs (fertilizers), level of employment, area of land under cultivation and level of mechanization. Thus, the model $F(y|X)$ provides for inclusion of conditional factors outside the farmers control like coherent social capitals in place such as conducive policies, extension services, prevailing climatic conditions and interaction of such factors. The results from this model gave different values for each condition calculated as follows and outputs summarized in Table 6.

$$V(y*X) = \int W(y)dF(y|Xi) = 1{,}517{,}631.995\, dF(y|Xrain)$$
$$= -118{,}942{,}175.29X\,0.001 = -118{,}942.175$$

Excessive rainfall increases food insecurity but also people in high rainfall areas had less number of meals since they spent most of their time out in the fields. However, it was also true that these people had heavier and more nutritious foods that were not captured in the model. The value at risk due to maximum temperature fluctuations was:

$$V(y*X) = \int W(y)dF(y|Xi) = -118{,}942{,}175.29\, dF(y|Xmax.temp)$$
$$= -118{,}942{,}175.29\,X0.033 = -3{,}925{,}091.785$$

This implies that Kenya would lose more in terms of becoming more food insecure when temperatures increased than when rainfall fluctuated. The result implies that a one unit

Table 6. The value at risk and food security (FS) at farm level.

S/N	Parameter	Effect on FS and wealth ('000)	Rank	Comment on each effect at farm level
1	Rainfall	-118.942	4	Excess rainfall depressed food security
2	Temperature	-3,925.092	3	Temperatures depressed food security
3	Education	-6,660.762	2	Education reduced food security at farm
4	Employment	-1.665	5	Off farm reduced on farm food security
5	Soil fertility	-0.001	6	Statistically insignificant
6	G-interaction	-6,898.646	1	Have insignificant effect on food security
7	I-interactive	122,034.672	A	Market parameters increased food security
8	Land area	832.595	D	Increased farm size increased food security
9	mechanization	3,116.285	C	Mechanization increased food security
10	Extension	3,972.669	B	Extension services increased food security
11	certified seed	118.942	E	Certified seed increased food security

increase in temperature caused USD 3.9 million worth of value to become at risk as compared to USD 118,942 worth when rainfall decreased by 1 mm. Overall, the value at risk in the Kenyan economy is USD 112.351 million, while the value at risk due to all other food security factors calculated in a similar way was recorded and ranked as Table 6.

The factors ranked 1 to 6 increased the probability to the risk for the loss of the value at risk and food insecurity by the magnitude indicated against each indicator, while those ranked A to E reduced it. They are associated with increasing the chances of improving the food security scenarios at farm levels. For example, increasing the level of education and employment would reduce the workforce at the farm levels and therefore aggravate the value at risk as opposed to lose in value. As such indication of the farm household to suffer hunger and its severity in terms of exposure can be calculated well in advance. Thus, it is possible for the government to clearly determine the current and in the near future by projection of those who may become food insecure and plan for mitigation and corrective action to improve the situation in the Kenyan communities. On the education and employment in Kenya, family labor is lost once a family member gets educated and employed because of the misconception that the education implies pulling out of the rural areas. Second, the family members (children and wife) who could otherwise be taking over from the aging generation also live in town with the educated person. In addition, the money earned may be sent to rural areas to hire labor but it may not completely carter for the four to five persons leaving in town away from the farm. The grand children are also withdrawn from the farms in pursuit of education. The sum total then is that the amount of labor is grossly reduced since the hired labor is never adequate and focused on the farm. Lastly the parents would be progressively growing older and weaker to continue providing the labor required. Then there is an aspect of land subdivisions fragmenting the parcels of land to tiny and economically unviable portions. Theoretically, education and employment can empower a farmer to hire labor but practically this has not been effective in Kenya. In addition, the hired labor in amounts does not take care of the quality of services offered by the family labor. Hired labor requires a lot of supervision which cannot be provided by the aged on the farm.

Conclusions

Climate change and variability, which occur in terms of changes in rainfall and temperature over time have major implications on farm household capacity to access food in a sustainable manner and in the required quantities and quality. Rising temperature is more critical as compared to reduced rainfall. A combination of temperature increases and decline in rainfall affects food security of households to a greater extent than individual effects of rain. climate change and climate variability cause both food insecurity and vulnerability to poverty. Addressing climate change and climate variability is therefore important to assure food security at household and national level. This can be achieved through the use of correct mitigation and adaptation measures to climate change and variability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Handling and utilization pattern of cattle milk and milk products in Northern Ethiopia

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A study was carried out in Enderta district, northern Ethiopia, with the objective of investigating traditional cattle milk and butter production, off take and traditional handling and utilization pattern of bovine milk and butter. Respondents who have milking cows and experience of dairying were purposively selected for the study. A semi structured questionnaire and checklist were prepared to collect the data through interview, farm visit and group discussion. Milk and butter were the most common dairy products that had different utilization patterns. Milk was used for household consumption, calf feed, accumulated on daily bases for fermentation and for sell; whereas, butter was used for household consumption as food, for cosmetic purpose and for sell. The overall average milk off take per day per household was 6.81±0.57 L of which, 62.94% was traditionally processed to butter and butter milk. The overall average butter production per household per week was 1.25±0.05 kg out of which, 80.2% was allocated to market and the remaining to cosmetic and sale. The dominant milking utensils in the area were 'Kordo', 'Gefho' and 'Jerican'. These were used for milking, fermenting and churning, respectively. Milk vessels were smoked for the purpose of increasing shelf life of milk and milk products. The interest of farmers engaging on dairy farming is increasing; but they lack awareness on keeping quality of milk and milk products. Almost half of the producers in the area did not use detergents to wash their milk vessels. Therefore, milk producers should be supported with strong extension service by way of introducing improved dairy technologies, improved milk handling and processing equipments.

Key words: Handling, utilization, milk vessels, transhumance, sedentary milk, butter.

INTRODUCTION

The Tigray National Regional State is the fourth cattle populated region in Ethiopia next to Oromiya, Amhara and Southern Nations Nationalities and People's (SNNP) regions. It is home to 2,622,166 cattle out of which 49% are male and 51% are female (MOARD, 2007). In spite of this huge resource, little work has been done on dairying;

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Table 1. Milk and butter production in Enderta District.

Variables	Transhumance	Sedentary	Over all	T	P-value
Average milk off take (liter/day):	N=45	N=105	N=150		
Mean ±SE	7.26±1.34	6.61±0.65	6.81±0.57	0.13	0.603
Average butter off take (kg/week):	N=45	N=105	N=150		
Mean ±SE	1.21±0.085	1.27±0.06	1.25±0.05	-0.47	0.641

N = number of observation.

except the work of characterization of urban dairy production in Mekelle (Negussie, 2006) and that of Improving Productivity and Market Success (IPMS) in Astbiwenberta and Alemata districts.

Enderta is one of the districts in south east zone of Tigray region, Ethiopia. The capital city of the region Mekelle with population of 169,207 (CSA, 2006), is encircled within Enderta district making it more advantageous from milk market proximity point of view. However, study on the current production, handling and utilization pattern of cattle milk and its products are not studied in the district. Such information is critically important for designing appropriate dairy development strategies, market orientations and providing base line information for further research and development. Moreover, agricultural knowledge and information are key components in commercial smallholder dairy developments as this play a significant role in improving productivity, linking producers to markets, improving competitiveness in markets and thus leading to improved livelihood, food security and national economies (Tesfaye et al., 2008).

In view of the above, the present study was designed to investigate cattle milk and butter production off take and their traditional handling and utilization pattern in Enderta district.

MATERIALS AND METHODS

Description of the study area

Location

The study was carried out in Enderta district, located about 760 km at 13°15`00`` N and 39°30`30`` E with an altitude ranging from 1500 to 2000 m above sea level in north of Addis Ababa.

Farming system

Two cattle production systems namely, sedentary and transhumance livestock production systems are practiced in Enderta district covering 70 and 30% of the total area in the district, respectively.

Sampling procedure

Four peasant associations “Dergeagen” from transhumance and the rest three “Didba”, “Kedemay-weyane” and “Arato” from sedentary livestock production systems were selected purposively based on their cattle population and milk production potential. Proportional probability to size (PPS) method was applied to select 150 respondents from the above specified areas in the district.

Data collection

A cross sectional study was conducted using semi structured questionnaire for interviewing respondents individually and also check list was developed to collect data through group discussion, interviewing key informants and farm visit.

Data management and statistical analysis

The data was entered into the data base management software Micro-soft-Excel computer program and analyzed using SPSS version 20. Descriptive statistics (frequencies and percentages) was used to summarize qualitative variables and means for quantitative variables were compared by independent samples t test. Chi square test was also applied to test qualitative variables.

RESULTS

Milk and butter production

As indicated in Table 1, the overall average milk off take recorded in this study was 6.81±0.57 L per day per household. This finding was in line with the report of Tesfaye (2008) for Metema district (6.3 L per household per day). But this result was lower than the result recorded (19.7±1.2 L per day per small farms) by Negussie (2006) in Mekelle zone. This might be related to the number of lactating cows and breed type they had as most of the households in the present study had local cows.

Milk and butter utilization

In this study, 8.45, 63.02, 14.12 and 14.37% of milk is utilized for household consumption, fermented for further processing, calf feeding and marketing, respectively (Table 2). This result is in agreement with the report of Abera (2008) who reported that 10 and 15% milk was allocated for consumption and sale in Kuyu district of Oromia regional state, respectively. The proportion of milk allocated for consumption in this study was quite smaller than the finding by Tesfaye (2009) in Metema district (23.3%). Milk allocated to fermentation in this

Table 2. Milk and butter allocation for different purpose.

Variables	Transhumance N=45		Sedentary N=105		Over all N=150	
	Sum	%	Sum	%	Sum	%
HH consumption	41	12.55	59	8.45	100	9.80
For fermentation	205	62.76	437.5	63.02	642.5	62.94
Calf feed	41	12.55	98	14.12	139	13.62
Marketed	38.62	11.82	99.75	14.37	138.37	13.55
Total milk (litter/day)	326.62		694.25		1020.88	
Consumed	2	3.71	5	3.91	7	3.85
As cosmetic	8	14.82	21	16.41	29	15.97
Marketed	44	81.48	101.95	79.68	145.60	80.2
Total butter (kg/week)	54		127.95		181.60	

Table 3. Milk and milk products handling and storing utensils in Enderta district.

Variables	Groups	Transhumance		Sedentary		*Overall*	
		N	%	N	%	N	%
MHU	Kordo/ plant source	25	55.6	38	36.2	63	42.0
	Plastic material	20	44.4	67	58.0	87	58.0
	Total	45		105		150	
YHU	Gefho/clay pot	30	66.7	62	59.0	92	61.3
	Jerican/plastic material	15	33.3	43	41.0	58	38.7
	Total	45		105		150	
BHU	Kucho/clay source	12	26.7	14	13.3	26	17.3
	Hamham/plant source	12	26.7	32	30.5	44	29.3
	Plastic material	8	17.8	30	28.6	38	25.3
	Kordo/plant source	13	28.9	29	27.6	42	28.0
	Total	45		105		150	
GHU	Kucho/clay source	28	62.8	56	53.3	84	56.0
	Plastic material	17	37.8	49	46.7	66	44.0
	Total	45		105		150	
Churner	Kucho/clay source	28	62.8	56	53.3	84	56.0
	Plastic material	17	37.8	49	46.7	66	44.0
	Total	45		105		150	

N= Number of respondents, MHU = milk handling utensil, YHU = yoghurt handling utensil, BHU = butter handling utensil, GHU = Ghee handling utensil.

finding (62.94%) was similar to that of Metema district (63.2%), but the milk for sale in this study (13.55%) is higher than that in the finding for Metema district (0.4%). This difference may show that, milk sale is more accustomed to the people of the current study area or there may be better market access.

Milk and milk products handling and storing utensils

As indicated in Table 3, 'kordo' and plastic materials were the dominant milking utensils in transhumance and sedentary production system, respectively.

On overall basis, 61.3 and 38.7% of the respondents use 'Gefho' and 'Jerican' to store milk for fermentation. 'Laga' and Jerican were also used as a churning material in 51.3 and 48.6% of the farms, respectively. Dairy farmers in north western Ethiopian highlands also use different milk utensils like gourd and clay pot for collecting, storing and processing milk (Ayenew et al., 2009). Tesfaye (2007) and Belete (2006) also found milk producers using traditional materials for milk handling, storing and churning in their respective study areas of Amhara region. 78.9% milk equipments in Bench maji

Figure 1. Milk and milk products handling equipments
Source: photo taken from study area

zone of Southwest Ethiopia were made of plastic materials (Gemechu and Amen (2017). The finding on milk producers using plastic materials for milking is similar to study of Tadesse et al. (2015) who reported that 54.3% of milk producers used plastic materials for milk handling in and around Hosanna Town, Hadya Zone of Southern Ethiopia (Figure 1).

Smoking of milk and milk products utensils

As indicated in Table 4, all (100%) of surveyed respondents applied smoking of milk utensils for different purposes. They had different reasons for smoking milk handling and storing utensils. The major reasons forwarded were: for good flavor of the products, for longer shelf life of the products, to have orange colored butter and to have attractive odor butter in order of importance. Practice of milk utensils smoking was also reported by Adebabay (2009), Kedja (2008) and Tesfaye (2007) in Bure, Meiso and Metema districts, respectively.

As presented in Table 5, 53.3% of the respondents used detergents like soap to wash milk vessels and 46.7% of them did not use detergents. The proportion of milk producers that used detergents to wash milk vessels in this study was higher than that of Eastern Ethiopia (17.5%) reported by Amentie et al. (2016). 96% of milk producers in Enderta district used pump water (clean water) to wash milk vessels. This result is in contrast to the findings of Bereda et al. (2013) who found that 57.2% of the producers in Gurage zone of Southern Ethiopia used water from river to wash their milk vessels. The study of Worku et al. (2014) showed that 28.8% of households in Borana Pastoral Community used water

Table 4. Experience of smoking and reasons given by the respondents for smoking milk vessels.

Variables	Groups	Transhumance		Sedentary		Overall	
		N	%	N	%	N	%
Smoking milk vessels	Total	45		105		150	
	Yes	45	100	105	100	150	100
	No	0	0	0	0	0	0
Reason for smoking milk vessels	Total	45		105		150	
	For good flavor of the product	15	33.3	65	61.9	80	53.3
	For longer shelf life of the products	14	31.1	34	32.4	48	32.0
	To have reddish color butter	7	15.6	5	4.8	12	8.0
	To have attractive odor butter	9	20.0	1	1.0	10	6.7

N = Number of respondents.

Table 5. Proportion of respondents using detergents to wash milk vessels, source of water and plants to smoke milk vessels.

Variables	Groups	Transhumance		Sedentary		Overall	
		N (45)	%	N (105)	%	N (150)	%
FCMU	Total	45		105		150	
	Once per day	4	8.9	2	1.9	6	4.0
	Twice per day	41	91.1	103	98.1	144	96.0
Using sanitizer	Total	45		105		150	
	Yes	24	53.3	56	53.3	80	53.3
	No	21	46.7	49	46.6	70	46.7
WSWMV	Total	45		105		150	
	Pump water	39	86.7	105	100	144	96
	River	6	13.3	0	0	6	4
Smoking plants:	Total	45		105		150	
	1st (*Acacia etbaica*)	45	100	105	100	150	100
	2nd (*Olean africana*)	45	100	105	100	150	100

N = Number of respondents, FCMU = frequency of cleaning milk vessel, WSWMV = water source for washing milk vessels.

from river to wash milk vessels. This may indicate the presence of better awareness of using clean water in the current study (Enderta district).

Farmers smoke their milk vessels by two commonly known plants in the district. The first and frequently selected plant to smoke milk vessels throughout the sampled respondents was *Acacia etbaica* locally called 'Seraw'. When this plant is not available, they use *Olean africana* locally called 'Awlie'. Different authors also indicated that milk producers in different parts of Ethiopia used plants to smoke their milk vessels. For instance, Lemma et al. (2005), Kedja (2008) reported that *Olea africana* is the most frequently used plant for smoking milk vessels in semi arid areas of Oremia region of the country. Similar to this study, Aysheshim et al. (2015) also reported that majority of the producers in urban and peri urban area of Dangila town of Western Amhara region used acacia species and *O. africana* to smoke milk vessels as a first and second choice, respectively.

CONCLUSION AND RECOMMENDATIONS

Milk production is one of the important sectors of livestock production in Enderta district. It is a source of income and employment. Out of the daily milk produced, the overall daily allocation of milk for household consumption, milk accumulated for fermentation, calf feed and for sell was 9.80, 62.94, 13.62 and 13.55%, respectively. And the overall weekly allocation of butter for household consumption, cosmetic and sell was 3.85, 15.97 and 80.2%, respectively.

In transhumance production system, 'kordo' was the dominant milking utensil, but in sedentary production

system, plastic materials were the dominant milking utensils. Majority of the respondents used ‘Gefho’ and others used ‘Jerican’ material to store milk for fermentation. ‘Laga’ and ‘Jerican’ were used as churning materials. Milk producers in the area selected *Acacia etbaica* locally called ‘Seraw’ for smoking milk vessels. Even if the interest of farmers engaged in dairy farming is increasing in the area, they lack awareness on keeping quality of milk and milk products. Nearly half of the producers did not use detergents to wash their milk vessels. Therefore, milk producers should be supported with strong extension service by way of introducing improved dairy technologies, improved milk handling and processing equipments in place of the plastic milk handling and storing utensils, as these plastics facilitate spoilage of the products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Foliar application of bio-fertilizers influenced the endogenous concentrations of phytohormones and amino acids in leaves and roots of olive trees (*Olea europaea* L. cv. Chemlali)

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The chemical composition of olive leaves and roots of Chemlali variety was evaluated regarding the contents of amino acids and endogenous phytohormones under foliar application of biofertilizers (F1: rich in nitrogen (N), phosphorus (P), potassium (K); F2: rich in calcium (Ca); F3: application of F1 and F2). The results of this study demonstrated significant effects of tested fertilizers on the concentrations of amino acids. Glutamic acid and asparagine had the highest concentrations in leaves and the lowest concentrations in roots under F1 and F3. Regarding the concentrations of endogenous phytohormones, high contents of indole-3-acetic acid and salicylic acid were found in leaves and roots under the application of F1 biofertilizer. Jasmonic acid (JA) was of higher concentration in root than leaves, while abscisic acid occurred in lower concentration. Moreover, the analysis of isoprenoid cytokinins by ultra-performance liquid-chromatography tandem mass spectrometry (UPLC-MS/MS) showed that these cytokinins were improved and became more abundant in roots under foliar treatments. Particularly, Zeatin-N7-glucoside and Dihydrozeatin-N9-Glucoside were enhanced under F1 and F3. The results of this study suggest that foliar application of biological fertilizers may improve physiological status of olive trees throughout the enhancement of some phytohormones and amino acids.

Key words: Foliar fertilization, olive trees, amino acids, indole-3-acetic acid, isoprenoid cytokinins.

INTRODUCTION

Olive tree (*Olea europaea* L.) is the most important tree in the countries of the Mediterranean basin. Deficiencies in mineral elements such as Nitrogen (N), Phosphorus (P), Potassium (K) and Calcium (Ca) are among the

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Table 1. Physico-chemical characteristics of the soil (0-40 cm) of the experimental orchard.

Depth (cm)	0 - 40
Clay (%)	20
Silt (%)	11
Sand (%)	69
pH	8.5
Electrical conductivity (mmhos/cm)	2.7
Organic carbon (%)	0.67
Organic matter (%)	1.15
Total nitrogen (%)	0.073
Available phosphorus (P_2O_5) (ppm)	5
Exchangeable potassium (K_2O) (ppm)	459

major factors which are negatively influencing olive tree growth. Foliar fertilization appeared among the most used methods against these deficiencies and aimed to help the plant during the development and ripening process. Various macronutrients and micronutrients not only play a nutritional role, but also have an additional independent function signaling their metabolism (Krapp and Castaings, 2012). In this case, the application of some known minerals is necessary to have a positive effect on the physiological status in different plants. For example, it has been shown that the main nitrogen source nitrate, could act as a signal molecule controlling gene expression and plant development (Krapp and Castaings, 2012). Recent studies showed how nitrogen and phytohormone signals are involved in the modification of plant physiology and morphology (Gu et al., 2018). Moreover, N is known to be a vital element incorporated in proteins, nucleic acids, chlorophyll and numerous plant metabolites including phytohormones. Also, P is a building block of many secondary metabolites of plants (Gan et al., 2015) and is involved in the growth and development of all crops (Zhu et al., 2018). K is also a necessary macronutrient for trees; it carries out vital functions in metabolic and stress adaptation (Armengaud et al., 2004). Moreover, Ca is necessary for membrane stability and, together with P, it plays a central role in maintaining osmotic homeostasis and cell signalling, coupled with stress tolerance and appropriate photosynthetic functions (Qu et al., 2012). It is involved also in plant growth and development, cytoplasmic streaming, cell division, and photosynthesis (Huang et al., 2017). It plays a crucial role in the heat stress, in the cold acclimation of plants, and in the pathogen attack in the reactive oxygen species (ROS) response (Sharma et al., 2017).

Some studies were reported on the effects of foliar fertilization on the amino acid content in different plant species (Gutiérrez-Gamboa et al., 2016; Hannam et al., 2015). However, nothing is known about the effects on the endogenous hormone levels. The plant hormones, including the classical auxins indole-3-acetic acid (IAA), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and cytokinins (CKs), are chemical messengers which play a vital role in the regulation of plant growth, development and responses to environmental conditions (O'Brien and Benková, 2013). The combined activities of several hormonal pathways, such as ABA and development-related hormones, such as CK and auxin resulted when a plant is exposed to abiotic stresses and are involved in plant development. Also, SA and JA are activated when a plant is exposed to pathogenic attack or various-stress conditions mainly low temperature, drought and salinity (Kiba et al., 2011). In fact, many studies showed good evidence for a relation between plant hormones and availability and/or deficiency of mineral elements, for example in maize (Gawronska et al., 2003; Zhao et al., 2016), *Betula pendula* and in *Acer pseudoplatanus* L. (Darrall and Wareing, 1981). However, there has been no detailed study that aimed to evaluate the response of the olive tree to an exogenous supply of mineral elements by foliar fertilization. Thus, the aim of this study was to assess the effects of foliar application of some fertilizers based on minerals (N, P, K and Ca) extracted from natural sources, on the content of amino acids and phytohormones in leaves and roots of olive trees (cv. Chemlali).

MATERIALS AND METHODS

Field experiment and treatments

The present study was carried out on mature olive trees of "Chemlali" cultivar grown in a rainfed orchard located at Jemmel, Tunisia (North latitude 35° 63′, East longitude 10° 68′ and Altitude: 44 m). The mean annual rainfall is 313 mm and the mean annual temperature is 18.2°C. The experimental soil has a sandy texture. A composite soil sample (0 to 40 cm depth) taken from the soil of the experimental farm was analyzed (Table 1). The experimental trees were planted at 10 × 10 m apart and arranged in a randomized block design with three blocks (9 trees in each block) and four treatments that consisted of the annual application of F0, F1, F2

Table 2. Mineral compositions (w/v: g/l) of the four foliar fertilizers used in this study.

Foliar treatment	N	P_2O_5	K_2O	CaO
F0	-	-	-	-
F1	40	20	60	-
F2	-	-	-	160
F3	40	20	60	160

and F3 (obtained from Agronutrition (France, Carbonne)) during two successive growing seasons (2014 and 2015):

F0: Control, without foliar fertilization (water spray).
F1: A ready liquid formulation made up of amino acids and extracts of Molasses beet (sources of nitrogen (N) and potassium (K)) and the edge of fish (source of phosphorus (P)). The mineral composition of this fertilizer is shown in Table 2. Foliar fertilization consisted of four applications with 15 day interval, starting from petal fall, with a dose of 5 L/ha.
F2: This is a liquid formulation of calcium concentrate (Table 2). Five applications with 15 days interval were conducted, starting from fruit set, with a dose of 2 L/ha.
F3: The trees received both F1 and F2 foliar fertilizers.

Plant

Fresh olive leaves and roots of *O. europaea* were collected in the second week of March (spring 2016, after two years of foliar fertilization). Leaves were collected from three random locations in each plot and root samples were collected at 10 to 15 cm depth. Fresh olive leaves and roots were washed with distilled water to eliminate any traces of dust. The samples were immediately protected in isothermal bins with dry ice and transferred to the laboratory. In order to stabilize the byproduct and to avoid quality losses and undesirable degradation during storage and transportation, plant materials were homogenized by grinding in liquid nitrogen and kept at -80°C before analysis of phytohormones and amino acids.

Determination of amino acids

Plant material was homogenized using a MagNA Lyser instrument (Roche Life Science, USA). Amino acids were extracted in formic acid (0.125 v%, 1 µl/mg fresh weight). Heavy labeled L-Glutamine-2, 3, 3, 4, 4-d5 (0.1 nmol, Sigma Aldrich, Overijse, Belgium) was added as an internal standard to quantify recovery and ionization efficiency. Prior to analysis, samples were filtered using a Chromafil AO20/3 (0.2 µm, 3 mm) Polyamine syringe filter (MV, Düren, Germany). Samples were analyzed using an UPLC system linked to an Acquity triple quadrupole detector (Waters, Milford, MA) equipped with an electrospray interface. Samples (6 µl) were injected on an ACQUITY BEH Amide Column (1.7 µm × 2.1 mm × 100 mm, Acquity UPLC, Waters, Milford, MA) and eluted with a gradient of solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in CAN) over 7.5 min at 0.5 ml/min. The effluent was introduced into the electrospray source (source temperature: 120°C, capillary voltage: 1.8 kV, component dependent cone voltages between 13 and 23 V). Quantification was done by multiple reactant monitoring (MRM) of the MH+ ion (dwell time: 0.016) and the proper product ion. This corresponded to 175>70 and 175>130 for arginine, 156>110 for histidine, 148>84 and 148>130 for glutamic acid, 147>84 and 147>130 for glutamine, 133>74 and 133>88 for asparagine, 133 > 70 for ornitine, 116>70 for proline, 106 > 70 and 106>88 for serine, 90>44 for alanine, and 152>89 and 152>135 for L-Glutamine-2,3,3,4,4-d5. All data were processed using Masslynx/Quanlynx software V4.1 (Waters, Milford, MA).

Measurement of phytohormones

Measurement of endogenous SA, JA, IAA and ABA levels

Extraction of SA, JA, IAA and ABA: Plant materials were extracted overnight in 80% methanol (10 µl/mg FW, -20°C, 16 h). 100 pmol of $C_6{}^{13}$-phenyl-IAA (Cambridge Isotope Laboratories Inc., Andover, Massachusetts, USA), 150 pmol of D6-ABA ([$^2H_6$](+)-*cis, trans* ABA (OlChemIm, Olomouc, Czech Republic)), 100 pmol of [2H4]-SA (OlChemIm) and 100 pmol of dehydro-Jasmonic acid (OlChemIm) were added as internal standards. After a purification step removing pigments on a C18 cartridge (Bond Elut C18 6 cc, 500 mg, Agilent, CA, USA) in 80% methanol, the extract was diluted and acidified with formic acid 6% so that hormones bind to a C18 cartridge. Diethyl ether was used to elute the hormones. The residual water was removed and the ether phase was evaporated under a stream of N_2 gas (Turbovac L V Evaporator, Hopkinton, MA, USA). After methylation with diazomethane (Schlenk and Gellerman, 1960), the samples were dried under N_2 gas and dissolved in 100% hexane (15µ l) for GC-MS/MS analysis of salicylic acid (SA) and jasmonic acid (JA). The remaining of the samples (after JA and SA analysis) were dried and redissolved in 50 µl of 10% for LC-MS/MS analysis of indole-3-acetic acid (IAA) and abscisic acid (ABA).

Analysis of SA and JA: Gas chromatography-mass spectrometry analysis was performed using a Waters Micromass Quattro micro GC (Waters; MA,USA): a triple quadrupole with an integrated Agilent 6890N gas chromatography oven, and using an electron impact (EI) ion source, positive ion mode, 70 eV, Collision Energy 10 eV, inter-channel delay 10 ms and inter-scan delay 10 ms. The gas chromatography column used was a 15 m × 0.25 mm Agilent J&W DB-5ms, film thickness: 0.25 um (Agilent Technologies; CA, USA), injection volume: 10 µl, Carrier gas: helium, flow rate: 1 ml min^{-1}. The oven started isothermally at 50°C for 2 min and increased linearly to 300°C at a rate of 25°C min^{-1}. 300°C was held for 3 min. The diagnostic ions used for quantification of SA and JA in MRM mode are 152> 20 m/z for MeSA, 156>124 m/z for D4-MeSA, 224>151 m/z for MeJA and 226>153 m/z for DHMeJA (dwell time: 0.010 s).

Analysis of IAA and ABA: IAA and ABA were analyzed by UPLC-MS/MS after methylation (Acquily TQD, TQD, Waters, Manchester, UK), 6 µl injection by partial loop, ACQUITY BECH18, 1.7 um column (WATERS), column temperature: 30°C, flow of 400. The solvent gradient was as follows: 0 to 2 min: 95/5 of 10% methanol in NH_4OAc 1 mM/methanol; 2 to 4 min: 10/90 of 10% methanol in NH_4OAc 1 mM/methanol; 4 to 6 min: isocratic 10/90 of 10% methanol in NH_4OAc 1 mM/methanol. MS conditions: Polarity MS ES(+), capillary: 2 kV, cone: 20 V, collision energy: 20 eV, source temperature: 120°C, desolvation temperature: 450°C, Cone gas

flow: 50 L/h, desolvation gas flow: 750 L/h, collision gas flow: 0.19 ml/min. The diagnostic ions used for quantification for IAA and ABA were: 190>130 m/z for Me-IAA, 196>136 m/z for Me-C^{13}-IAA, 279>173 m/z for Me-ABA and 285>179 m/z for d_6-Me-ABA (dwell time: 0.02 s). Methanol and water used for MS were UPLC grade form Biosolve (Valkenswaard, the Netherlands). Data were expressed in pmol per gram of fresh weight (pmol. g^{-1} FW).

Measurement of endogenous CK levels

Cytokinin extraction: Plant material was extracted overnight in 80% methanol (10 µl/mg FW, -20°C, 16 h). [$^2H_3$]Dihydrozeatin (d-DHZ), [$^2H_3$]Dihydrozeatin Riboside (d-DHZR), [$^2H_6$]N^6-*Iso*pentenyladenine (d-*i*P), [$^2H_6$]N^6-*Iso*pentenyladenosine (d-*i*PA), [$^2H_5$]Zeatin-*7*-Glucoside (d-Z*7*G), [$^2H_5$]Zeatin-*9*-Glucoside (d-Z*9*G), [$^2H_6$]N^6-Isopentenyladenine-*7*-Glucoside (d-*i*P*7*G) and $^2H_6$]N^6-*Iso*pentenyladenine-*9*-Glucoside (d-*i*P*9*G) (10 pmol each, OlChemIm) were added as internal standards. After centrifugation (20000 g, 15 min, 4°C, 5810R, rotor FA-45-30-11 Eppendorf, Hamburg, Germany), the supernatant was passed over a C18 cartridge (500 mg, Varian) to retain pigments and was consecutively filtered (Chromafil Xtra PA-20/25, 0.2 µm, ϕ 25 mm, MN, Duren, Germany). Samples were dried in a SpeedVac (Christ RNC2-25 vacuum concentrator, with KNF N860.3FT.40.18 pump, Freiburg, Germany), and redissolved in 50 µl of 10% methanol for analysis.

Cytokinin analysis: Isoprenoid cytokinins were analyzed by UPLC-MS/MS (WATERS): 6 µl injection by partial loop, ACQUITY BEH-C18, 1.7 µm column (WATERS), column temperature: 30°C, flow 400, solvent gradient: 0 to 0.5 min: 95/5 of 10% methanol in NH_4OAc 1 mM/methanol; 0.5 to 3 min: 75/25 of 10% methanol in NH_4OAc 1 mM/methanol; 3 to 5 min: isocratic 75/25 of 10% methanol in NH_4OAc 1 mM/Methanol; 5 to 6 min: 5/95 of 10% methanol in NH_4OAc 1 mM/methanol; 6 to 6.5 min: isocratic 5/95 of 10% methanol in NH_4OAc 1 mM/methanol. MS conditions: Polarity MS ES(+), capillary: 2 kV, cone: 20V, collision energy: 20 eV, source temperature: 120°C, desolvation temperature: 400°C, Cone gas flow: 20 L/h, desolvation gas flow: 800 L/h, collision gas flow: 0.22 ml/min). The diagnostic ions used for quantification for isoprenoid cytokinins were: 222>136 m/z for DHZ, 220>136 m/z for Z, 353>222 m/z for DHZR, 352>220 m/z for ZR, 204>136 m/z for *i*P, 336>204 m/z for *i*PA, 384>222 m/z for DHZ*7*G and DHZ*9*G, 382>220 m/z for Z*7*G and Z*9*G, 366>204 m/z for *i*P*7*G and *i*P*9*G, 225>136 m/z for d-DHZ, 356>225 m/z for d-DHZR, 210>136 m/z d-*i*P, 342>210 m/z for d-*i*PA, 389>227 m/z for d-Z*7*G and d-Z*9*G, 372>210 m/z for d-*i*P*7*G and d-*i*P*9*G. Methanol and water used for MS were UPLC grade from Biosolve. Data were expressed in pmol per gram of fresh weight (pmol.g^{-1}FW).

Statistical analysis

All statistical values were calculated using SPSS for Windows v. 18.0 and expressed as mean ± standard deviation (SD) of three independent measurements. The post hoc Duncan's test was applied. The statistical significance level was fixed at P≤ 0.05.

RESULTS

Changes in amino acid concentrations in leaves and roots following foliar fertilization treatments

Under F1, this total increase is merely due to the clear increase of asparagine and glutamic acid, whereas under F3, the increase is due also to a minor increase of ornithine and serine in combination with a major increase of asparagine and glutamic acid. These results indicated that after N, P and K supply, leaves accumulated more amino acids than in control leaves or in those treated with F2.

Although, it was observed that the concentrations of amino acids were approximately 6 times higher in roots than in leaves, lower amino acid concentrations were observed in roots after fertilization as compared to those in control conditions (Table 3). Especially, glutamic acid, glutamine and to a lesser amount proline, became significantly lower when fertilizers were applied.

Variation of ABA, IAA, SA and JA concentrations in leaves and roots after foliar fertilization

In this experiment, ABA, IAA, JA and SA concentrations in leaves and roots were determined under the different treatments (Figure 1a to d). In leaves, a significant decrease of the ABA concentration was observed after foliar fertilization. The SA level increased significantly from 3900 pmol/g in F0 to 4700 in F1 and 7500 pmol/g in F3. Compared to F0, a significant increase in IAA concentrations were also observed in F1 (by about 73.33%) and in F2 (by 60%). These results were accompanied by an increase of the JA concentration in F3 and a clear decrease in F1 and F2 as compared to F0.

In roots, foliar fertilization did not significantly affect the endogenous ABA concentration. In contrast, the IAA root concentration increased only under F1 as compared to control (by about 59.47%). The concentration ranges of root IAA were comparable to leaf ones. IAA level was remarkably enhanced under F1 and F2 fertilization. Although the SA concentration in roots was 10 times lower than those observed in leaves, a slight increase of SA could be observed after F1 fertilization (560 pmol/g) as compared to F0 (350 pmol/g). Regarding JA level, being 10 times higher in roots than in leaves, JA concentration in roots decreased significantly under F1, F2 and F3.

Cytokinin concentrations in leaves and roots were differently affected under foliar fertilization treatments

The chemical structure of all the isoprenoid Cks analyzed (DHZR: Dihydrozeatin-N*9*-Riboside, DHZ: Dihydrozeatin, *trans*-ZR: *trans*-zeatin-N*9*-Riboside, *cis*-ZR: *cis*-zeatin-N*9*-Riboside, Z-N*7*-G: Zeatin-N*7*-glucoside, Z-N*9*-G: Zeatin-N*9*-glucoside, *i*P: *iso*pentenyladenine, *i*PA: *iso*pentenyladenosine, *i*P-N*7*-G: N6-*iso*pentenyladenine-N*7*-Glucoside, *i*P-N*9*-G: N6-isopentenyladenine-N*9*-Glucoside, *trans*-Z: *trans*-zeatin, *cis*-Z: *cis*-zeatin, DHZ-N*7*-G: Dihydrozeatin-N*7*-Glucoside, DHZ-N*9*-G:

Table 3. Effects of the foliar treatments on amino acid concentration (10^3 pmol/g FW) in leaves and roots of olive trees.

Amino acids (10^3pmol/g FW)	Leaves			
	F0	F1	F2	F3
Arginine	2.07±0.03^{b}	2.01±0.05^{b}	2.55±0.61^{ab}	3.06±0.52^{a}
Histidine	3.32±0.04^{c}	4.10±0.15^{b}	3.70±0.59^{bc}	6.14±0.02^{a}
Glutamic acid	28.79±10.73^{c}	71.79±7.17^{a}	50.95±0.22^{b}	108.99±38.79^{a}
Glutamine	5.17±0.67^{c}	9.86±1.60^{b}	9.96±1.30^{b}	15.69±0.13^{a}
Asparagine	103.44±15.01^{b}	199.10±25.33^{a}	89.58±6.65^{b}	167.36±45.57^{a}
Ornithine	10.32±0.59^{b}	8.43±1.70^{b}	7.65±3.86^{b}	44.78±2.81^{a}
Proline	6.15±0.25^{c}	11.53±0.82^{a}	8.41±1.24^{b}	5.99±0.12^{c}
Serine	19.43±0.21^{c}	16.01±1.72^{d}	41.97±0.51^{a}	32.45±6.85^{b}
Alanine	10.63±0.54^{a}	10.23±0.76^{a}	8.97±1.23^{a}	9.08±1.27^{a}
		Roots		
Arginine	69.55±1.50^{a}	65.90±5.50^{a}	70.78±4.12^{a}	61.22±27.48^{a}
Histidine	19.47±0.06^{a}	15.00±1.74^{a}	22.86±2.37^{a}	21.38±16.66^{a}
Glutamic acid	479.74±20.29^{a}	301.10±37.99^{c}	357.71±3.08^{b}	240.02±95.06^{c}
Glutamine	695.92±53.43^{a}	234.95±13.98^{d}	327.72±0.51^{b}	303.23±1.63E-05^{c}
Asparagine	180.90±2.89^{a}	82.78±16.53^{c}	128.74±2.69^{b}	81.93±37.18^{c}
Ornithine	92.89±4.45^{a}	28.01±7.40^{c}	75.08±0.31^{ab}	85.12±6.81^{a}
Proline	5.07±0.35^{a}	2.44±0.44^{c}	3.66±0.02^{b}	4.49±1.07^{ab}
Serine	64.83±2.02^{a}	37.37±7.36^{c}	56.07±4.35^{b}	53.58±2.50^{b}
Alanine	20.86±0.94^{a}	9.95±0.69^{c}	17.01±1.25^{b}	15.11±0.76^{b}

Values are means± SD (n=3). Different letters indicate significantly different values at p≤0.05 according to Duncan test. Amino Acids not listed were below detection limit.

Dihydrozeatin-N*9*-Glucoside) are as shown in Figure 2. The effects of foliar biofertilization on the concentrations of these Cks are shown in Table 4. The results showed also that *i*P-N*9*-G was the most abundant cytokinin metabolite in both leaves and roots (Table 4). In leaves, the foliar treatments decreased considerably the level of *i*P-N*9*-G (by about 14.54% in F1, 10.90% in F2 and 9% in T3 comparing to control) and the level of *trans*-ZR (by about 42.10% in F1, 32.63% in F2 and 57.9% in F3 as compared to control). In roots, *i*P-N*9*-G decreased also under biofertilization with the three treatments. However, DHZR, *trans*-ZR, *i*PA, Z-N7-G, DHZ-N*9*-G and *i*P-N7-G were enhanced under foliar fertilization, especially with F1 and F3 spraying (Table 4).

DISCUSSION

Foliar biofertilization influenced amino acid compositions in olive leaves and roots

In this study, absorption of nutrients by foliar sprays resulted in an increase of amino acid levels in leaves, especially under F1 and F3 applications. This may be due to the action of the fertilizer based on nitrogen, phosphorus and potassium that served as nitrogen and phosphorus donors for the biosynthesis of all essentially amino acids, nucleic acids, and other nitrogen-containing compounds. Nitrogen contributes to the synthesis of the first amino acids glutamate and glutamine and thereafter, the synthesis of other amino acids (Hirel and Lea, 2011). Asparagine, aspartate, glutamine and glutamate are carriers to translocate inorganic nitrogen from source organs to sink organs (Peoples and Gifford, 1990). This may explain the increase of glutamic acid and asparagine in leaves and not in roots. Gutiérrez-Gamboa et al. (2016) studied the effect of different foliar nitrogen applications by organic source treatments on the amino acid composition in vineyard. They reported that these treatments improved the amino acid content and increased glutathione concentration.

The findings showed an increase in the content of proline under F1 (N, P and K-based fertilizer) and F2 (Ca^{2+}-based fertilizer). These results agree with those obtained by Gutiérrez-Gamboa et al. (2016) who observed an increase in proline after foliar urea application to grapevines. This is a positive feature for olive trees because it has been reported that the elevated proline concentration may act either as a compatible solute to protect plants from dehydration or contribute to stabilizing subcellular structures and scavenging free radicals, thus conferring tolerance of plants to abiotic stress (Ashraf and Foolad, 2007). Moreover, Zhao et al. (2009) found that nitric oxide (NO), where nitrogen is one

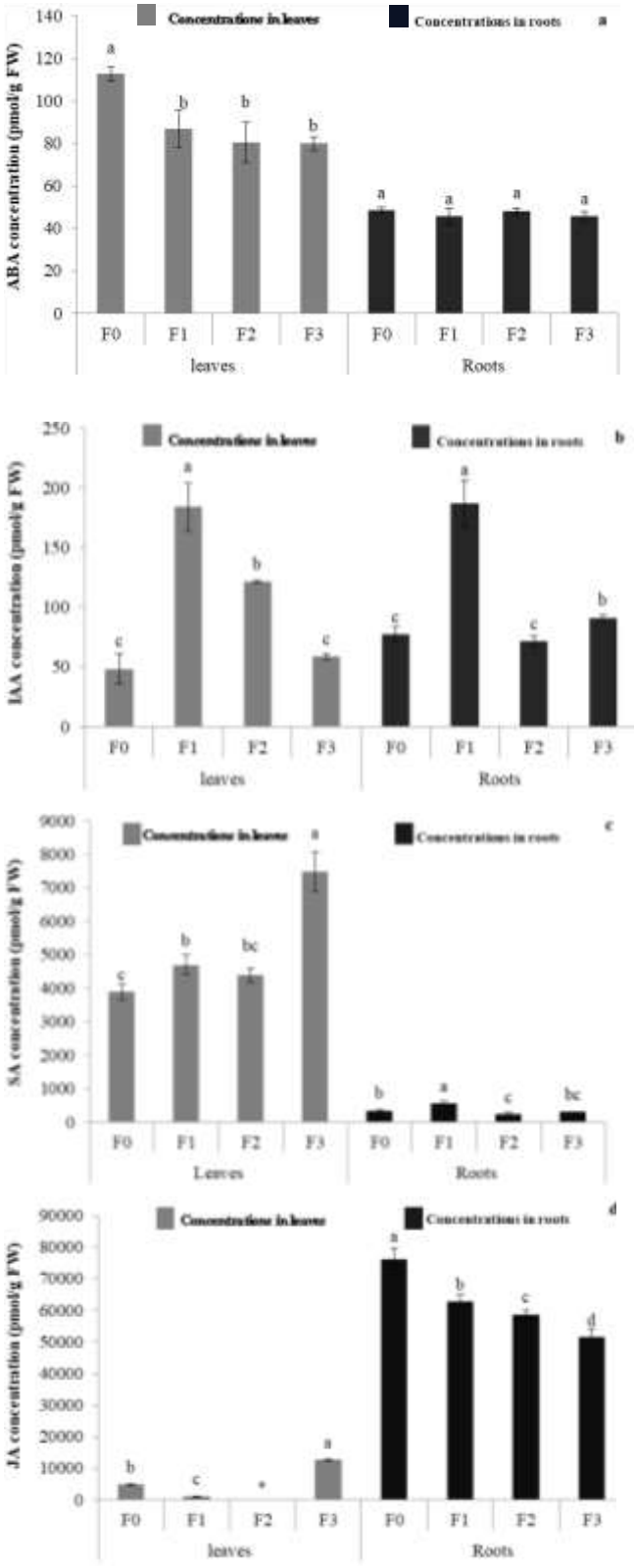

Figure 1. Effect of foliar treatments on the abscisic acid ABA) (a), indole-3-acetic acid (IAA) (b), salicylic acid (SA) (c) and jasmonic acid (JA) (d) concentrations (pmol/g FW) in leaves and roots of olive trees. Values are expressed in means ± SD (n=3). Different letters indicate significantly different values at p≤0.05 according to Duncan test. *Below detection limit.

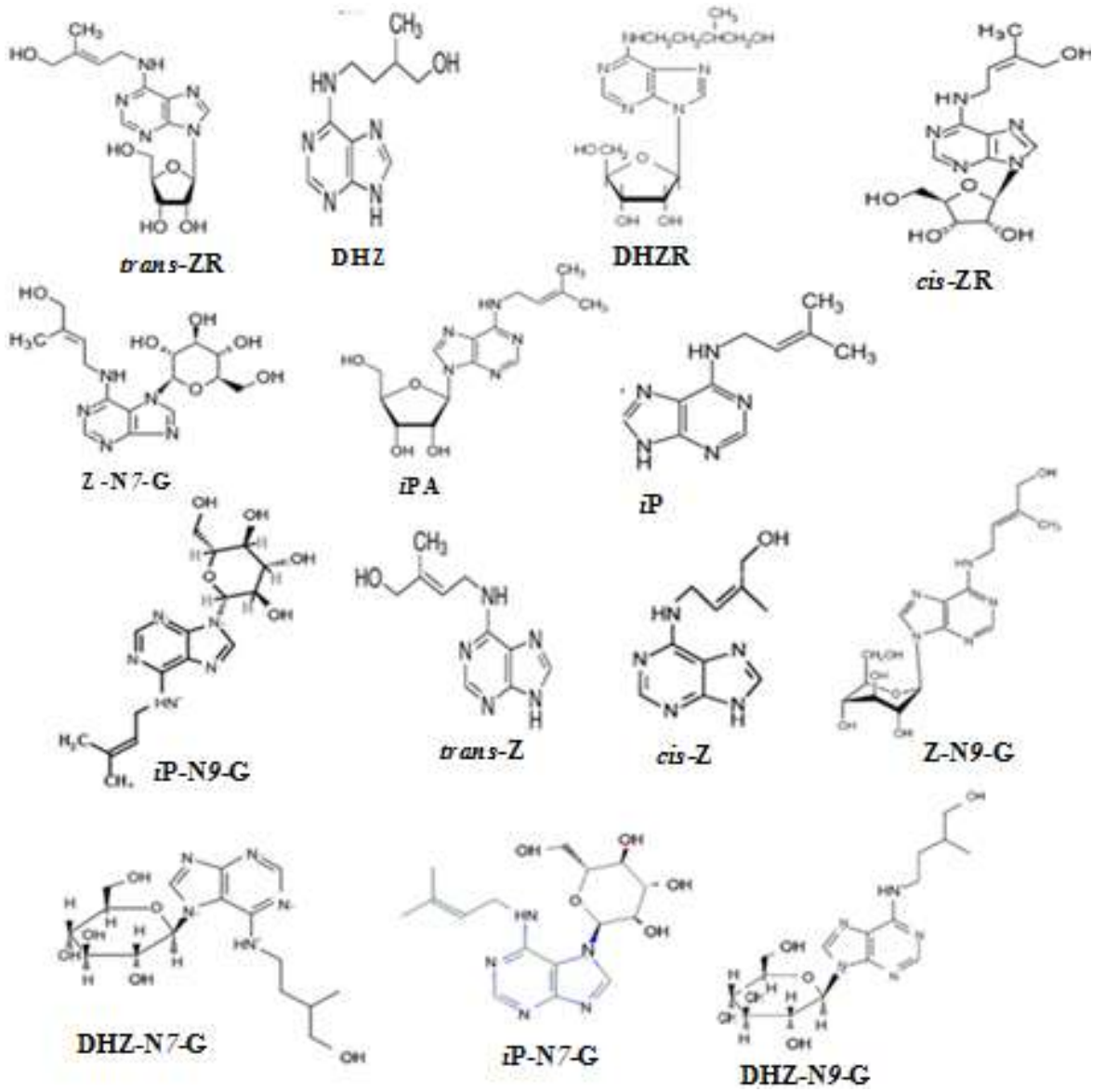

Figure 2. Chemical structures of the tested isoprenoid cytokinins. DHZR: Dihydrozeatin-N9-riboside, DHZ: Dihydrozeatin, *trans*-ZR: *trans*-zeatin-N9-riboside, *cis*-ZR: *cis*-zeatin-N9-iboside, Z-N7-G: Zeatin-N7-glucoside, Z-N9-G: Zeatin-N9-glucoside, *i*P: *iso*pentenyladenine, *i*PA: *iso*pentenyladenosine, *i*P-N7-G: N^6-*iso*pentenyladenine-N7-glucoside, *i*P-N9-G: N^6-*iso*pentenyladenine-N9-glucoside, *trans*-Z: *trans*-zeatin, *cis*-Z: *cis*-zeatin, DHZ-N7-G: Dihydrozeatin-N7-glucoside, DHZ-N9-G: Dihydrozeatin-N9-glucoside.

of component, is a highly active and an important signaling molecule involved in diverse pathophysiological processes and may be associated with the accumulation of proline by transcriptionally promoting proline synthesis and suppressing proline degradation, thus contributing to enhanced abiotic stress tolerance in plant. The results discussed here support the hypothesis that exogenous application of nutrients, particularly nitrogen, may play an important role in the way of proline signaling.

In addition to N, K is known to have a positive effect on amino acid content. According to Tovar-Méndez et al. (2003), in pea seedling, pyruvate dehydrogenase kinase activity is stimulated by NH_4^+ and K^+. Furthermore, the calcium cation Ca^{2+} acts as a regulator. It activates pyruvate dehydrogenase phosphatase by other divalent cations, which in turn activates the pyruvate dehydrogenase component of the pyruvate dehydrogenase complex (Tovar-Méndez et al., 2003). Therefore, this increases the rate of reaction of several cycle steps, and thus increases the flow of compounds through this metabolic pathway such as amino acids.

F1, F2 and F3 foliar applications increase clearly the individual amino acids in leaves. This beneficial effect in plant leads to improvement in the primary metabolism

Table 4. Isoprenoid cytokinin concentrations (pmol/g FW) in olive leaves and roots under different foliar treatments.

Variable	Leaves				Roots			
	F0	F1	F2	F3	F0	F1	F2	F3
DHZR	b.d	b.d.	b.d.	b.d.	4.0±0.4^{b}	5.2±0.8^{a}	b.d.	b.d.
trans-ZR	95±7^{a}	55±4^{b}	64±3^{b}	40±9^{b}	12.2±0.3^{d}	18.2±0.3^{a}	13.5±0.2^{c}	15.4±0.3^{b}
cis-ZR	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.
DHZ	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.
trans-Z	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.
cis-Z	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.
iPA	b.d.	b.d.	b.d.	b.d.	14.8±0.2^{b}	14.8±0.6^{b}	18±2^{a}	16.01±0.09^{ab}
*i*P	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.
DHZ-N7-G	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.
Z-N7-G	b.d.	b.d.	b.d.	b.d.	67±2^{b}	81±9^{a}	62±4^{b}	89±1.8^{a}
DHZ-N9-G	0.8±0.8^{a}	b.d.	b.d.	b.d.	111.±16^{b}	170±20^{a}	110±10^{b}	160±6^{a}
Z-N9-G	b.d.	b.d.	b.d.	b.d.	31±8^{a}	b.d.	b.d.	37±9^{a}
*i*P-N7-G	b.d.	b.d.	b.d.	b.d.	8.4±1.1^{c}	16.4±0.2^{a}	13.9±1.2^{b}	8.9±0.7^{c}
*i*P-N9-G	550±21^{a}	470±6^{b}	490±22^{b}	500±13^{b}	149±6^{a}	130±12^{b}	130±4^{b}	119±12^{b}

DHZR: Dihydrozeatin-N9-riboside, DHZ: dihydrozeatin, *trans*-ZR: *trans*-zeatin-N9-riboside, *cis*-ZR: *cis*-zeatin-N9-riboside, Z-N7G: Zeatin-N7-glucoside, Z-N9G: Zeatin-N9-glucoside, *i*P: *iso*pentenyladenine, *i*PA: Isopentenyladenosine, *i*P-N7-G: N^6-Isopentenyladenine-N7-glucoside, *i*P-N9-G: N^6-*iso*pentenyladenine-N9-glucoside, *trans*-Z: *trans*-zeatin, *cis*-Z: *cis*-zeatin, DHZ-N7-G: Dihydrozeatin-N7-glucoside, DHZ-N9-G: Dihydrozeatin-N9-glucoside. b.d. = below detection limit. Values are means± SD (n=3). Different letters indicate significantly different values at p≤0.05 according to Duncan test.

(protein synthesis) and polyamine biosynthesis playing an important role at flowering, fruit set and the plant development (Gény et al., 2007).

Foliar fertilization modified the phytohormone profile in olive trees

The observed increase of IAA level in both leaves and roots may be due to the action of some minerals known to have a positive effect on the IAA synthesis, such as N that is one of the chemical components of IAA. Furthermore, IAA seems to be translocated from leaves to root in response to foliar nitrogen-based fertilization. The supply of nitrogen will therefore allow the plant to multiply cells and thus stimulates vegetative growth by the formation of IAA which promotes the proliferation of buds and slows down the formation of root tips (Tu et al., 2017). Furthermore, potassium status may also affect IAA accumulation in the plant. According to Tu et al. (2017), K fertilization increased the contents of IAA and ZR, but also reduced ABA content consistently in vegetable soybean seeds. The results were not in agreement with those of Armengaud et al. (2004), who observed a reduction in the expression of genes controlling IAA biosynthesis when resupplying potassium of potassium-deprived *Arabidopsis* plants. On the other hand, it was suggested that under low-nitrate conditions, soybean and *Arabidopsis* plants accumulated higher levels of IAA in the root when compared with plants grown under high-nitrate conditions (Rubio et al., 2009). Studies on the effects of nutrient supply on JA and SA signaling are very limited. A significant reduction of JA was observed in leaves and roots of olive trees fertilized with F1, which is in agreement with the results of Armengaud et al. (2004) who reported that K^+ starvation allows an increase in the levels of JA by increasing transcription of proteins such as lipoxygenase while after a K^+ resupply, JA decreased rapidly. This indicates that foliar treatments rich in K mitigated the transcriptional response of JA biosynthesis genes (Armengaud et al., 2004). On the other hand, no JA-responsive genes were identified in response to nitrogen and phosphorus starved plants (Hammond et al., 2003; Wu et al., 2003). JA plays a role in plant defense responses against pathogen attack (Kunkel and Brooks, 2002). One might hypothesize that the observed increase of JA in F3 may contribute to the protection of olive trees against insect herbivores and fungi attack.

Regarding SA, the most remarkable thing is that there is an increase in the level of this phenolic compound in leaves and roots after foliar supply with N, P and K. Del Río et al. (2003) observed that N supply caused the rise of leaf phenolic compound concentrations in olive trees. Besides, in some cases SA and JA act by antagonism, where the SA blocks JA biosynthesis or negatively control the expression of genes regulated by JA. In other cases, the signals act synergistically to activate defense responses (Calatayud et al., 2013). The production of SA increases locally and systemically after infection by various pathogens. Similarly, JA, as a signal molecule in defense, increases also. Both SA and JA led to the

production of plant defensive compounds to activate the plant defensive pathways (War et al., 2015; Ibrahim et al., 2018). In the other side, SA and Ca play crucial roles in plant development and mediate plant response to biotic and abiotic stress (Guo et al., 2018). Thus, the increase of SA especially after F1 and F3 fertilization may promote the natural defense mechanism of olive trees against abiotic stress.

ABA acts as an internal signal enabling plants to survive under adverse environmental conditions (Keskin et al., 2010). In agreement with the results of the present study, Kiba et al. (2011) observed that ABA content in *Arabidopsis* roots did not show a significant difference between seedlings grown under high nitrate or low nitrate conditions. In leaves, the reduction in the ABA content is in agreement with the results reported by Gawronska et al. (2003) for maize (*Zea mays* L.) showing that the ABA concentration in tissue is negatively affected by nitrogen availability. The study of Gawronska et al. (2003) also demonstrated that ABA content in leaves and xylem sap was negatively correlated with nitrogen availability. ABA and IAA are known to be possible effectors for cytokinins (Gawronska et al., 2003). In fact, the decrease of the ABA content in leaves may be related to the cytokinin increase in the roots after foliar bio-fertilization, because of the antagonistic relationship between ABA and CKs. In the literature, it was shown that under water stress, plants reduced the level of CKs and increased the content of ABA (Pospíšilová, 2003). This meant that the ratio of cytokinins to ABA in the leaves and the roots substantially changes by the availability of nitrogen sources.

CKs level increased slightly under F1 and F3 in roots (Table 4). It has been reported that for maize and *Arabidopsis*, a nitrate supply caused an accumulation of CKs in roots followed by a flow of these cytokinins to the aerial parts (Takei et al., 2004). Darrall and Wareing (1981) have found in *B. pendula* that a supply of ammonium, nitrate, or ammonium nitrate showed the highest level of cytokinin-like activity, implying that CKs are a nitrogen status signal (Kiba et al., 2011). Also, Gu et al. (2018) noted that CKs interacted with N coordinating various metabolic processes and developmental programs in plants. The increase in CK levels, in turn, enhances growth and developmental processes in many plants (Nishiyama et al., 2011; Kang et al., 2012). Therefore, it is suggested that the increase of CKs under foliar bio-fertilization could be a positive feature regarding its implication on olive tree growth.

Conclusion

This study revealed that nutrient availability by foliar fertilization is an important factor determining the amino acid synthesis and the profile of phytohormone in both leaves and roots of olive tree. It was also shown that foliar bio-fertilization induced increase significantly on the production of endogenous phytohormones and amino acids in Chemlali cv. This study shows that the use of foliar bio-fertilizers (N, P, K and Ca-based) separately or in combination enhances the adaptation against biotic factors (microbes or herbivores) by increasing the SA and JA and improving the olive tree performances by increasing the amino acid, auxin and cytokinins. Thus, it is suggested that foliar bio-fertilizers have an important role as secondary signals. It can initiate a cascade of signaling events which lead to the modification and translocation synthesis of metabolites in trees. As an enticing area of future study, application of similar treatments is viewed in the olive tree under stressful conditions such as drought stress to determine if the foliar bio-fertilizers tested give usually the same effects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Multivariate analysis of phenotypic variability in Tef [*Eragrostis tef* (Zucc.) Trotter] genotypes from Ethiopia

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Tef [*Eragrostis tef* (Zucc.) Trotter] is an important food crop in Ethiopia. The present research was conducted to characterize the phenotypic variability of 68 tef genotypes collected from Ethiopia. Where a Bi-replicated 7×10 alpha lattice design was used to evaluate the 70 tef genotypes at Holetta and Debre Zeit Research Centers during 2015. Based on the results of cluster analysis (CA), genotypes were grouped into twelve clusters and twenty nine genotypes formed a single cluster; whereas, nine clusters comprised of five or few genotypes. The first five principal components (PC) with eigenvalue greater than one accounted for 80% of the total genetic variation, height related traits, the diameters of the two basal culm internodes, and number of spikletes and primary branches per main panicle were traits that chiefly contribute for the total variance accounted for by the first PC. The second PC gross variation originated due mainly to variations in yield and yield related traits like grain yield, total biomass, straw yield and harvest index. In addition, genetic distances (D^2) which ranged from 326.22 to 25.07 were measured among the 12 clusters. Thus, indicates their chance of giving better genetic recombination and segregation of progenies.

Key words: Cluster analysis, genetic distance, multivariate, principal component, Tef.

INTRODUCTION

Tef (*Eragrostis tef* (Zucc.) Trotter) is traditionally grown as a staple cereal crop in Ethiopia and it is produced by more than 6.5 million small scale farmers (CSA, 2015). The grain is ground into flour, which is used to make a pancake-like local bread called "*injera*" (Ketema, 1997).

The grain is also used to make a local drink. In addition, tef has been used as a forage or pasture crop for cattle in some parts of the world (Assefa et al., 2009). The straw also serves as bedding material, mulch and domestic fuel source (Assefa et al., 2001b). Tef is better adapted to excessive or low soil moisture conditions than other cereals and often sown as a rescue crop (Tefera and Ketema, 2001).

Therefore, tef is considered an important food security crop. In Ethiopia, tef shows low productivity, because of the lack of lodging resistant varieties, low yielding varieties under a wide range of cultivation, pest problem, drought and labor intensive nature of cultivation (Assefa

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et al., 2013). The development of improved tef varieties had been successful (Assefa et al., 2013; MoARD, 2016). The existence of genetic variability is an important factor in the development and selection of improved varieties. Therefore, estimating the genetic variation among landraces will enhance breeding activities (Assefa et al., 2015; Kefyalew et al., 2000). Tef is an ancient crop in Ethiopia and cultivated across a wide range of environments, which can contribute to greater genetic variation. The Ethiopian Biodiversity Institute (EBI) currently holds 6000 tef landraces mostly from altitudes ranging from 800 to 3200 m.a.s.l. (Tesema, 2013). Hence, multivariate analysis is a useful tool for characterization and classification of plant genetic resources evaluated for several pheno-morphic and agronomic traits (Assefa et al., 2003). The present study was conducted to study variability of newly collected local tef genotypes.

MATERIALS AND METHODS

The experiment was planted during 2015 growing season at Holetta (9°·03'N and 38°30'E) and Debre Zeit (8°44'N and 38°58' E). Sixty-eight locally collected genotypes along with two checks genotypes were evaluated in a 7×10 alpha lattice designs with two replications (Table 1). Tef accessions were initially (each contain 50-100 panicles selected from individual plants) collected from farmers' field within 15 km interval and also sown in separate rows for purification at Debre Zite Agricultural Research Center during the 2013 and 2014 main-cropping seasons and the 2015 off-season.

Genotypes were planted in a plot area of 1 m^2 (1 m × 1 m). A spacing of 0.2, 0.5 and 1.5 m were used between rows, plots and replications, respectively. For the seven month (from June to December) growing season, average rainfall, minimum and maximum temperatures of Holetta and Debre Zeit were: 710 mm, 5.6°C, 19.5°C and 73 mm, 18.8°C, 24.9°C, respectively.

Data collections were made on eighteen traits. Days to heading, days to maturity, days to grain filling period, lodging index, total biomass (g), grain yield (g), straw yield (g), thousand seed weight (g), and harvest index (HI) where taken on plot base. In contrast, plant height (cm), panicle length (cm), culm length (cm), number of total tillers per plant, number of fertile tillers per plant, number of spikelets per panicle, number of primary branches per main panicle, first basal culm internode diameter (mm), and second basal culm internode diameter (mm) were recorded on five random sample individual plants.

For multivariate analysis, the mean data of the 70 test genotypes for each of the traits were the first pre-standardized to mean zero and variance unity to avoid bias due to differences in measurement scales.

Multivariate statistical analysis methods included cluster analysis (CA) and principal component analysis (PCA) using the MINITAB statistical computer package, version 14.00 (MINITAB, 2003). Points where local peaks of the pseudo F statistic join with small values of the pseudo t^2 statistic followed by a larger pseudo t^2 for the next cluster fusion were observed to decide the number of clusters (SAS Institute, 2002).

Genetic distance between clusters was computed using the generalized Mahalanobis's D^2 statistics formula as suggested in Singh and Chaudhary (1996) and distance analysis was computed using the SAS computer software (SAS Institute, 2002). It was also made based on the mean values for the 18 quantitative traits and 70 tef genotypes over the two locations.

$D^2_p = (Xi-Xj)' S^{-1} (Xi-Xj)$.

where D^2_p= total generalized distance based on p characters, Xi and Xj are the p mean vectors of 70 test genotypes I and j, respectively, S^{-1}=pooled error variance and co-variance matrix.

The D^2 value obtained for pairs of clusters was considered as the calculated value of Chi-square and was tested for significance at 5 and 1% levels of probability against the tabulated values of X^2 at 'q' degrees of freedom, where q represents the number of traits studied (Fikreselassie, 2012).

RESULTS AND DISCUSSION

Cluster analysis

Using a 73% similarity level, the genotypes formed 12 clusters (C). The number of genotypes in each cluster ranged from 1 to 29 (Figure 1 and Table 2). The largest cluster (C-3) contains different tef germplasm collected from all zones, while C-6 was the second largest cluster and it comprised 14 germplasm accessions of which 13 were from Jimma and Horo Gudru Zones of Oromya, while the remaining tef germplasm lines were from North Wello Zone of Amhara Region. The third big cluster (C-5) constituted the improved variety Quncho (DZ-Cr-387) and other 8 local germplasm accessions of which two (Oro-ACC#8-L13 and Oro-ACC#9-L45) were from Jimma zone and the remaining 6 were equally distributed between North Wello and West Shewa zones of Amhara and Oromya, respectively. Beside those major clusters, each of clusters 7 and 2 comprised 5 and 3 tef germplasm lines, respectively. Regarding their origin, cluster 7 comprised of tef germplasm entirely collected from North Wello and North Shewa Zones of Amhara Region, while those in cluster 2 originated from North Wello Zone of Amhara. In addition, clusters 1, 4 and 9 each comprised two germpalsm accessions, with the former two containing types from North Wello of Amhara and West Shewa of Oromya Region, while the latter one contained accessions collected from Jimma Zone of Oromya. Four of the twelve clusters comprising single genotype including the germplasm accessions Oro-ACC#8-L30 (C-8), Oro-ACC#4-L18(C-10), Oro-ACC#4-L25) (C-11), and the released variety "*Tseday*" (C-12). Those unclear patterns of genotypes grouping in respect to their origin could be a result of free exchange of genotypes and the expansion of improved tef varieties.

In line with the present results, Assefa et al. (1999) categorized 320 tef lines into 14 major complexes consisting of 1 to 183 tef lines. Previous cluster analyses with different sets of tef materials have also demonstrated variable groupings of tef genotypes based on similarity (Assefa et al., 2000, 2001a, 2003).

The cluster mean comparison for the 18 traits evaluated depicted that the first cluster consisted of tef germplasm lines with early panicle emergence and maturity, short grain filling period, thin first and second basal culm internodes. In contrast, this cluster is

Table 1. Tef genotypes used in the study and area of collection.

S/N	Name	Collection zones	No.	Name	Collection zones
1	Amh-ACC#1-L50	North Wello	36	Oro-ACC#16-L38	Jima
2	Amh-ACC#1-L51	North Wello	37	Oro-ACC#16-L48	Jima
3	Amh-ACC#1-L56	North Wello	38	Oro-ACC#16-L51	Jima
4	Amh-ACC#1-L59	North Wello	39	Oro-ACC#16-L52	Jima
5	Amh-ACC#5-L4	North Wello	40	Oro-ACC#7-L1	Horo Gudru
6	Amh-ACC#5-L63	North Wello	41	Oro-ACC#7-L15	Horo Gudru
7	Amh-ACC#6-L5	North Wello	42	Oro-ACC#7-L19	Horo Gudru
8	Amh-ACC#6-L11	North Wello	43	Oro-ACC#9-L2	Horo Gudru
9	Amh-ACC#6-L41	North Wello	44	Oro-ACC#9L5	Horo Gudru
10	Amh-ACC#8-L13	North Wello	45	Oro-ACC#9-L26	Horo Gudru
11	Amh-ACC#8-L20	North Wello	46	Oro-ACC#9-L28	Horo Gudru
12	Amh-ACC#8-L51	North Wello	47	Oro-ACC#9-L38	Horo Gudru
13	Amh-ACC#8-L61	North Wello	48	Oro-ACC#1-L1	South WestShewa
14	Amh-ACC#9-L4	North Wello	49	Oro-ACC#1-L21	South WestShewa
15	Amh-ACC#9-L45	North Wello	50	Oro-ACC#1-L37	SouthWestShewa
16	Amh-ACC#11-L13	North Wello	51	Oro-ACC#4-L18	South WestShewa
17	Amh-ACC#11-L44	North Wello	52	Oro-ACC#4-L25	South WestShewa
18	Amh-ACC#11-L22	North Shewa	53	Oro-ACC#4-L47	South WestShewa
19	Amh-ACC#11-L36	North Shewa	54	Oro-ACC#8-L10	South WestShewa
20	Amh-ACC#12-L2	North Shewa	55	Oro-ACC#8-L17	South WestShewa
21	Amh-ACC#12-L4	North Shewa	56	Oro-ACC#8-L25	South WestShewa
22	Amh-ACC#12-L29	North Shewa	57	Oro-ACC#15-L8	South WestShewa
23	Amh-ACC#14-L21	North Shewa	58	Oro-ACC#15-L12	South WestShewa
24	Amh-ACC#14-L23	North Shewa	59	Oro-ACC#15-L30	South WestShewa
25	Amh-ACC#14-L24	North Shewa	60	Oro-ACC#16-L42	WestShewa
26	Oro-ACC#8-L13	Jima	61	Oro-ACC#16-L49	West Shewa
27	Oro-ACC#8-L30	Jima	62	Oro-ACC#19-L32	West Shewa
28	Oro-ACC#8-L32	Jima	63	Oro-ACC#19-L36	West Shewa
29	Oro-ACC#8-L5	Jima	64	Oro-ACC#27-L3	West Shewa
30	Oro-ACC#9-L34	Jima	65	Oro-ACC#27-L17	West Shewa
31	Oro-ACC#9-L37	Jima	66	Oro-ACC#30-L7	West Shewa
32	Oro-ACC#9-L45	Jima	67	Oro-ACC#30-L14	West Shewa
33	Oro-ACC#11-L15	Jima	68	Oro-ACC#30-L29	West Shewa
34	Oro-ACC#11-L26	Jima	69	Quncho (DZ-Cr-387)	Released variety (2006*)
35	Oro-ACC#11-L36	Jima	70	Tsedey (DZ-Cr-37)	Released variety (1984*)

*Year of release.

characterized by tef materials having high harvest index, lodging index and grain yield. On the other hand, except relatively high values of tiller number (total and fertile) and harvest index, the remaining characters of the genotypes included in the second cluster scored small values (Tables 3 and 4).

However, most quantitative traits of tef germplasm lines measured within C-3, 5, 6 and 10 showed relatively high values. Unlike their common characters, C-3, 5 and 6 contained the largest number tef germplasm lines, while C-10 contained a locally collected single tef germplasm line (Oro-ACC#4-L18). In addition, both C-5 and C-6 showed lower tiller numbers (total and fertile) and lodging index values. On top of this, relatively lower values of harvest index were exhibited by cluster 10. Days to maturity and grain filling period showed the highest mean value in C-7, but the lowest cluster mean values of total biomass and grain yield, lower value of straw yield, lodging index, number of fertile and total tillers were noted for this cluster. Cluster 4 is characterized by tef germplasm lines which have relatively small number of primary panicle branches, high total tiller number, longer grain filling period, late maturity, and high values of total biomass, grain yield and straw yield. The lowest cluster mean values of most traits were noted for C-8, which contained the single tef germplasm line (Oro-ACC#8-L30)

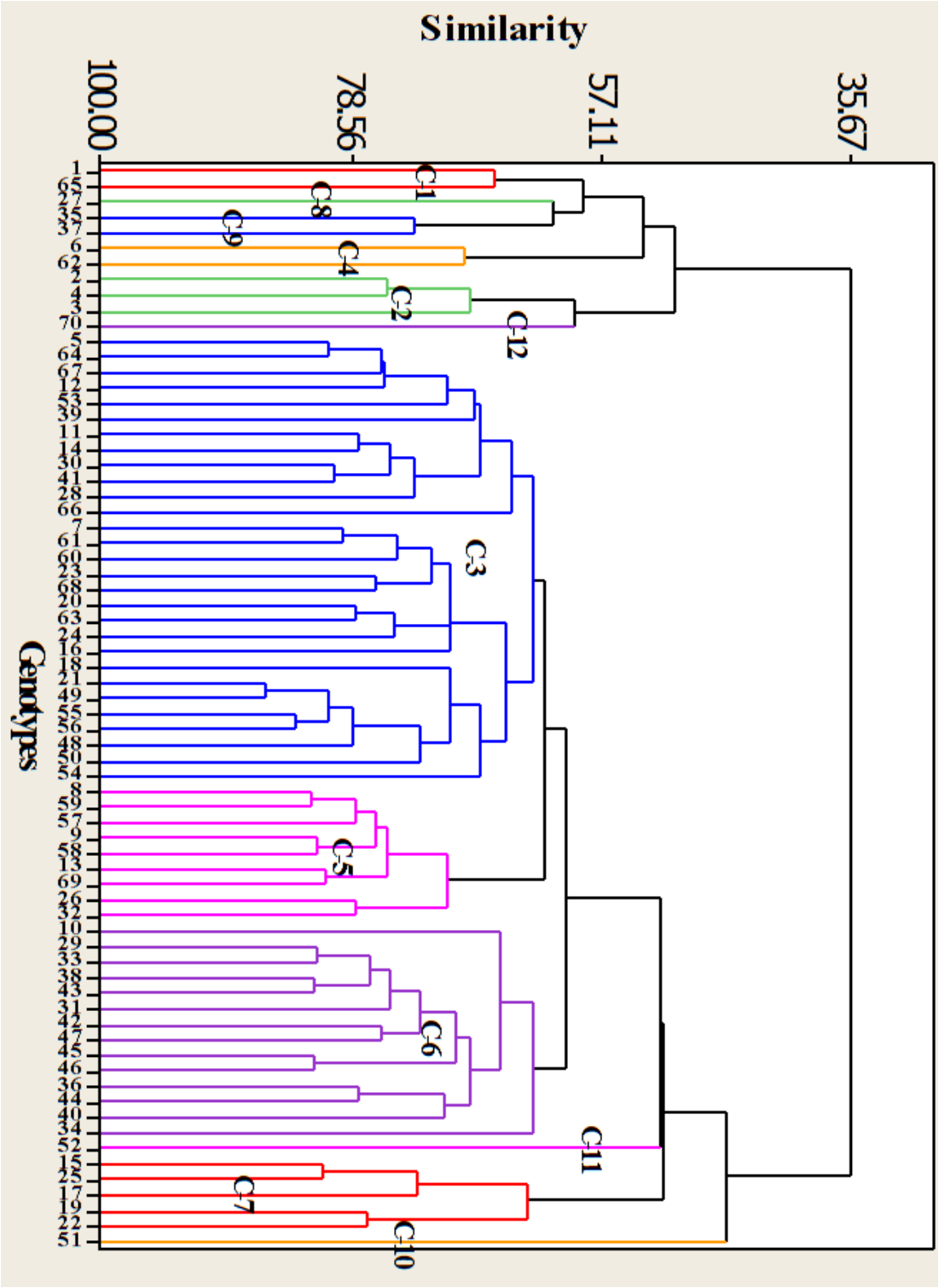

Figure 1. Dendrogram of seventy genotypes based on average linkage and Euclidean distance of 18 traits evaluated at two locations.

exhibiting the lowest value of height related traits (plant height, panicle length and culm length), basal culm diameters, number of total tillers, and lower number spikletes per main panicle and number of fertile tillers. On the other hand, these traits scored similarly low values in C-9. Contrary to this, the highest mean grain yield and

Table 2. List of tef genotypes grouped in 12 clusters (average linkage Euclidean distance cluster analyses) using 18 traits evaluated at two locations.

Cluster	No. of genotypes	Tef genotypes
1	2	Amh-ACC#1-L50 and Oro-ACC#27-L17
2	3	Amh-ACC#1-L51, Amh-ACC#1-L56 and Amh-ACC#1-L59
3	29	Amh-ACC#5-L4, Amh-ACC#6-L5, Amh-ACC#8-L20, Amh-ACC#8-L51, Amh-ACC#9-L4, Amh-ACC#11-L13, Amh-ACC#11-L22, Amh-ACC#12-L2, Amh-ACC#12-L4, Amh-ACC#14-L21, Amh-ACC#14-L23, Oro-ACC#8-L32, Oro-ACC#9-L34, Oro-ACC#16-L52, Oro-ACC#7-L15, Oro-ACC#1-L1, Oro-ACC#1-L21, Oro-ACC#1-L37,Oro-ACC#4-L47, Oro-ACC#8-L10, Oro-ACC#8-L17, Oro-ACC#8-L25, Oro-ACC#16-L42, Oro-ACC#16-L49, Oro-ACC#19-L36, Oro-ACC#27-L3, Oro-ACC#30-L7, Oro-ACC#30-L14 and Oro-ACC#30-L29
4	2	Amh-ACC#5-L63 and Oro-ACC#19-L32
5	9	Amh-ACC#6-L11, Amh-ACC#6-L41, Amh-ACC#8-L61, Oro-ACC#8-L13, Oro-ACC#9-L45, Oro-ACC#15-L8,Oro-ACC#15-L12, Oro-ACC#15-L30 and Quncho (DZ-Cr-387)
6	14	Amh-ACC#8-L13, Oro-ACC#8-L5, Oro-ACC#9-L37, Oro-ACC#11-L15, Oro-ACC#11-L26, Oro-ACC#16-L38,Oro-ACC#16-L51, Oro-ACC#7-L1, Oro-ACC#7-L19, Oro-ACC#9-L2, Oro-ACC#9L-5, Oro-ACC#9-L26,Oro-ACC#9-L28 and Oro-ACC#9-L38)
7	5	Amh-ACC#9-L45, Amh-ACC#11-L44, Amh-ACC#11-L36, Amh-ACC#12-L29 and Amh-ACC#14-L24
8	1	Oro-ACC#8-L30
9	2	Oro-ACC#11-L36and Oro-ACC#16-L48
10	1	Oro-ACC#4-L18
11	1	Oro-ACC#4-L25
12	1	Tsedey (DZ-Cr-37)

higher total biomass and straw yield means were noted for C-8. Similarly, C-9 holds tef materials which passed higher lodging index but lower total biomass and straw yield mean values.

Cluster 11 which comprised the solitary tef germplasm line Oro-ACC#4-L25, scored the lowest number of primary branches per main panicle and harvest index (Tables 3 and 4). Additionally, this cluster is characterized by tef genotype which had high mean value for height related traits, longer grain filling period and low grain yield. The last cluster (C-12) which contained only the single released variety "*Tseday*", scored the lowest values in phenological traits (days to maturity and grain filling period) and the highest value in total and fertile tiller numbers. Similarly, higher harvest index value, earliness in panicle emergence, lower total biomass, and straw yield were the characteristics features of this cluster. In addition, this specific character of the tef variety "*Tseday*" is in agreement with the inherent nature of the variety, because this variety is manly released for use in low moisture stress areas, and it possesses characteristics of earliness in phenological traits to escape terminal drought. Finally, most of tef germplasm lines which included in the two big clusters (3 and 5) had best performance with respect to most important traits under consideration. Those genotypes, therefore, can be recommended for further evaluation.

Inter cluster distances (D^2)

Most inter cluster distances showed highly significant ($P<0.01$) differences, while there were no significant inter-cluster distances between C-3 and C-5 and C-3 and C-6 (Table 5). In addition, the shortest ($D^{2=}25.07$) inter-cluster D^2 values were estimated between C-3 and C-5, while the largest ($D^{2=}326.22$) was estimated between C-8 and C-10, each of which contain one local tef germplasm line Oro-ACC#8-L3 and Oro-ACC#4-L18, respectively. Similarly, C-8 and C-11 comprised the second most divergent ($D^{2=}275.22$) groups and in this case Oro-ACC#8-L30 formed far inter-cluster distance with Oro-ACC#4-L25. In addition, the other clusters (C 8 and C 12) which in that order contain the solitary local tef germplam

Table 3. Means for the 12 clustered for the 18 quantitative traits of 70 Tef genotypes clustered into 12 groups.

Cluster	DH	DM	GFP	PH	PL	CL	NSPP	NPB	FBCD
C-1	40.38^{e}	93.50^{c}	53.13^{cd}	88.02^{de}	33.09^{def}	54.93^{de}	322.25^{bcd}	21.35^{cdef}	1.67^{def}
C-2	44.42^{cd}	97.17^{c}	52.75^{cd}	79.40^{f}	28.33^{fg}	51.07^{ef}	242.66^{d}	19.63^{ef}	1.59^{efg}
C-3	48.79^{b}	112.04^{ab}	63.25^{ab}	99.68^{bc}	37.12^{abcd}	62.56^{abc}	397.97^{abc}	25.76^{abc}	2.12^{abc}
C-4	47.75^{bc}	110.88^{ab}	63.13^{ab}	88.24^{de}	32.73^{def}	55.51^{de}	295.61^{cd}	20.33^{def}	1.93^{bcde}
C-5	49.44^{ab}	106.00^{b}	56.56^{bcd}	108.63^{a}	41.30^{a}	67.34^{a}	479.97^{a}	26.96^{ab}	2.28^{ab}
C-6	48.80^{b}	112.96^{ab}	64.16^{ab}	105.55^{ab}	38.74^{abc}	66.81^{a}	431.57^{ab}	28.60^{a}	2.37^{a}
C-7	47.30^{bc}	113.90^{a}	66.60^{a}	90.63^{d}	34.89^{cde}	55.75^{cde}	341.21^{bcd}	25.09^{abcd}	2.26^{abc}
C-8	44.25^{cd}	105.75^{b}	61.50^{ab}	74.99^{f}	27.13^{g}	47.85^{f}	247.35^{d}	21.80^{cdef}	1.25^{g}
C-9	47.13^{bc}	105.75^{b}	58.63^{bc}	81.49^{ef}	30.50^{efg}	50.99^{ef}	298.99^{cd}	21.16^{cdef}	1.54^{fg}
C-10	52.75^{a}	112.25^{ab}	59.50^{abc}	104.45^{ab}	37.70^{abcd}	66.75^{a}	486.97^{a}	23.54^{bcde}	1.90^{cdef}
C-11	44.75^{cd}	108.25^{ab}	63.50^{ab}	104.49^{ab}	40.50^{ab}	63.99^{ab}	383.71^{abc}	17.44^{f}	1.99^{bcd}
C-12	41.25^{de}	91.25^{c}	50.00^{d}	95.54^{cd}	35.80^{bcde}	59.74a^{bcd}	384.70^{abc}	23.70^{bcde}	1.67^{def}
LSD at 0.05	3.73	7.83	7.74	8.45	5.36	6.95	116.87	4.82	0.37

Entry	SBCD	LI	NTT	NFT	TSW	TBM	GY	SY	HI
C-1	1.71^{def}	75.35^{abc}	4.25^{c}	3.78^{cd}	0.28	11750^{cde}	4115^{a}	7635de	34.50^{a}
C-2	1.73^{def}	66.00^{bcd}	5.92^{ab}	5.46^{b}	0.29	9458^{ef}	3288^{bcd}	6171^{e}	34.87^{a}
C-3	2.28^{ab}	69.45^{abcd}	4.07^{c}	3.57^{cd}	0.30	13519^{abc}	3994^{ab}	9525^{bc}	29.64^{bc}
C-4	1.86^{def}	68.38^{bcd}	5.46^{b}	4.42^{c}	0.30	14125^{abc}	4187^{a}	9939^{abc}	29.49^{bc}
C-5	2.40^{a}	65.86^{bcd}	3.48^{c}	3.07^{d}	0.30	14861^{a}	4310^{a}	10551^{ab}	29.02^{bc}
C-6	2.46^{a}	59.09^{d}	3.58^{c}	3.24^{d}	0.31	11813^{cde}	3145^{cd}	8668^{cd}	26.60^{cd}
C-7	2.26^{abc}	63.75^{cd}	3.58^{c}	3.16^{d}	0.30	8800^{f}	2569^{d}	6231^{e}	28.61^{bc}
C-8	1.55^{f}	73.75^{abc}	3.25^{c}	3.10^{d}	0.31	14375^{ab}	4484^{a}	9892^{abc}	31.62^{ab}
C-9	1.61^{ef}	77.25^{ab}	3.88^{c}	3.66^{cd}	0.27	9875^{def}	3157^{cd}	6719^{e}	31.90^{ab}
C-10	2.02^{bcd}	81.50^{a}	6.15^{ab}	5.70^{ab}	0.30	15250^{a}	3671^{abc}	11579^{a}	23.99^{d}
C-11	1.93^{cde}	72.00^{abc}	4.21^{c}	3.34^{d}	0.36	12125^{bcd}	2789^{d}	9336^{bcd}	22.88^{d}
C-12	2.02^{bcd}	73.50^{abc}	6.75^{a}	6.50^{a}	0.30	9250^{f}	3114^{cd}	6136^{e}	34.63^{a}
LSD at 0.05	0.34	12.54	1.20	1.03	NS	2400.4	815.38	1830	4.02

Genotypes followed by the same letter are not significantly different at $P < 0.05$.

Table 4. The Generalized Squared inter cluster distance of 18 quantitative traits of 70 tef genotypes evaluated over two locations

Cluster	1	2	3	4	5	6	7	8	9	10	11
1	0										
2	54.06**	0									
3	94.01**	140.73**	0								
4	70.74**	87.85**	52.40**	0							
5	133.33**	219.18**	25.07ns	117.65**	0						
6	180.63**	232.30**	26.76ns	131.45**	32.29*	0					
7	125.67**	144.76**	36.70**	76.78**	97.44**	46.22**	0				
8	100.46**	124.82**	127.34**	103.99**	216.47**	233.95**	155.42**	0			
9	48.40**	45.35**	80.04**	55.41**	158.72**	159.90**	73.87**	66.81**	0		
10	190.02**	228.77**	112.33**	161.78**	95.21**	132.37**	202.01**	326.22**	200.02**	0	
11	145.08**	210.71**	86.13**	138.72**	89.65**	84.52**	110.17**	275.22**	166.59**	86.23**	0
12	106.84**	62.87**	190.60**	188.98**	226.55**	250.08**	214.67**	273.28**	131.61**	184.92**	199.21**

lines Oro-ACC#8-L30 and the released tef variety "*Tseday*" constituted the third most divergent (D^2=273.28) group, while the fourth most divergent (D^2 = 250.08) groups were cluster C-6 which constituted local tef

Table 5. Eigenvectors and eigenvalues of the first five principal components for 18 traits of 70 tef genotypes evaluated at Debre Zeit and Holetta during the 2015 main cropping season

Traits	PC1	PC2	PC3	PC4	PC5
Days to heading	-0.223	-0.001	-0.159	-0.074	-0.401
Days to maturity	-0.229	0.157	-0.539	-0.117	-0.042
Grain filling period	-0.163	0.19	-0.57	-0.103	0.153
Plant height	-0.328	-0.077	0.196	-0.002	0.09
Panicle length	-0.295	-0.109	0.157	0.054	0.129
Culm length	-0.298	-0.043	0.192	-0.039	0.049
Number of spikletes per panicle	-0.286	-0.114	0.193	-0.13	-0.076
Number of primary panicle branches	-0.259	0.087	0.232	-0.146	0.062
First basal culm diameter	-0.305	0.182	0.109	-0.099	0.169
Second basal culm diameter	-0.311	0.127	0.122	-0.186	0.12
Lodging index	0.099	-0.33	-0.245	0.019	0.318
Number of total tillers	0.193	-0.184	0.029	-0.634	-0.046
Number of fertile tillers	0.200	-0.169	0.063	-0.632	-0.059
Thousand seed weight	-0.123	-0.023	-0.136	-0.179	0.639
Total biomass	-0.202	-0.456	-0.132	0.052	-0.152
Grain yield	-0.084	-0.523	-0.077	0.162	0.078
Straw yield	-0.233	-0.389	-0.144	0.002	-0.235
Harvest index	0.214	-0.213	0.117	0.142	0.368
Eigenvalue	7.1661	2.8857	1.6609	1.4726	1.1191
Percent of total variation explained	39.8	16	9.2	8.2	6.2
Cumulative percent of total variance explained	39.8	55.8	65.1	73.3	79.5

germplasm lines mostly collected from Jimma and Horo Gudru Zones of Oromya region and C-12 containing the released variety "*Tseday*".

Overall, the released variety "*Tseday*" and the locally collected tef germplasm line (Oro-ACC#8-L30) had large genetic distance with most of the other clusters in this experiment. On top of this, the high inter-cluster distances noted among different clusters may result from locations in which those tef germplasms were collected and different genetic background of those tef materials (released vs. local tef germplasm lines). Generally, a wide generalized squared distance (D^2) serves as a better indicator for selecting crossing materials. Consequently, most divergent clusters noted in this study are expected to give maximum genetic recombination and genetic variation in the subsequent segregating generations.

Principal components analysis

The first five principal components (PCs) having a minimum eigenvalue of one accounting for 80% of the total variability observed among the 70 tef test genotypes (Table 6). Of these, the first PC alone explained about 40% of the total variance mainly due to the variations in height related traits (that is, plant height, panicle length, and culm length), first and second basal culm internode diameters, and number of spiklets and primary branches per main panicle. On the other hand, even if relatively lower percent variation was explained by PC 1 in the studies of Assefa et al. (1999, 2000, 2001a, b), most of the traits responsible for variation in PC 1 showed similarity with the current study. In addition, another experiment of Assefa et al. (2003) with seventeen traits of 60 tef germplasm population showed similarity in both percent variation explained, and the traits contributing to the variation in PC 1. However, the first PC in the studies of Adnew et al. (2005) and Jifar et al. (2015) explained relatively high proportion of the variation than that in this study.

Unlike, the first PC, most yield related traits like grain yield, total biomass, straw yield, harvest index and lodging index contributed to about 16% of the gross variation accounted for by the second PC (Table 6). This is line with results of the second PC of Assefa et al. (2000). However, slightly larger variability was reported by Assefa (1999, 2001b, 2003) in other studies, whereas Assefa et al. (2001a) and Adnew et al. (2005) reported that the second PC, respectively explained 7.1% more and 5.6% less variability than that in the current study. Furthermore, about 9, 8 and 6% of the total genotype variance was explained on the basis of the third, fourth and fifth PCs, respectively (Table 6). The former was largely due to the variations in phenological traits (that is, days to maturity and grain filling period), lodging index and number of primary panicle branches, whereas, number of total and fertile tillers were the primary

Table 6. Eigenvectors and eigenvalues of the first five principal components for 18 traits of 70 tef genotypes evaluated at Debre Zeit and Holetta during the 2015 main cropping season.

Traits	PC1	PC2	PC3	PC4	PC5
Days to heading	-0.223	-0.001	-0.159	-0.074	-0.401
Days to maturity	-0.229	0.157	-0.539	-0.117	-0.042
Grain filling period	-0.163	0.19	-0.57	-0.103	0.153
Plant height	-0.328	-0.077	0.196	-0.002	0.09
Panicle length	-0.295	-0.109	0.157	0.054	0.129
Culm length	-0.298	-0.043	0.192	-0.039	0.049
Number of spikletes per panicle	-0.286	-0.114	0.193	-0.13	-0.076
Number of primary panicle branches	-0.259	0.087	0.232	-0.146	0.062
First basal culm diameter	-0.305	0.182	0.109	-0.099	0.169
Second basal culm diameter	-0.311	0.127	0.122	-0.186	0.12
Lodging index	0.099	-0.33	-0.245	0.019	0.318
Number of total tillers	0.193	-0.184	0.029	-0.634	-0.046
Number of fertile tillers	0.200	-0.169	0.063	-0.632	-0.059
Thousand seed weight	-0.123	-0.023	-0.136	-0.179	0.639
Total biomass	-0.202	-0.456	-0.132	0.052	-0.152
Grain yield	-0.084	-0.523	-0.077	0.162	0.078
Straw yield	-0.233	-0.389	-0.144	0.002	-0.235
Harvest index	0.214	-0.213	0.117	0.142	0.368
Eigenvalue	7.1661	2.8857	1.6609	1.4726	1.1191
Percent of total variation explained	39.8	16	9.2	8.2	6.2
Cumulative percent of total variance explained	39.8	55.8	65.1	73.3	79.5

contributors to the variation explained by PC4. Likewise, the contribution of PC5 resulted chiefly from variations in characters like thousand seed weight, days to heading, harvest index, lodging index, and straw yield.

Conclusion

The grouping of tef genotypes into twelve clusters at 73% similarity level confirmed the existence of important trait variability among tef genotypes that could be recommended for further evaluation and regarding conservation of the indigenous tef genetic resources in Ethiopia, unclear patterns of genotypes grouping in respect to their origin in this experiment showed the importance to address each tef growing zones of the country. Height related traits (that is plant height, panicle length and culm length), first and second basal culm internode diameters and number of spiklets and primary branches per main panicle contributed more for the 40% variation explained by the first PC. In addition, most of tef germplasm lines which were included in the two big clusters (3 and 5) had best performance with regard to most important traits under consideration. Moreover, the higher mean values of most yield related traits of Oro-ACC#8-L30 (C-8) and earliness in maturity, higher tiller number and harvest index of "*Tseday*"(C-12), in line with their large genetic distance with most of the other clusters could make them source of elite materials for future use.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Identification of important morphological traits in Mozambican sorghum [*Sorghum bicolor* (L.) Moench] germplasm using multivariate analysis

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Classification of sorghum [*Sorghum bicolor* (L) Moench] breeding material based on multiple crucial characters is important towards the possible formation of homogeneous groups of genotypes and groups that can be exploited in the identification of parents for use in a breeding program. The objective was to determine the morphological characters that distinguish desirable breeding material and group the genotypes of sorghum according to similarity. Principal component analysis (PCA) and cluster analysis were used to establish the relationships among germplasm and the Shannon Diversity index was used to quantify the level of diversity. The experiment involving 26 sorghum genotypes was conducted at Sussundenga Research Station across two seasons and laid out in a 13 × 2 alpha lattice design with four replications. Cluster analysis grouped genotypes into four clusters based on 15 evaluated traits. Five principal components cumulatively accounting for 58.5% of the total variation were estimated from the PCA analysis. The results showed that genotypes 150B, IS 14257R, LARSVYT 46B, TX 631B, TX 630B and 8601B were the early maturity while for late maturity genotypes were MA 6B, A 6352R, ICSA 19B and MZ 30R. The genotypes IS 7179R, SPL 9B, A 6353R, SPL 38B, SDS 6013R and MZ 2R showed a potential for grain yield improvement. Other genotypes presented potential for drought tolerance and birds attack. The multivariate analyses clearly showed the grouping of the genotypes according to the characters outlined in the study. These results have implications in selecting parents for use in sorghum breeding program.

Key words: Cluster analysis, diversity index, principal component analysis and sorghum improvement.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop worldwide that is used for food, feed and biofuel. There are different types of sorghum depending on intended use, *viz*. grain sorghum, dual purpose (grain and fodder) sorghum, fodder sorghum, and sweet stalk sorghum (Kumar et al., 2008; Reddy et al., 2012). Classification of sorghum is also based on its uses and importance.

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Table 1. List of sorghum lines used in the study.

Genotype No.	Line	Genotype No.	Line
1	150B	14	MA6B
2	8607B	15	MACIA
3	860IB	16	MZ 2R
4	A6352R	17	MZ 30R
5	CK 60B	18	MZ 37R
6	ICSA 12B	19	SDS 260R
7	ICSA 19B	20	SDS 6013R
8	ICSA 21B	21	SPI 38B
9	IS 14257R	22	SPL9B
10	IS 21458R	23	TX 623B
11	IS 7179R	24	TX 628B
12	LARSVYT 19R	25	TX 630B
13	LARSYT46B	26	TX 631B

Central Africa is the origin of sorghum and is where it was domesticated and cultivated (House, 1995). The cultivated and wild sorghums demonstrate greatest genetic diversity of this crop (Ayana and Bekele, 1999). In Ethiopia, the centre of diversity of sorghum, 15 cultivated sorghum races have been reported (Mengesha, 1975). Within these 15 races, 5 races are primary (*bicolor*, *caudatum, guinea, durra* and *kafir*) and 10 races are intermediates of primary races (Harlan and De Wet, 1972). The most cultivated race in Southern and Eastern Africa is *guinea* (Folkertsma et al., 2005; Lacy et al., 2006). However, Ramathani et al. (2011) reported that all five primary races are cultivated in sub-Saharan Africa. Therefore, it is important to classify the germplasm used in breeding programmes to make it easy for plant breeders to identify and select valuable genetic resources to improve farmers preferred traits in a variety.

There are many mathematical methods that permit grouping of organisms and/or species according to their characteristics. The common methods are the multivariate analysis that includes principal component analysis (PCA) and cluster analysis, which are used to establish the relationship among germplasm and Shannon diversity index which is used to determine the level of diversity. The PCA is a strong tool, which reduces the dimensions of the data before applying clustering (Derksen et al., 1995; Yeung and Ruzzo, 2001). Additionally, cluster analysis is used for pattern recognition and as a discriminant method that reveals structure and relationships in the data (Anderberg, 2014). The Shannon diversity index measures unequal weights through decomposing the measurements into expressive components such as independent alpha and beta components (Jost, 2007). One of the differences between principal component analysis and cluster analysis is that the few PCs containing most of the variation do not capture most of the cluster structure (Yeung and Ruzzo, 2001). This implies that the two methods can complement each other and help breeders better identify proper germplasm for use in their breeding programs.

The diversity of different germplasm is used as a possible source of genes that can be used to improve the performance of cultivars in terms of phenotypic and genetic make-up (Geleta et al., 2006). The use of multivariate approaches such as cluster and principal component analysis may help to estimate the magnitude of diversity among germplasm. These methods use the morphological characters to provide information about the similar groups and the information generated can be used to identify genotypes that have desirable characters for breeding purposes such as hybridization for pedigree breeding. Chikuta et al. (2015) used multivariate analysis approaches to select sorghum genotypes exhibiting high levels of grain and fodder traits from morphological and agronomic data, while Mujaju and Chakauya (2008) used multivariate analysis to categorise agro-morphological characters of sorghum landraces to explain production factors and uses of sorghum at farmers' level.

Several studies have evaluated genetic diversity through phenotypic data (Ganesamurthy, 2013; Chikuta et al., 2015; Fernandez et al, 2014) and molecular marker data (Muraya, 2014; Uttam et al., 2017). However, there is a need to validate information on the genetic diversity of Mozambican sorghum germplasm using similar approaches. The objective of this study was to identify important morphological traits that distinguish desirable breeding material in the National Sorghum breeding programme.

MATERIALS AND METHODS

Plant

Fifteen cytoplasmic male sterile (CMS) lines and ten male fertile (restorer-R) lines of sorghum were used in this study (Table 1). These breeding lines were sourced from International Crops

Table 2. Characteristic of the location and season used for evaluation of germplasm.

Location	Season	Code	Latitude (°S)	Longitude (°E)	Altitude (m)	Rainfall* (mm)
Sussundenga	2015/2016	Sus16	19°18'	33°15'	635	522
Sussundenga	2016/2017	Sus17	19°18'	33°15'	635	989

*Rainfall referred to the amount received during the crop growing season.

Research Institute for the Semi-Arid Tropics (ICRISAT) and from the Sorghum National program. Maintainer lines (B-lines) were planted next to the A-lines to facilitate grain formation by male sterile lines, thereby enabling collection of data for panicle and grain traits.

Location and experimental design

The experiment was conducted at Sussundenga Research Station (SRS), Manica State, Mozambique over two seasons. The lines were planted in January 2015 and December 2016. This location covered the mid-altitude mega-environment. Table 2 summarizes the location and annual average rainfall per season. The maximum temperature of 29.5°C and minimum of 17.6°C characterize the location (MAE, 2014). The SRS is located at longitude 33.28° and Latitude -19.4° and an altitude of 579 m above sea level (WA. 2018). The soil type in SRS is majorly red clay soil but sandy soil is also found in some areas (MAE, 2014).

The trial was laid out in a 13 × 2 alpha lattice design with four replications. Each plot had four rows that were 4 m long and spaced 80 cm apart, with an in-row spacing of 25 cm. The crop management was according to recommended practices (Bias et al., 2010).

Data collection

Morphological characterization was done using International Board for Plant Genetic Resource (IBPGR) and International Crops Research Institute for the Semi-Arid Tropics IBPGR and ICRISAT (1993) descriptor list. The characteristics used for phenotypic characterization are described in Table 3. The data were collected and recorded from the two middle rows of each plot. Six plants per accession were randomly selected for observations and measurements.

Data analysis

The analysis of variance for the characters was used to estimate the mean squares effects using the GLM procedures in SAS software version 9.3 (SAS, 2011), according to the model:

$$P_{ijkl} = \mu + g_i + r_j + b_k + t_l + \varepsilon_{ijkl}$$

where P_{ijk} is the phenotypic value of the i^{th} accession, μ is the grand mean, g_i is the genetic effect for the i^{th} accession, r_j is the replication effect, b_k is the block effect in each replication, t_l is the effect of season and ε_{ijk} is the residual error.

The phenotypic variances for the characters were estimated according to the following model:

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

where σ_p^2 is the phenotypic variance, σ_g^2 is the genotypic variance, σ_e^2 is the environment variance (the mean square of residual error).

$$\sigma^2_g = MSg - Mse / r$$

where MSg is the mean square of genotypes, MSe is the mean square of error and r is the number of replications.

The broad sense heritability (h_b^2) was calculated as:

$$h^2_b = \sigma^2_g / \sigma^2_p \times 100$$

where σ_p^2 is the phenotypic variance and σ_g^2 is the genotypic variance.

Cluster analysis was performed using unweighted pair-group method with arithmetic average (UPGMA) and dendrogram constructed using the GenStat statistic software version 18^{th} (Payne et al., 2016). Principal component analysis (PCA) was performed using the R statistics software (R Team, 2014) where the biplot of multivariate data was constructed.

The diversity among germplasm was determined from morphological frequencies using the method suggested by Grenier et al. (2000). The characters observed were used to calculate Shannon-Weaver index of diversity (H') from the frequency distribution for the lines and grouped into different classes according to Perry and McIntosh (1991). The calculation was done as:

$$H' = 1 - \sum_{i=1}^{n} pi\ Log\ e\ pi$$

where *H'* is Shannon Diversity Index; p_i is the proportion of lines in the i^{th} class of n-class character; n is the number of phenotypic classes of traits.

The H' estimates were done using GenStat statistic software version 18th (Payne et al., 2016) and Microsoft Excel.

RESULTS

Analysis of variance

Analysis of variance showed highly significant differences ($p \leq 0.01$) among lines for most of the characters measured except for the grain colour, glume colour and presence of awns (Table 4).

Variability and heritability of the characters

The genetic variance, phenotypic variance and heritability estimates are shown in Table 5. The phenotypic variance

Table 3. Descriptors used for morphological characterization of sorghum germplasm.

Characteristic	Descriptor and code
Stay green	Very slight senescent (1), leaves senescent 25% (2), leaves senescent 50% (3), leaves senescent 75% (4) and complete senescent (5) at harvest stage
Seed size	Small< 5 mm (1), medium< 5-10 mm (2), large> 10 mm (3)
Leaf rolling	Non-rolled leaf (1), 25% leaves rolled (2), 50% leaves rolled (3), 75% leaves rolled (4) and all leaves rolled (5)
Panicle exsertion	Slightly exserted <2 cm (1), exserted 2-10 cm (2), well exserted >10 cm (3), peduncle re-curved (4)
Leaf colour	Dark green (1) and light green (2)
Leaf orientation	Erect (1) and dropping (2)
Inflorescence compactness	Very loose erect (1), very loose dropping (2), loose erect (3), loose dropping (4), semi loose erect (5), semi loose dropping (6), semi compact elliptic (7), compact elliptic (8), compact oval (9), half broom corn (10) and broom corn (11)
Head shape	Elliptical (1), oblong (2), round (3), semi-loose (4) and loose (5)
Midrib colour	White (1), dull green (2), yellow (3), brown (4) and purple (5)
Grain colour	Red (1), yellow (2), brown (3), white (4), light orange (5), white with orange (6) and white and red (7), cream (8)
Awns	Absent (1) and present (2)
Glume colour	White (1), red (2), purple (3), black (4), grey (5), brown (6), dark brown (7)
Glume cover	25% grain covered (1), 50% grain covered (2), 75% grain covered (3), 100% grain covered (4) and glume longer than grain (5)

Source: Adapted from IBPGR/ICRISAT (1993).

Table 4. Analysis of variance for the genotypes characters measured across seasons.

Source	Sum Square	Mean Square	CV	SE	LSD
Days to 50% flowering	19119.74	764.79***	5.8	5	5.77
1000 seed weight	699.72	27.99***	14.0	2.55	2.52
Stay green	56.31	2.25***	8.8	0.15	0.15
Grain colour	539.08	21.56	4.6	0.28	0.57
Seed size	38.77	1.55***	17.9	0.39	0.39
Panicle exertion	91.58	3.66***	22	0.45	0.44
Midrib colour	90.39	3.62***	9.4	0.16	0.15
Leaf rolling	50.24	2.01***	27.9	0.43	0.43
Leaf orientation	44.79	1.79***	11.8	0.19	0.18
Leaf colour	26.88	1.08***	24.1	0.37	0.37
Inflorescence	334.04	13.37***	11.6	0.86	0.85
Awn	7.69	0.31	3.2	0.02	0.1
Head shape	95.69	3.87***	15.6	0.47	0.47
Glume colour	260.0	10.40	1.6	0.19	0.37

Table 4. Contd.

Glume cover	44.08	1.76***	13	0.18	0.18
Degree of freedom (DF)					
Rep	3	-	-	-	-
Season	2	-	-	-	-
Lines	25	-	-	-	-
Error	179	-	-	-	-
Total	207	-	-	-	-

***, **, * significant at 0.1, 1 and 5%, respectively.

Table 5. Mean squares and variability parameters for various characters of sorghum genotypes.

Character	MS	GV	PV	h^2_b	H'
Days to 50% flowering	764.79***	182.66	216.83	84.2	3.25
1000 seed weight (g)	27.99***	5.37	11.88	45.2	3.25
Stay green	2.25***	0.56	0.58	96.2	3.21
Grain colour	7.43	1.73	2.24	77.2	3.21
Seed size	1.55***	0.35	0.50	69.1	3.24
Panicle exertion	3.66***	0.87	1.06	81.3	3.20
Midrib colour	3.62***	0.90	0.92	97.4	3.19
Leaf rolling	2.01***	0.46	0.64	71.1	3.21
Leaf orientation	1.79***	0.44	0.47	92.8	3.21
Leaf colour	1.08***	0.23	0.37	63.2	3.23
Inflorescence	13.36***	3.16	3.90	81.0	3.24
Awn	1.78	0.19	1.23	15.1	3.25
Head shape	3.83***	0.90	1.12	80.1	3.23
Glume colour	16.06	3.76	4.79	78.5	3.21
Glume cover	1.76***	0.43	0.47	92.8	3.21

***, **, *Significant at 0.1, 1 and 5% respectively. MS: Mean square, GV: genotypic variance, PV: phenotypic variance, h2b: heritability broad sense and H': Shannon-Weiner Diversity index.

was higher than the genetic variance for all characters. Higher estimates were observed for days to 50% flowering, thousand seed weight and inflorescence compactness. The other characters such as stay green, seed size, leaf rolling, leaf colour and glume cover presented lower estimates.

Very high heritability estimates were obtained for stay green and midrib colour with 96.2 and 97.4%, respectively. Glume cover and leaf orientation also had very high heritability estimates of 92.8% each. The characters with heritability estimates below 50% were thousand seed weight and presence of awns with 45.2 and 15.1%, respectively.

Morphological characterization

Days to 50% flowering ranged from 81 days (line 150B) to 116 days (lines A6352R and MA6B). For the thousand-seed weight, line ICSA21B had a weight of 13.7 g representing the lowest whereas IS 7179B and SP 9B recorded 21.5 and 21.1 g, respectively, representing the highest values (Table 6).

Regarding the stay green character, 65.4% of the lines had 25.0% of their leaves senesced, 30.8% of the lines had very slight senescence, whereas 3.8% had 50.0% of their leaves senesced. The most senesced genotype was IS 7179R whereby harvesting time, 50% of the leaves were senesced. The majority of lines (65%) had white grain colour, 11% had creamy grains, while the remaining lines had red (8%), brown (8%) and light orange (8%) grains. In respect to seed size, most lines were medium size although lines SDS 6013R, SPL 38B, MZ 2R and IS 7179R were on average, large seeded. Panicle exsertion was mostly between 2 and 10 cm (42.3%), however, some had more than 10 cm (34.6%) and fewer exserted below 2 cm (23.1%) (Table 6).

Furthermore, midrib colour presented dull green colour

Table 5. Mean squares and variability parameters for various characters of sorghum genotypes.

Character	MS	GV	PV	h^2_b	H'
Days to 50% flowering	764.79***	182.66	216.83	84.2	3.25
1000 seed weight (g)	27.99***	5.37	11.88	45.2	3.25
Stay green	2.25***	0.56	0.58	96.2	3.21
Grain colour	7.43	1.73	2.24	77.2	3.21
Seed size	1.55***	0.35	0.50	69.1	3.24
Panicle exertion	3.66***	0.87	1.06	81.3	3.20
Midrib colour	3.62***	0.90	0.92	97.4	3.19
Leaf rolling	2.01***	0.46	0.64	71.1	3.21
Leaf orientation	1.79***	0.44	0.47	92.8	3.21
Leaf colour	1.08***	0.23	0.37	63.2	3.23
Inflorescence	13.36***	3.16	3.90	81.0	3.24
Awn	1.78	0.19	1.23	15.1	3.25
Head shape	3.83***	0.90	1.12	80.1	3.23
Glume colour	16.06	3.76	4.79	78.5	3.21
Glume cover	1.76***	0.43	0.47	92.8	3.21

***, **, *Significant at 0.1, 1 and 5% respectively. MS: Mean square, GV: genotypic variance, PV: phenotypic variance, h2b: heritability broad sense and H': Shannon-Weiner Diversity index.

Table 6. Means for the morphological characters used in the study for each genotype.

Line	DF	SW	SS	PE	MC	LR	LO	LC	IF
150 B	81	18.1	2	3	2	2	2	2	8
8607 B	101	17.1	3	2	1	1	2	1	7
860I B	88	16.1	2	3	2	2	1	2	8
A6352 R	115	20.9	2	3	2	2	1	2	8
CK 60 B	107	17.6	2	2	2	2	1	1	5
ICSA 12 B	106	19.1	2	3	2	2	2	1	7
ICSA 19 B	114	16.7	2	3	2	1	2	2	7
ICSA 21 B	104	13.7	2	3	2	1	2	2	8
IS 14257	87	17.2	3	1	2	2	2	2	8
IS 21458	107	20.1	2	2	1	2	2	2	6
IS 7179	94	21.5	3	3	1	1	1	2	8
LARSVYT 19 R	97	16.4	2	2	1	2	2	1	8
LARSYT46 B	87	20.3	2	2	1	2	2	2	8
MA6 B	116	18.2	2	2	1	1	1	1	7
MACIA	94	16.4	2	3	1	1	1	2	8
MZ 2	106	18.4	3	2	2	1	2	1	9
MZ 30	112	16.5	2	1	1	2	1	1	8
MZ 37	101	16.8	1	2	2	1	2	2	7
SDS 260 R	94	18.7	2	2	4	2	1	1	3
SDS 6013 R	106	16.8	3	1	1	2	2	2	7
SPI 38 B	98	18.2	3	3	2	1	1	2	7
SPL9 B	104	21.1	2	2	2	1	2	1	8
TX 623 B	103	20.3	2	1	1	1	2	2	8
TX 628 B	105	20.2	2	2	2	3	2	1	9
TX 630 B	88	19.2	2	1	2	2	1	2	9
TX 631 B	87	17.6	2	1	1	2	2	2	9
LSD	5.8	2.5	0.4	0.4	0.2	0.4	0.2	0.4	0.8
CV (%)	5.8	14.0	17.9	22.0	9.4	27.9	11.8	24.1	11.6

Table 6. Contd.

SED	2.9	1.3	0.2	0.2	0.1	0.2	0.1	0.2	0.4

DF: Days to flowering, SW: 1000 seed weight, SS: seed size, PE: panicle exsertion, MC: midrib colour, LR: leaf rolling, LO: leaf orientation, LC: leaf colour, IF: inflorescence compactness.

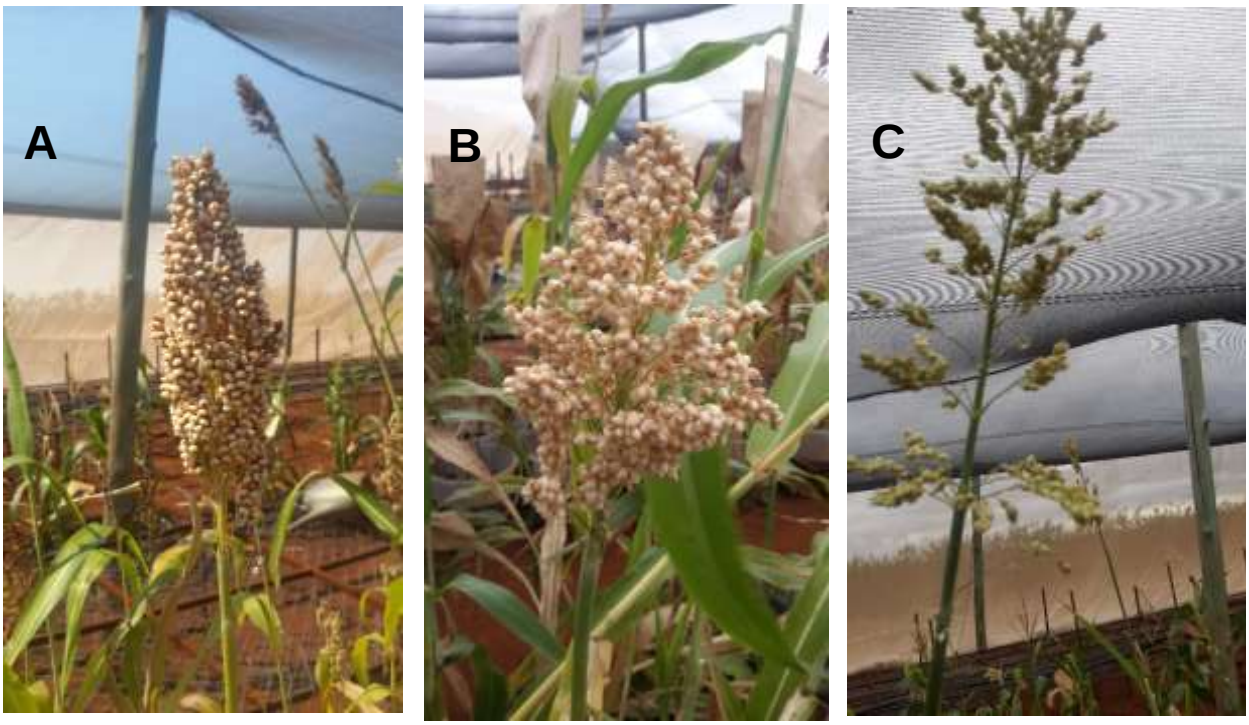

Figure 1. Inflorescence compactness of different genotypes: compact elliptic (a), semi compact elliptic (b) and semi loose erect (c).

in the majority of lines (53.8%), while white midrib colour was present in 42.3% of the lines and 3.8% were brown. Similar percentages were observed for leaf rolling characteristic, where 53.8% had their leaves rolled by 25%, 42.3% had non-rolled leaves, and 3.8% had leaves rolled by 50%. Likewise, regarding leaf orientation and colour, the majority of genotypes had dropping and dark green leaves (61.5%), whilst some had erect and light green leaves (38.5%).

For inflorescence compactness (Figure 1 and Table 6), the compact elliptic form was the most abundant (46.2%), followed by the semi compact elliptic (26.9%), compact oval (15.4%), and semi loose dropping, semi loose erect and loose erect each with 3.8%. On the other hand, 42.3% of the lines had round shaped heads, followed by semi loose shape (38.5%) and oblong shape (19.2%). About 96.2% lines in this study had no awns whereas 3.8% displayed awns as observed in line IS 7179R. Furthermore, different glume colours and glume covering percentages were observed. Most of the lines displayed grey glume colour (73.1%) while other lines presented red glumes (11.5%), black glumes (7.7%) and brown glumes (7.7%). The grain glume covering was 25% for the majority (53.8%) of lines, and other lines (46.2%) had 50% covering (Table 6).

Cluster analysis

The results of cluster analysis are presented in Figure 2 (genotypes names in Table 1). Lines 8 (ICSA 21B), 23 (TX 623B), 22 (SPL 9B), 20 (SDS 6013R), 24 (TX 628B), 10 (IS 21458R), 16 (MZ 2R), 6 (ICSA 12B) and 5 (CK 60B) were grouped together (Cluster I). The second group (Cluster II) constituted lines 17 (MZ 30R), 7 (ICSA 19B), 14 (MA 6B) and 4 (A6352R). The third group (Cluster III) included lines 19 (SDS 260R), 15 (Macia), 11 (IS 7179R), 21 (SPL 38B), 12 (LARSVYT 19R), 18 (MZ 37R) and 2 (8607R). The fourth group (Cluster IV) comprised lines 25 (TX 630B), 13 (LARSVYT 46B), 26

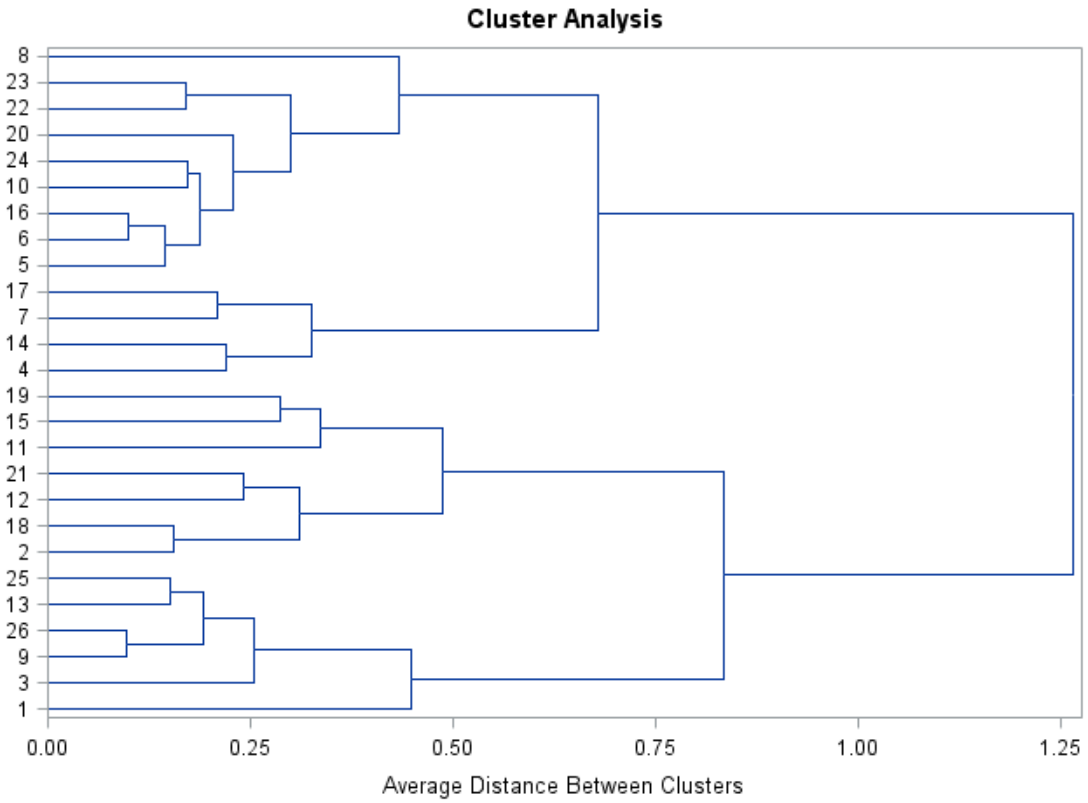

Figure 2. Dendrogram of 26 sorghum lines showing genetic similarity based on morphological characters (genotypes names are shown in Table 1).

(TX 631B), 9 (IS 14257R), 3 (8601B) and 1 (150B).

Cluster I contained the largest number of B and R lines from different groups, followed by cluster III which was made up of only R lines. Cluster IV grouped the majority of B lines and only one IS 14257R line. The least number of lines was found in cluster II.

Figure 2 shows cluster means for the various characters that were measured or observed. Cluster I had lines with an average of 105 days for 50% flowering. Average seed weight in this cluster was 18.6 g per thousand grains, with medium seed size and plants with 25% senesced leaves at harvesting maturity. Panicle exsertion was 2 to 10 cm with dark green and dropped leaves. The leaves were 25% rolled and midrib colour was dull green. The head was round, semi compact elliptic inflorescence, awn less with white grains. The grains were 25% covered with purple glumes.

Cluster II contained lines with the longest duration to 50% flowering. The lines had an average of 114 days for flowering days and mean of 18.1 g for thousand seed weight. The stay green character rating was on average 25% leaves senesced at harvesting maturity. Seed size was medium and panicle was exserted between 2 and 10 cm. Additional characters included non-rolling and erect leaves with a dull green midrib. The leaves were dark green and inflorescence compactness was the semi compact elliptic category. Head shape was on average round with white grains, awn less with purple glumes covering 25% of the grain (Table 7).

Days from planting to 50% flowering averaged 97 in cluster III and plants produced medium sized seed weighing on average 17.9 g per thousand grains. The plants stayed green until harvesting maturity (25% senesced leaves) with dull green midrib and no rolling leaves, erect oriented and light green. The panicles were2 to 10 cm exserted with semi compact elliptic inflorescence that was round in shape. The grains were white with no awns but covered 25% with black glumes (Table 7).

Cluster IV consisted of early flowering group with an average of 86 days to 50% flowering. The size of seeds was medium with an average weight of 18.1 g per thousand grains and plants having 25% senesced leaves at harvesting maturity. The leaves were light green, 25% rolled, dropped with a dull green midrib. The panicles were exserted 2 to 10 cm with round and compact elliptic inflorescence, and awn less. The grains were light orange and covered 50% with grey glumes (Table 7).

Principal component analysis

The PCA analysis showed that 58.5% of the total variation

Table 7. Cluster means for characters measured in the 26 genotypes.

Character	Cluster			
	I	II	III	IV
	n=9	n=4	n=7	n=6
Days to 50% flowering	105	114	97	86
1000 seed weight (g)	18.6	18.1	17.9	18.1
Stay green	2	2	2	2
Seed size	2	2	2	2
Panicle exsertion	2	2	2	2
Midrib colour	2	2	2	2
Leaf rolling	2	1	1	2
Leaf orientation	2	1	1	2
Leaf colour	1	1	2	2
Inflorescence compactness	7	7	7	8
Head shape	3	3	3	3
Glume cover	1	1	1	2
Glume colour	5	3	4	5
Grain colour	4	4	4	5
Awn	1	1	1	1

Table 8. Principal components and eigenvector loadings for the morphological characters.

Principal Components	Component 1	Component 2	Component 3	Component 4	Component 5
Eigen vectors (loadings)	-	-	-	-	-
Head shape	0.35	0.33	-0.25	0.15	0.11
Stay green	-0.17	-0.48	-0.37	-0.33	0.11
Leaf rolling	-0.22	-0.63	0.20	-0.26	0.14
Panicle exsertion	0.13	0.38	0.11	-0.40	-0.14
Leaf colour	-0.41	0.23	0.18	-0.12	0.37
Leaf orientation	-0.36	0.11	0.26	0.15	-0.20
Inflorescence compactness	-0.35	-0.34	0.18	-0.16	-0.30
Midrib colour	0.28	0.30	0.31	-0.27	0.17
Grain colour	-0.28	0.24	0.38	-0.25	-0.19
Awn	-0.28	0.30	-0.43	-0.15	-0.14
Glume colour	-0.28	0.36	0.21	0.19	0.41
Glume cover	-0.24	0.20	-0.18	0.16	0.46
Seed size	-0.20	-0.17	0.27	-0.77	0.20
Days to 50% flowering	0.31	-0.26	0.30	0.62	0.17
1000 seed weight (g)	-0.18	0.19	-0.18	0.53	0.17
Proportion of variance (%)	0.150	0.139	0.122	0.095	0.079
Cumulative proportion (%)	0.150	0.289	0.410	0.506	0.585

was accounted for by five components (Table 8) and the first component had the major contribution of 15% to the variation. Variation in the first component was mainly from the positive eigenvector loadings of head shape, days to 50% flowering and negative loadings of leaf colour, leaf orientation and inflorescence compactness. The second component contributed 13% to the variation mainly from the positive loadings of head shape, panicle exsertion, midrib colour, glume colour and negative loadings of leaf rolling and inflorescence compactness (Figure 3).

The variation in the third component (12.2%) was due to positive loadings of midrib colour, grain colour and days to 50% flowering while the negative eigenvector loadings were due to stay green and presence of awns. Positive loadings of days to 50% flowering and the

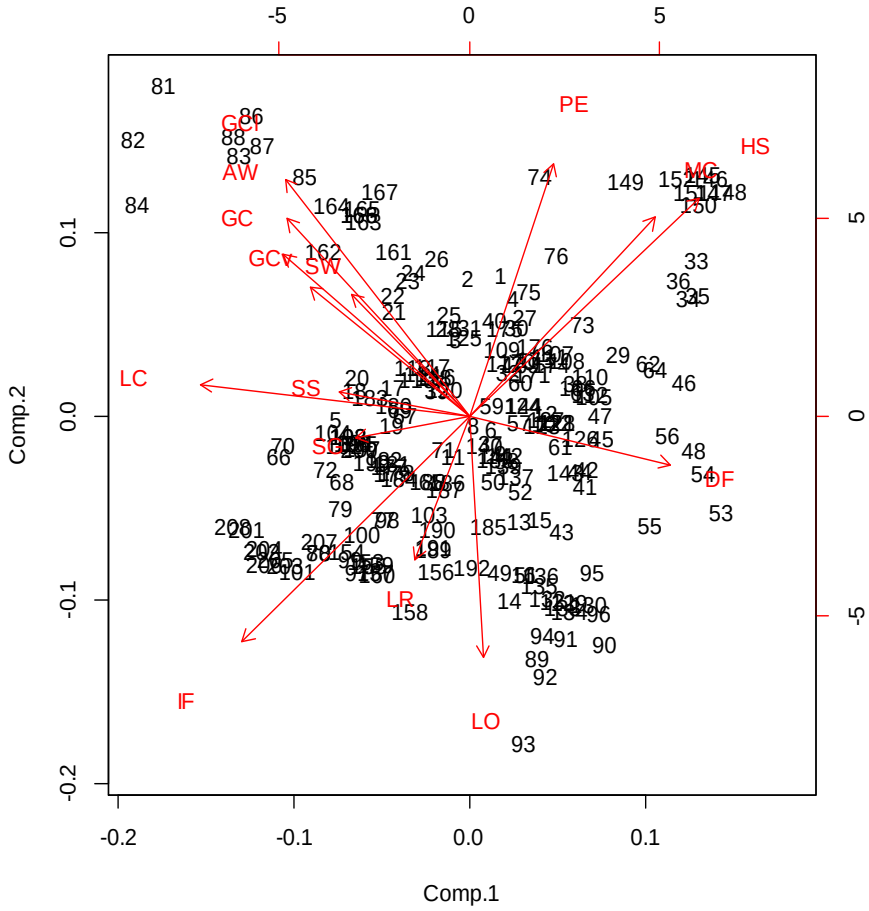

Figure 3. Biplot of the first and second principal components (Comp. 1 and Comp. 2) of morphological characters in the study. DF: Days to flowering, SW: 1000 seed weight, SG: stay green, GC: grain colour, SS: seed size, PE: panicle exsertion, MC: midrib colour, LR: leaf rolling, LO: leaf orientation, LC: leaf colour, IF: inflorescence compactness, Aw: presence of awns, HS: head shape, GCl: glume colour, GCv: glume cover

thousand-seed weight contributed 9.5% to the total variation of the fourth component with high negative loadings of seed size. The fifth component variation (7.9%) was due to positive loadings of glume colour, glume cover and negative loadings of inflorescence compactness and leaf colour (Table 8).

The characters such as head shape, midrib colour, panicle exsertion, glume colour, presence of awns, grain colour, glume cover, and thousand seed weight were positively correlated. Negative correlations were found between the characters days to 50% flowering, leaf colour, seed size, stay green, grain colour, thousand seed weight, awn presence, glume colour and inflorescence compactness (Figure 3). A strong positive correlation was found between the characters glume cover, glume colour, presence of awns and thousand seed weight. On the other hand, there was a strong positive correlation between the characters head shape, midrib colour and panicle exsertion. A strong negative correlation was found between inflorescence compactness, head shape, midrib colour and panicle exsertion. The negative correlations were found between the characters head shape and inflorescence compactness as well as between the days to 50% flowering and seed size.

Diversity index

The Shannon diversity index (H') was estimated to compare the morphological characters used in the study (Table 5). The mean of Shannon diversity index of the characters was 3.22. The H' of stay green, panicle exsertion, midrib colour, leaf rolling, leaf orientation, glume colour, glume cover and grain colour were on par with the mean. Days to 50% flowering, thousand seed weight, seed size, leaf colour, inflorescence compactness, head shape and presence of awns were found to have H'

greater than mean. The last seven characters showed high diversity when compared with the first eight characters (Table 5).

DISCUSSION

The flowering period among genotypes was almost a month and this might be attributed to a mixture of genotypes with different genetic backgrounds and different responses to environmental conditions. A study by Craufurd and Peacock (1993) characterised genotypes on responses to temperature and photoperiod, and they found that variation in flowering period was affected by photoperiod and environment adaptation. Other author found sorghum genotypes that are insensitive during winter in India (Shinde et al., 2013). Water stress also has an influence on days to flowering in as much as it increased the period between panicle initiation and flowering by retarding the rate of panicle development (Craufurd et al., 1993; Zelalem et al., 2015). The uncertain rainfall across seasons and differences in planting dates showed difference in flowering and maturity period for the genotypes. This showed the importance of planting at the beginning of January in Southern Africa particularly Central Mozambique.

The seed size in this study was mostly medium size although some lines had large seeds. The fact that most of the genotypes were medium sized imply lack of variation in seed size. There is, therefore, no evidence in this study to suggest that seed weight was influenced by seed size. Seed weight has been reported to be positively correlated to seed size and yield (Evans and Bhatt, 1977; Ezeaku and Mohammed, 2006). Seed size may be considered as an important factor when selecting seed due to plasticity associated with the seed to complete different growth stages (Sadras, 2007) and also associated to seed weight, are important elements of yield (Tao et al., 2017).

As far as leaf orientation and leaf rolling are concerned, the majority of lines had dropped orientation and rolled leaves. These characteristics might be influenced by a short period of drought during the growth season. Sorghum shows different mechanism to tolerant drought and water use during dry periods (Roby, 2016). Water deficit, high air temperature and sunlight affect leaf rolling in plants (Kadioglu and Terzi, 2007). Regarding the stay green character, 65.4% of the lines had low levels of senescence (25% of their leaves senesced) under optimal growing conditions. Additionally, Burke et al. (2010) found that the best way to identify stay green line (BTx642) is to evaluate in well-watered environments. However, stay green trait could be affected by pre-flowering or post-flowering drought stress (Burke et al., 2013). The stay green trait is an important component when breeding for drought tolerant crop and photosynthesis components (Thomas and Smart, 1993). It also improves adaptation to drought and respond to yield under different agro-ecological conditions of sorghum (Borrell et al., 2000; Borrel et al., 2014). The majority of grain exhibited white colour and it was observed as one of the preferred characteristics by farmers in a PRA study (Mulima, unpublished). White grain colour was indicated by farmers to be associated with preferences of porridge colour and taste (Vom Brocke et al., 2010). Seed size and seed colour are the important traits to farmers during variety selection (Odendo et al., 2001). Although, the difference among their germplasm is small due to frequent seed exchange among farmers (Desmae et al., 2016). Therefore, selection of a variety has to meet specific farmer requirements in order to cater for local food industrial requirements as preferred by the final consumer (Dicko et al., 2006).

Inflorescence compactness was dominated by the compact elliptic type with round head shape. The inflorescence structure is an essential element for breeders due to the contribution of it to the yield, stability and quality of the grain (Brown et al., 2006). Additionally, it was observed that most lines had no awns and seed were covered 25% by grey glume. The presence of strong awns in the seed may be used as a protection against bird's attack. According to Upadhyaya et al. (2010), glume cover and glume colour may be utilized for screening grain mould resistance. Panicle compactness is used as a racial indicator and it is influenced not only by a number of branches and elongation but also by abortions in a branch (Brown et al., 2006; Wang et al., 2015).

Variability and heritability of the characters

The phenotypic variance was higher than the genotypic variance for all characters. Higher phenotypic estimates were observed for days to 50% flowering, thousand seed weight glume colour and inflorescence compactness. The other characters such as stay green, seed size, leaf rolling, leaf colour and glume cover presented lower estimates. The phenotypic expression could be influenced by rainfall and temperature difference between the seasons as recorded with 522 mm during 2016 and 989 mm in 2017. Similar findings from Chikuta et al. (2015) and Ayana et al. (2000) indicate that gradient of rainfall, temperature and growing sites are important for genotype variation. The phenotypic expression can infer genetic variability and consequences of phenotypic variation due to changes in the environment (Abubakar and Bubuche, 2013). Contrary, Seetharam and Ganesamurthy (2013) found that a narrow difference between the phenotypic and genetic variation are an indication of little environmental influence. Variability in characters such as stay green, leaf rolling, and leaf orientation implies that the traits can be used to exploit drought tolerance. An

extensive collection of genetic variability can be used in the improvement of drought tolerance in grain sorghum (Abdalla, 2014; Idris et al., 2015). Yazici and Bilir (2017) reported that there could be many environmental factors in the variability.

High heritability estimates were obtained for stay green, midrib colour, glume cover and leaf orientation. The characters such as thousand seed weight and presence of awns had heritability estimates below 50%. The low heritability estimates have implication in breeding because phenotypic selection cannot be based on those traits with low heritability values. Similar results of low heritability were observed in sorghum for ear head length and breadth (Arunkumar, 2013). According to Bello et al. (2007), traits that are related to grain yield and yield components might have low heritability due to direct or indirect effects of the several components while Obilana and Fakorede (1981) described that heritability estimates tend to be low for the traits that are influenced by environment (quantitative traits).

It is said that the characters with higher heritability estimates may reflect the utility of the characters in a breeding strategy. This result is in agreement with Warkad et al. (2008) who observed low heritability estimates for grain and fodder yield, thousand seed weight and presence of awns in sorghum. Similar results were obtained by Seetharam and Ganesamurthy (2013) for 50% flowering and Liang et al. (1972) for 50% flowering, plant height and seed weight. The inflorescence has higher heritability in the primary branch than secondary and tertiary branches (Brown et al., 2006). High heritability suggests that the main genes for those characters may have an additive gene effect and consequently indicate the importance of those characters for selection.

Cluster analysis

Cluster analysis was able to group the lines according to flowering period, with cluster II having members taking longest time to flowering; clusters I and III were intermediate flowering groups and cluster IV was the earliest to flower. This grouping revealed that information about flowering period among the lines may be useful in order to identify parents for different maturity groups. The success of any crop breeding programme is based on the knowledge and availability of genetic variability for efficient selection (Ali et al., 2008). The characters, thousand seed weight, stay green, seed size, panicle exsertion, midrib colour, head shape and presence of awns showed similar characteristics in all clusters. Characters such as leaf rolling, leaf orientation, leaf colour, inflorescence compactness, glume covering, glume colour and grain colour were the most distinguishing traits between the clusters. Leaf rolling and leaf orientation were clustered in the same pattern in clusters I and IV as well as II and III. The clusters that were paired together were I and II, III and IV for leaf colour. Inflorescence compactness, glume cover and grain colour clustered together I, II and III. Grouping the genotypes according to the characteristics might reveal that the lines have similarity in one or more traits. Seetharam and Ganesamurthy (2013) reported that promising genotypes can be identified from cluster means recorded for each trait. A better understanding of genetics of morphological characteristics is required by the breeder to increase the efficiency of selection of more diverse and adapted parents for crop improvement (Billot et al., 2013). These clusters suggested that there is a large amount of allelic diversity in the germplasm in this study, assuming that it could be divided into four groups.

Principal component analysis

In the first component, maximum weight should be given to the traits with high magnitude and positive eigenvector loadings, namely head shape and days to 50% flowering and traits with high magnitude negative loadings *viz*. leaf colour, leaf orientation and inflorescence compactness. In a separate study, days to 50% flowering was found as one of the most important characters contributing to the first principal component (Ayana and Bekele, 1999; Jain and Patel, 2016), hence its importance has been confirmed in this study. The second principal component explained 13.9% of the variation and in this component maximum importance should be attached to traits with high positive loadings specifically head shape, panicle exsertion, midrib colour and glume colour and those with high magnitude negative loadings *viz.* leaf rolling and inflorescence compactness. In the third component, maximum importance should be attached to traits with high positive loadings, namely, midrib colour, grain colour and days to 50% flowering; and those traits with high negative loadings, that is, stay green and presence of awns. The traits, days to 50% flowering and the thousand seed weight (with positive loadings), and stay green, panicle exsertion and seed size (with negative loadings) should be given maximum importance in the fourth principal component. Ayana and Bekele (1999) also observed that thousand seed weight was one of the important traits in the fourth principal component. In the fifth component, maximum weight should be attached to with positive loadings, namely, leaf colour, glume colour and glume cover and those with negative loadings, specifically, inflorescence compactness and leaf colour. The dull green midrib colour and dark green leaf colour were suggested to be associated with pithy stems meaning juicy stems (Ngugi and Maswili, 2010) while days to 50% flowering was found to be strongly correlated with 95% maturity (El Naim et al., 2012).

Positive strong correlation was found between the characters glume cover and glume colour, presence of

awns and thousand seed weight. Also, there was a strong positive correlation between the characters head shape, midrib colour and panicle exsertion. The negative strong correlation was found between inflorescence compactness, head shape, panicle exsertion and midrib colour. The opposite correlations (positive) were found between the characters head shape and inflorescence compactness as well as between the days to 50% flowering and seed size. These results aligned with PCA result, whereby the positively correlated characters are the same with positive contribution under PCA. These results showed that there is a correlation between some morphological characters measured in the study. Tesfamichael et al. (2015) reported agronomic scores to be positive correlated to stay green, grain yield and harvest index while days to 50% flowering, maturity and panicle length are negative correlated. Additionally, the negatively correlated characters were also similar to PCA result. This suggested that those characters should be taken into consideration when doing the selection for crop improvement. Grouping morphologically similar germplasm is useful for selecting parents for crossing (Ayana and Bekele, 1999; Iannucci et al., 2011) and evaluating the F_1. According to Rahim et al. (2010), F_1 hybrids from genotypes with maximum distance result in high yield, achieving maximum heterosis.

Diversity index

The Shannon diversity index (H') values for stay green, panicle exsertion, midrib colour, leaf rolling, leaf orientation, glume colour, glume cover and grain colour were on par with the mean. This indicated that the traits were less diverse. Days to 50% flowering, thousand seed weight, seed size, leaf colour, inflorescence compactness, head shape and presence of awns were found to have H' greater than mean. A low H' shows lack of genetic diversity and an extremely unbalanced frequency classes for an individual trait (Upadhyaya et al., 2010). Highly diverse genotypes are important in a breeding programme as they may be useful in predicting the potential of hybrid progenies when combined with other genotypes (Seetharam and Ganesamurthy, 2013). Additionally, it would be interesting and fruitful to see the extent of segregation for different traits generated by those crosses (Upadhyaya et al., 2010). This results showed to be relevant for grouping the germplasm according to their similarity and it might be influenced by the characters under evaluation and environment used.

Conclusion

The results of the Mozambican sorghum germplasm diversity study have provided important information that is useful in improvement of the genotypes. The traits that are not strongly related could be exploited in recombination breeding in future. The multivariate analyses clearly showed the grouping of the genotypes according to the characters outlined in the study. Diversity index additionally confirmed the diversity in the traits which can be used in hybridization. Therefore, these results have implications in selection of parents for use in sorghum improvement programme. For example, genotypes that are early in maturity, 150B, IS 14257R, LARSVYT 46B, TX 631B, TX 630B and 8601B could be used for improving earliness, while for late maturity genotypes MA 6B, A 6352R, ICSA 19B and MZ 30R could be used when late cultivars are desired. Moreover, grain yield can be increased using genotypes that produce seed with good weight such as IS 7179R, SPL 9B and A 6353R and those associated with large seed size as observed in lines SPL 38B, SDS 6013R and MZ 2R. On the other hand, lines ICSA 21B, 8610B, MZ 37R, 150B and MZ 2R can be exploited for drought tolerance variety deployment due to the intense stay green character. The line IS 7179R can be used for hybridization to reduce the bird attack due to the presence of awns. Additionally, for mould resistance, lines 8601B and TX 630B can be used. Morphological characteristics identified will assist breeders in understanding the importance of the germplasm diversity, and also help identify important characters that are highly preferred by farmers such as earliness, grain yield, plant height and grain colour.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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