African Journal of Plant Science

Volume 11 Number 7, July 2017
ISSN 1996-0824

Academic Journals

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African Journal of Plant Science

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academicJournals

Vol. 11(7), pp. 264-275, July 2017
DOI: 10.5897/AJPS2016.1477
Article Number: EFC42ED64744
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Urban and peri-urban crop farming in Central Uganda: Characteristics, constraints and opportunities for household food security and income

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Received 5 October, 2016; Accepted 30 December, 2016

Urban and peri-urban farming has a potential to address challenges related to food insecurity among city and town dwellers. It provides the urban population with food, nutrition and a source of income and employment, thus reducing on poverty and food scarcity. It has the advantage of proximity to urban markets which saves on transportation costs, thereby increasing farmers' profitability. This study was carried out to establish the current characteristics and trends of urban and peri-urban crop farming in Central Uganda. To accomplish this, a household survey was conducted in Kampala, Wakiso and Masaka districts, Central Uganda. A total of 297 farming households were interviewed on a number of aspects including cropping practices, income sources, home gardening techniques, marketing, irrigation and household waste management. Focus group discussions were also held in each district. Cropping activities were found to contribute on average 40% to the income of farming households, complementing other livelihood sources such as transport business, livestock production, formal employment and other trade. The major crops grown were vegetables, maize, beans, bananas and avocado. A number of home gardening techniques were identified among farmers, for instance, growing crops on food towers, in buckets and bags (sacks). Irrigation and fertilizer application were practiced by 60% of households, mainly on vegetables. Sixty-four percent of the households recycled waste and of these, 75% converted kitchen waste into manure for crop production. We recommend farmers' training on use of household biodegradable waste in home gardening for improved nutrient use efficiency, economical irrigation water management strategies, and other agronomic and marketing aspects of crops that are commercially viable in urban areas, particularly horticultural crops.

Key words: Urban and peri-urban farming, cropping practices, food security, income.

INTRODUCTION

The level of urbanization in Uganda currently stands at 12%, growing at a rate of 4.7% and it is estimated to reach 30% (20 million people) by the year 2030 (UN Habitat, 2011; Lwasa et al., 2014). With population growth comes more need for food for the urban dwellers, some of which can be supplemented through practicing urban agriculture. Farming in the urban areas, referred to as urban agriculture, can complement livelihoods of mainly the urban poor. According to Stewart et al. (2013), urban agriculture refers to "agriculture located within and around cities whose products are at least partly destined for the city and for which alternatives exist between the agricultural and non-agricultural uses of resources". An estimated 40% of agricultural products consumed in urban areas are produced from within urban areas (Draft UNUP, 2013). Urban and peri-urban (UAP) farming provides income and employment opportunities to the population. It supplements the sources of food supply at an affordable price thereby contributing to food security.

Urban agriculture, if managed well, can also contribute to waste management, urban greening and beautification. For instance, 33% of total agricultural production in the Netherlands comes from urban agriculture. Similarly, 10% of the total urban population in the United States of America participates in urban agricultural activities (Brown and Carter, 2003; Indraprahasta and Agustina, 2011). According to Zezza and Tasciotti (2010), urban agriculture has potential to improve livelihoods, particularly in much of sub Saharan Africa and in all those countries where agriculture provides a substantial share of income for the urban poor. Zezza and Tasciotti (2010) found fairly consistent evidence of a positive statistical association between engagement in urban agriculture and dietary adequacy indicators.

In order for urban agriculture to make meaningful contribution to urban livelihoods and avert environmental degradation, there is need to identify production practices that are economically viable and environmentally sustainable. The challenge with urban agriculture is how to ensure that it contributes to sustainable livelihoods without compromising human and environmental health standards of cities. Currently, there is scanty information about crop farming practices in UAP areas of Uganda, including its key characteristics and how these relate to the demographic and environmental aspects of the urban environment. This study provides data from a typical developing country perspective on the nature and extent of urban and peri-urban agriculture. This is crucial information given on-going development of the Uganda National Urban Policy. This policy process could lend support to other cities in the region that may develop similar policies in future. The objectives of this study were to determine the characteristics of current crop production practices within UAP farming systems in Central Uganda and to identify the opportunities and major constraints to further sustainable development of UAP farming in the region.

MATERIALS AND METHODS

Study area

Three districts within Central Uganda were selected on the basis of their location in UAP settings; Kampala (00°19'N, 32°35'E), Wakiso (00°24'N, 32°29'E) and Masaka (00°22'S, 31°42'E) (Figure 1). Kampala and Wakiso were chosen to represent the urban areas, while Masaka district represented the peri-urban area. Kampala is the Capital City of Uganda and it is located within the Central Region of the country. Wakiso is the second most urbanized district in Uganda after Kampala and it borders most of Kampala district (Figure 1).

Stratified random sampling was used to select sub-counties, parishes/wards and villages from each district according to the extent of cropping practices and intensity of settlements (Table 1). Within each district, two sub counties (or divisions) were selected on the basis of existence of significant amounts of agriculture on the advice of local key informants (Local Government Officials and technical personnel).

Data collection

Focus group discussions (FGDs) were held in each division/sub county with a minimum of 12 household heads who were involved in crop production. This was done with the help of local council leaders. This was followed by face-to-face interviews of farmers selected randomly from farmer lists provided by the local council leaders in those villages. A total of 297 farming households were interviewed using a pre-tested structured questionnaire on a number of aspects including cropping practices, income sources, home gardening techniques, marketing, irrigation and household waste management among others.

Data entry and analysis

Data were entered into Microsoft Excel (Microsoft Office, 2007) where preliminary cleaning and exploration was done. Descriptive statistics were performed using the Statistical Package for Social Scientists (SPSS) v19 (SPSS, 2007). Binary logistic regression analysis was used to model determinants of waste recycling and use of manure for home gardening. Weighted ranks were also obtained using the Microsoft excel program. Graphs and charts were generated from the data using SPSS and Microsoft excel

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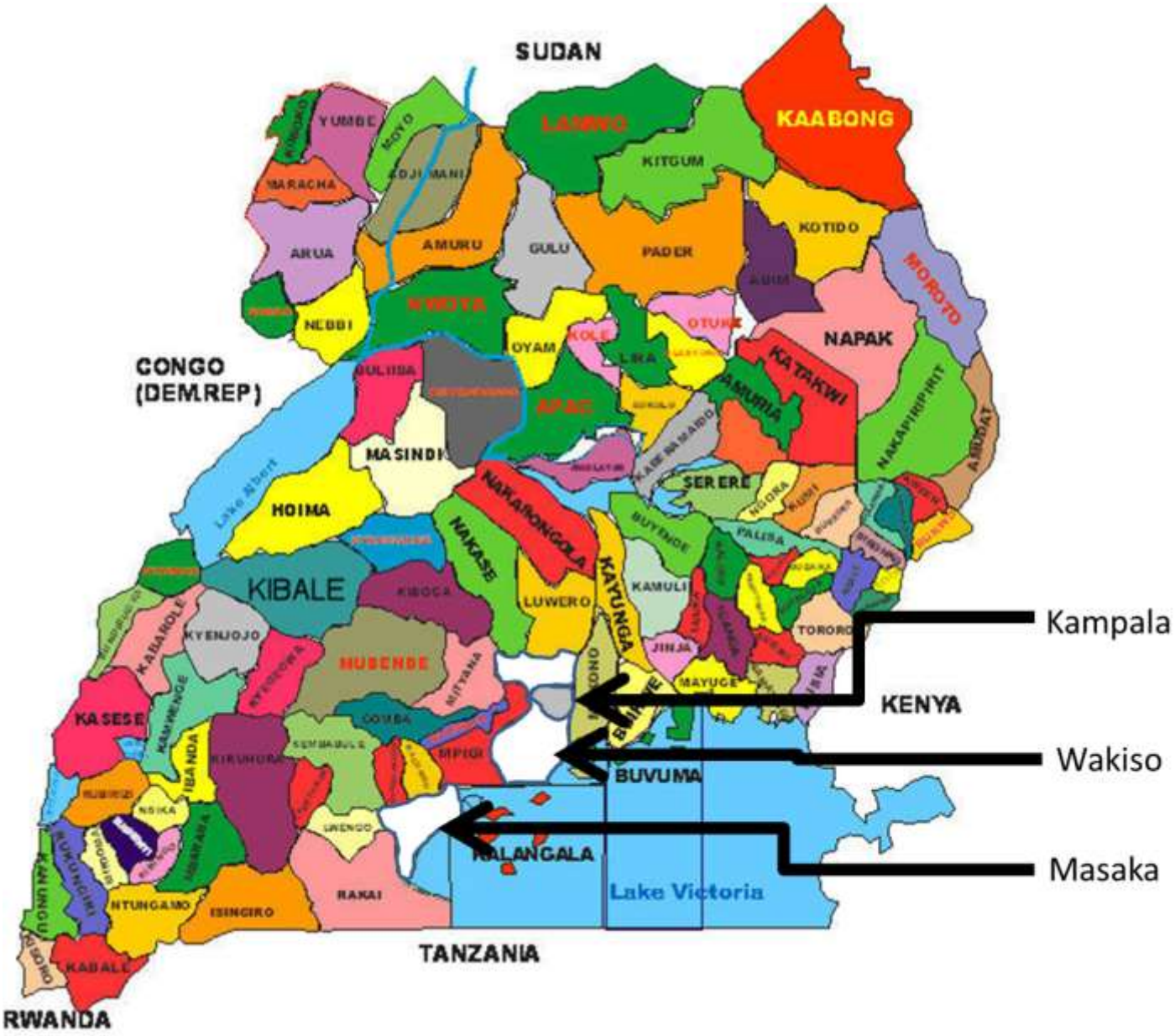

Figure 1. Map of Uganda showing the location of the study districts in Central Uganda.

Table 1. Sites where the household surveys and focus group discussions were held.

No.	District	Sparsely built-up	Densely built-up
1	Kampala	Makindye division (Salaama and Makindye Parishes)	Mutundwe division (Kabowa, Mutundwe and Luwafu Parishes)
2	Wakiso	Nsangi sub county (Nsangi, Musale and Buddo Prishes)	Entebbe Division B (Kiwafu and Kigungu parishes)
3	Masaka	Mukungwe (Kalagala, Samalia and Nyendo Prishes)	Nyendo-Senyange (Senyange A and Senyange B Parishes)

Table 2. Demographic characteristics of UAP farming households in Central Uganda.

Characteristics	Categories	Masaka	Kampala	Wakiso	All	% of All
Sex of household head	Male	61	66	63	190	64.2
	Female	37	30	29	96	32.4
Age of household head	Mean	54.1	55.6	51.5	53.7	-
	Std. Deviation	14.7	15.2	13.36	14.4	-
Education level of household head	No formal education	2	4	0	6	2.2
	Primary	30	18	29	77	28.1
	Ordinary level	21	25	29	75	27.4
	Advanced level	11	10	13	34	12.4
	Certificate graduate	12	4	0	16	5.8
	Diploma graduate	10	15	6	31	11.3
	Degree graduate	11	19	5	35	12.8
Marital status of household head	Single	9	6	9	24	8.9
	Married	54	65	58	177	65.6
	Divorced	1	3	3	7	2.6
	Widowed	21	20	9	50	18.5
	Separated	6	1	5	12	4.4
*Age distribution of household members	M <5	29	51	57	137	6.3
	M 5-15	72	97	77	246	11.3
	M 16-35	112	135	121	368	16.9
	M 36-65	80	62	67	209	9.6
	M >65	21	13	13	47	2.2
	F <5	41	57	49	147	6.8
	F 5-15	89	93	80	262	12.1
	F 16-35	196	135	133	464	21.4
	F 36-65	102	73	76	251	11.6
	F >65	12	21	8	41	1.9

*M=Male, F=Female.

software.

RESULTS AND DISCUSSION

Household demographics

Approximately 33% of the sampled households were female-headed and the average age of the household heads was 54±2 years (Table 2). The education level of most household heads (~70%) was ordinary level, one quarter of whom had either diplomas or were university graduates. Two thirds of the sampled household heads were married and close to 25% were widowed. About 60% of the household members were within the active age groups (16 to 65 years) and the distribution between males and females was comparable. This presents an opportunity for enhanced production and marketing within the study areas especially since most household members are within the active age groups.

Income sources

The study only ranked, not directly quantified the financial contribution of urban agriculture to livelihoods. However, on a percentile scale, the rankings of agriculture showed it to approximate between 30 and 50% contribution to livelihoods. Overall, crop production was ranked the most important livelihood source in all the districts surveyed.

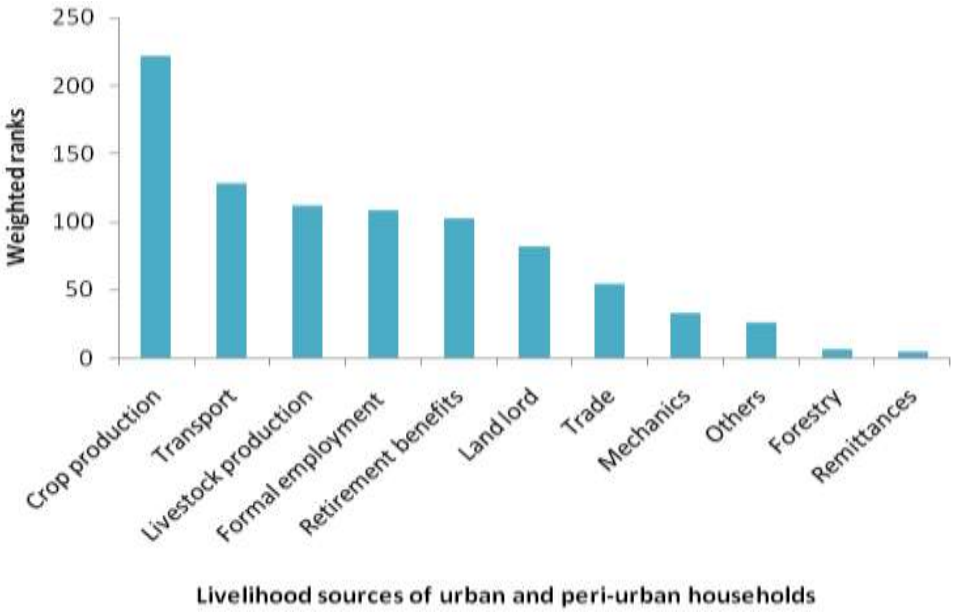

Figure 2. Weighted ranks of livelihood sources of UAP households in Central Uganda.

Table 3. Ranking of crops for food security in UAP areas of Central Uganda.

Rank	Vegetables		Perennial		Fruit trees		Annual	
	Crop	Score	Crop	Score	Crop	Score	Crop	Score
1	Nakati	113	Bananas	1009	Avocado	120	Maize	528
2	Tomatoes	89	Cassava	532	Oranges	18	Beans	448
3	Cabbage	72	Coffee	92	Mangoes	17	S.Potatoes	406
4	Sukuma	46	Sugarcane	52	Pawpaws	15	Yams	84

This was followed by livestock production while transportation came fourth (Figure 2). Generally, transport, livestock production, formal employment and retirement benefits/pension ranked high among the livelihood sources. Previous studies by Zezza and Tasciotti (2010) showed that compared to cities in developed countries where agriculture contributes less than 5% to household income, in developing countries, UAP agriculture contributes a high proportion (30 to 71%) to the income of households engaged in it. It is therefore not surprising that crop production ranked high in its contribution to household income in UAP households in Uganda. The high contribution of transport in Wakiso could be related to the emergence of motorcycle transport, locally known as "*Boda boda"*, which employs a large number of youth who seek for housing in the outskirts of the town and nearby capital city, Kampala.

Crop production

Crop production was ranked according to whether the main objective was food or cash. Bananas remain high in priority for both food and cash (Table 3). Banana is an important crop in Central Uganda because it is both a food and cash crop. Besides, it can be grown in compounds for aesthetic value while contributing to food security in the household. This possibly explains the overall high ranking of this crop. Cassava, which is increasingly becoming an important food security crop in Uganda, was ranked highly especially in Wakiso district. Vegetables generally were also a highly ranked group due to the relatively small spaces required to cultivate them, together with the relatively high contribution they make to food and nutrition security at household level. They are also capable of being cultivated throughout the year, providing regular income to the households that grow them. Avocado, a common fruit tree, emerged strongly as a cash crop in UAP areas of Central Uganda (Table 4). Despite the limited land available to farmers in Urban and peri-urban areas, planting one or two fruit trees such as avocado could possibly make a difference providing not only food but also income for these families. However, this might not be possible for those with very

Table 4. Ranking of crops for income security in UAP of Central Uganda.

Rank	Cash crops	Scores			
		Masaka	Kampala	Wakiso	Over all
1	Avocado	259	136	251	646
2	Bananas	244	126	262	632
3	Beans	181	98	214	493
4	Bitter berries	114	52	114	280
5	Cabbage	55	40	47	142
6	Carrots	24	4	0	28
7	Maize	14	0	0	14

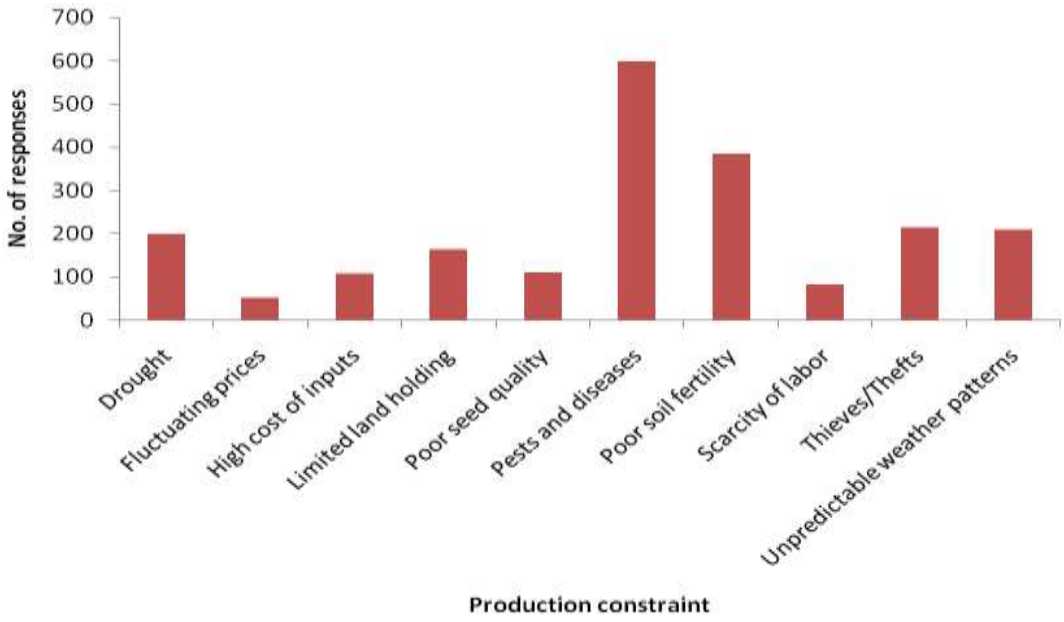

Figure 3. Major constraints to crop production among UAP farmers in Central Uganda.

small pieces of land.

Farmers identified and ranked several production constraints. The most prominent of these were incidence of pests and diseases particularly banana bacterial wilt. Unfavorable weather was also prominent in Wakiso and Kampala areas (Figure 3).

Home gardening techniques

Home gardening techniques existed in several forms including food towers, pots, sacks, polythene bags and ridges in farmers' backyards. The major reasons advanced for use of these techniques included the ease to establish and manage them, space optimization, no special skills required and affordability by many farmers. Other additional benefits such as food security and aesthetic value of the homestead were also mentioned. The major crops preferred for home gardening were mainly vegetables (Figure 4).

Eighty percent (234) of the farmers that were involved in home gardening practiced irrigation, fertilization or fertigation (combination of irrigation and fertilizer application). The practice of irrigation, fertilization and fertigation among farmers having home gardens varied across sites (Figure 5) with Masaka and Wakiso exhibiting higher rates of irrigation compared to Kampala. Ironically, more farmers in Kampala used fertilizers compared to either Masaka or Wakiso.

Irrigation is used mainly for vegetables including *nakati*, cabbage, tomatoes and *sukuma wiki* in that order. On the other hand, fertilizer is reportedly applied to mainly bananas (90), maize (34), nakati (23), tomatoes (19) and cabbage (17). The various attributes of farmers practicing

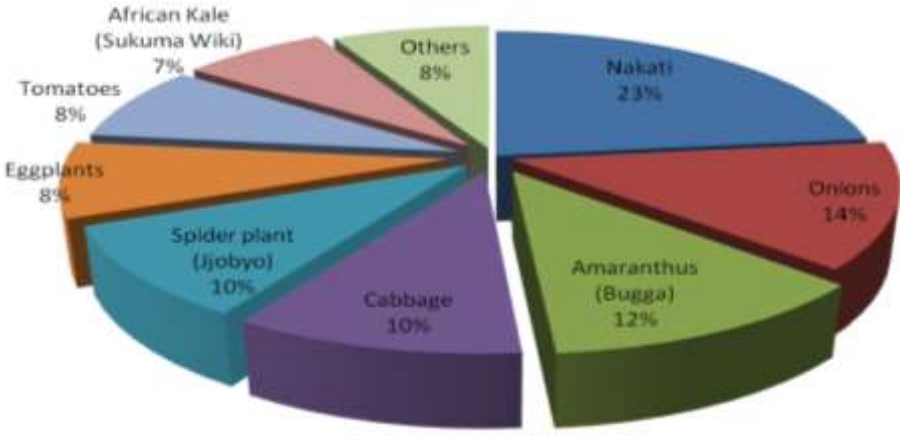

Figure 4. Preferred crops for home gardening in UAP farming in Central Uganda.

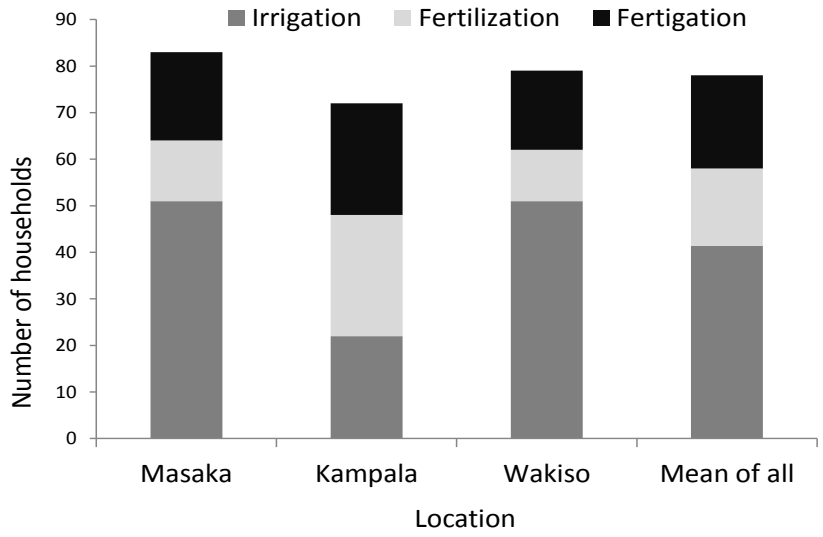

Figure 5. Use of irrigation, fertilization and fertigation among UAP farmers in Central Uganda.

irrigation in home gardens are indicated in Table 5.

Waste management

It was determined whether households recycle waste or not, and for those who use it for agricultural production, whether the main type is kitchen or animal manure. Sixty four percent (64%) of the households recycled waste in one way or another. Of those who use manure for gardening, 75% used kitchen waste. The major problem with the waste was broken glasses which were usually disposed of by burying in the ground while plastics were burnt to ashes. The most common type of waste, however, is kitchen and animal waste, which some farmers use as manure for cropping.

In the logistic regression, the household-level factors affecting waste recycling were assessed as well as the major type of manure used in home gardening. Table 6 shows the factors that were hypothesized to affect waste recycling and major type of manure used in small gardening technologies. The regression analysis showed that households in Masaka were more likely to recycle waste compared to those in either Kampala or Wakiso, and that those in Wakiso were the least likely to recycle household waste (Table 7).

It was also found that the higher the level of education of the household head, the more likely the household would recycle waste. This was with the exception of the primary-level educated household heads; that had less tendency to recycle compared to those with no formal education. Larger household sizes are also less likely to

Table 5. Irrigation attributes in UAP farming in Central Uganda.

Attribute	Water sources							
	Tap water	Rain	Bore hole	Wells	Ponds	Swamps	Rivers	Lakes
Site								
Masaka	45	22	2	15	0	2	0	0
Kampala	32	16	0	1	0	0	0	0
Wakiso	22	22	1	15	2	9	1	6
Uses								
Gardening only	2	4	3	8	5	12	7	16
Gardening & livestock	2	4	0	1	2	9	0	2
Multiple uses	19	13	2	12	0	1	0	1
Harvesting method								
Trenches	0	0	0	1	0	0	3	0
In-situ	5	4	0	2	0	1	0	0
Gutters	8	48	0	0	0	0	0	0
Drainage channels	2	2	0	1	0	0	0	1
Taps	11	0	0	0	0	0	0	0
Jerry cans	10	0	2	12	0	0	1	3
Pipes	3	0	0	0	0	0	0	0
Storage methods								
Jerry cans	21	4	1	18	2	4	1	3
Water drums/tanks	34	34	2	9	0	0	0	0
Underground reservoir	12	19	0	1	0	1	0	0

Table 6. Variables used in the Binary logit models for waste recycling, and manure types used in UAP agriculture in Central Uganda.

Variable name	Category	Category code	Logit1: Waste recycling		Logit2: Main manure type	
			Frequency	%	Frequency	%
District	Masaka	1	58	29.1	43	39.4
	Kampala	2	83	41.7	45	41.3
	Wakiso	3	58	29.1	21	19.3
Education level of household head	None	0	5	2.5	4	3.7
	Primary	1	55	27.6	31	28.4
	Ordinary	2	70	35.2	33	30.3
	Tertiary	3	69	34.7	41	37.6
Main livelihood source	On-farm	0	119	59.8	64	58.7
	Off-farm	1	80	40.2	45	41.3
Practice home gardening	Yes	1	124	62.3	80	73.4
	No	2	75	37.7	29	26.6
Main source of labor for farm activities	Family	1	94	47.2	48	44.0
	Hired	2	12	6.0	4	3.7
	Both	3	93	46.7	57	52.3
Use of irrigation and/or fertilizer	Yes	1	166	83.4	90	82.6
	No	2	33	16.6	19	17.4

Table 6. Contd.

Participation in training	Yes	1	103	51.8	70	64.2
	No	2	96	48.2	39	35.8
Access to credit	Yes	1	74	37.2	46	42.2
	No	2	125	62.8	63	57.8

Table 7. Logistic regression analysis of factors affecting (a) recycling of household waste and (b) major type of manure used in home gardening in UAP areas in Central Uganda.

Factor	Logit 1: Recycling of household waste						Logit 2: Major source of manure for home gardening					
	B	S.E.	Wald	df	*p*	Exp(B)	B	S.E.	Wald	df	*P*	Exp(B)
District	-	-	20.98	2	0.000	-	-	-	5.59	2	0.061	-
District(1)	-1.58	0.52	9.33	1	0.002	0.21	-1.17	0.74	2.49	1	0.114	0.31
District(2)	-2.01	0.49	16.98	1	0.000	0.13	-1.58	0.69	5.26	1	0.022	0.21
Educhhd2	-	-	4.48	3	0.214	-	-	-	4.23	3	0.237	-
Educhhd2(1)	-1.52	1.70	0.00	1	0.099	0.00	-1.37	1.31	1.10	1	0.295	0.26
Educhhd2(2)	0.56	0.49	1.34	1	0.246	1.76	-0.88	0.58	2.29	1	0.130	0.41
Educhhd2(3)	0.93	0.44	4.48	1	0.034	2.54	-0.99	0.55	3.23	1	0.072	0.37
Hhsize	-0.07	0.06	1.45	1	0.229	0.93	-0.03	0.07	0.19	1	0.662	0.97
Lisource12(1)	-0.07	0.38	0.04	1	0.846	0.93	-0.21	0.53	0.16	1	0.689	0.81
Sgarden(1)	-0.15	0.42	0.13	1	0.721	0.86	0.34	0.59	0.34	1	0.557	1.41
Irrifert(1)	0.15	0.52	0.09	1	0.766	1.17	0.64	0.63	1.02	1	0.312	1.89
Labour	-	-	2.80	2	0.247	-	-	-	2.40	2	0.301	-
Labour(1)	0.40	0.40	1.03	1	0.311	1.49	0.72	0.47	2.35	1	0.125	2.06
Labour(2)	1.14	0.72	2.52	1	0.112	3.13	0.72	1.17	0.37	1	0.541	2.05
Training(1)	-0.41	0.49	0.71	1	0.400	.66	0.31	0.69	0.20	1	0.654	1.36
Associatn(1)	-1.42	0.53	7.22	1	0.007	.24	0.68	0.68	0.98	1	0.321	1.97
Credit(1)	0.31	0.48	0.42	1	0.518	1.36	-0.26	0.56	0.22	1	0.640	0.77
Constant	0.89	0.82	1.19	1	0.276	2.43	0.36	1.01	0.13	1	0.721	1.44

recycle household waste. As would be expected, there are higher chances of recycling waste if the major livelihood source for the household is on-farm rather than off-farm. Compared to use of only family labour, use of either hired labour or a combination of hired and family labour increased the probability that a household would recycle waste. This suggests that large household sizes do not necessarily imply more farm labour availability, as is expected in UAP areas. Access to farmer training and membership to farmer groups all increased chances of households recycling household waste.

Marketing

Approximately 60% of the households market more than 40% of their crop produce. However, the proportion of produce sold varies widely by crop and by location. Overall, vegetables ranked high among the marketed crops, the proportion ranging from 0.5 to 1. Farm gate, road side, sub-county and urban markets are the major markets to which farmers sell their produce. Approximately, one third of the farmers (131) sell their produce at farm gate. Some of the farmers use multiple market channels such as roadside kiosks and direct transport to market centers. Most farmers prefer selling at farm gate reportedly because they avoid having to incur transport costs and taxes in the markets besides saving time for other activities.

Information access

Seventy six percent of the households reported that they

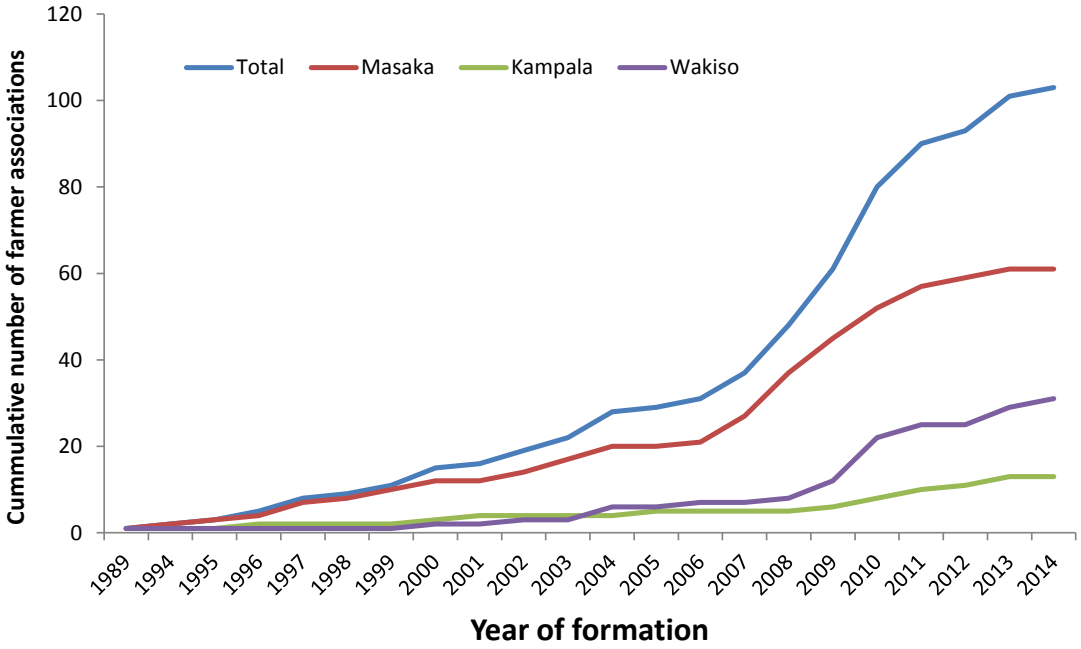

Figure 6. Cumulative number of farmer associations formed over the last 25 years in Central Uganda.

had access to some agricultural information. Of those who accessed information, 19, 17, 12 11 and 11% reported to have accessed information on crop agronomy and production, animal production, pest and disease control, banana production and vegetable production, respectively. These results are consistent with those reported by FAO (2014). The proportion of farmers accessing information was higher in Masaka, followed by Wakiso and least in Kampala.

Public extension, the former National Agricultural Advisory Services (NAADS), was reported to be the most frequently used source of information acquisition (39%), followed by radio, television and farmer-to-farmer knowledge transfer. This pattern was consistent for all the studied districts. Only few (<10%) of the farmers reported to have obtained agricultural information from such sources as farmer associations, newspapers/print media, the National Agricultural Research Organization (NARO), NGOs, study tours, the internet and agro-input shops. The percentage of households where at least one member had received training was 44%. More farmers in Masaka had received training (53%) than those in either Wakiso (30%) or Kampala (16%). A range of topics were offered for training to UAP farmers. The major topics were crop production, pest and disease management and vegetable production.

Membership to associations

Overall, membership to farmers' associations in UAP areas of Central Uganda stood at 44%. Disaggregating membership by districts revealed that Masaka has significantly higher membership (61%) compared to Kampala (11%) and Wakiso (29%) districts. The number of associations has been growing since 1989 and saw a dramatic rise in 2007 (Figure 6). All groups have some form of membership fee payable annually. The fees vary from US$ 10 to 20 depending on the nature of the group.

These results suggest that use of farmer groups, as is recommended, may not yield as good results as would be in Masaka. Ninety three percent of the former associations were registered at various levels including sub-county, district and national. Participation in farmer groups depended on the nature of the activity, with males dominating where saving and credit activities were the main focus of the group compared to other activities like training, marketing and group farming (Figure 7).

Access to credit services

Thirty seven percent of the farmers reported that they had accessed credit within the past year. There were a number of sources from which farmers accessed their credit and the amount obtained per farmer ranged from approximately US$ 100 to 2,000 (Figure 8). This usually serves to rent office space, pay registration fees to local governments and sometimes provide quick loans to members in case of emergencies.

CONCLUSIONS AND RECOMMENDATIONS

In this study, we set out to understand the characteristics

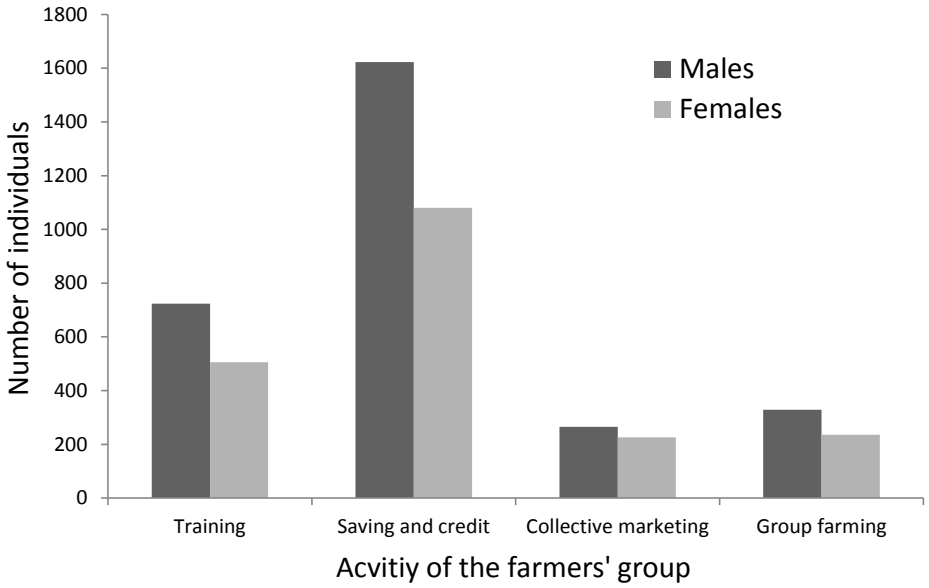

Figure 7. Sex-disaggregated data for participation in group activities among urban and peri-urban farmers in Central Uganda.

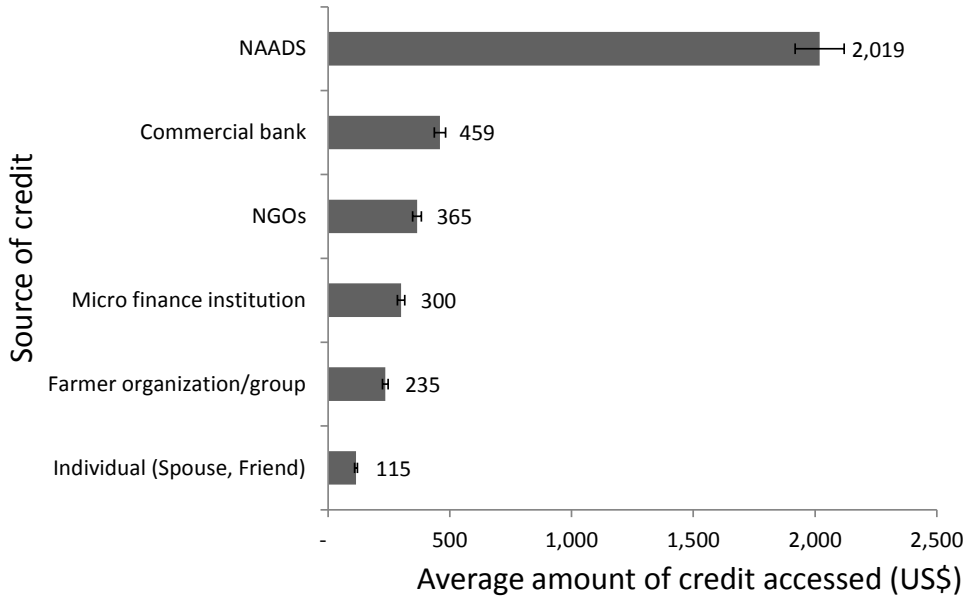

Figure 8. Amount of credit accessed from various sources by urban and peri-urban farmers in Central Uganda. Error bars are standard deviation (N=296).

and current trends in crop productivity within urban and peri-urban farming systems in Central Uganda. It was found that agriculture contributes to the livelihoods of urban and peri-urban farmers to a fairly good extent. However, they are faced with a number of constraints. The major ones include theft, weather changes (unpredictable weather pattern), pests and diseases, high cost of inputs and poor seed quality. There is opportunity to recycle household biodegradable waste for use as manure for enhancing copping practices. Overcoming investment costs for rain water harvesting infrastructure can be achieved through micro-credit schemes to ensure continued production of high value crops, particularly vegetables. This can enhance household food security

and income using small space technologies such as kitchen technologies and backyard gardens.

In order to improve UAP agriculture in Central Uganda, there is need to train farmers on aspects such as pests and disease control, use of household organic waste as manure, use of high yielding varieties, irrigation, and marketing aspects of commercially viable crops, particularly vegetables. Farmers within Kampala should be targeted since the study had shown that they have had less exposure to training compared to their counterparts in Wakiso and Masaka study areas. In addition, improving access to credit can help farmers establish critical infrastructures such as water reservoirs and agro-inputs, which would facilitate urban farming. Studies are required on improved use of household biodegradable waste in home gardening and economical irrigation water management strategies for increased crop yields. This would lead to enhanced productivity and economic viability of urban and peri-urban cropping practices in Central Uganda.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was funded by the World Bank ATAAS project through the Government of Uganda and the National Agricultural Research Organisation. All who participated in data collection including Maria Nampeera, Teopista Namirimu, Kyampeire Barbara, Godfrey Biso, Robert Mutyaba, Kyambadde Kyeyune, Robinah Gafabusa and George Kituuka were acknowledged. The officials of Wakiso Local Government, Masaka Local Government and Kampala Capital City Authority were also appreciated for their corporation and support during the entire course of the study.

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academicJournals

Vol. 11(7), pp. 276-281, July 2017
DOI: 10.5897/AJPS2015.1330
Article Number: 2C9AFA764748
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Effect of different rate of nitrogen fertilizer on the growth and yield of cabbage (*Brassica Oleraceae*) at Debre Markos, North West Ethiopia

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Received 7 July, 2015; Accepted 29 September, 2015

A research was conducted at Debre Markos, North West Ethiopia in 2015. The objective of the study was to evaluate different rate of nitrogen fertilizer on growth and yield of cabbage. The experment was laidout in randomized complete block design with four treatments and three replication. Nitrogen fertilizer have no significant effect on plant height and number of outer leaves, while leaf length, leaf width, head diameter, head fresh weight and head dry weight had significant effect on cabbage. The widest leaf width (21.86 cm) was recorded from 150 kg/ ha and followed by 100 kg/ ha, while the narrowest leaf width (16.93 cm) was from 0 kg/ ha N. The highest leaf length (20.1 cm) obtained was from 150 kg/ ha and the lowest leaf length (16.166 cm) was obtained from 0 kg/ ha. The highest head diameter (11.043 cm) was obtained from 150 kg/ ha, while the lowest head diameter was 8.696 cm noticed from 0 kg/ha. The highest fresh weight (0.771 kg/plant) was recorded from 150 kg/ ha and the lowest (0.442 kg/plant) was from 0 kg/ ha. The highest dry weight (0.114 kg/plant) was recorded from 150 kg/ ha while the lowest (0.0437 kg/plant) dry weight was obtained from 0 kg/ha of Nitrogen.

Key words: Cabbage, nitrogen, yield.

INTRODUCTION

Cabbage (*Brassica oleracea L. var. capitata*) belongs to the family cruciferae and it is a biennial crop with a very short stem supporting a mass of overlapping leaves from a compact head. It originated from wild non-headed type 'colewart' (crambecordifolias) from Western Europe and northern shore of Mediterranean (Semuli, 2005). It has been domesticated and used for human consumption since the earliest antiquity. It is a cool season crop that is popular with gardeners and commercial producers.

Cabbage is known for its nutritional importance and it

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is rich in mineral and vitamins like A, B1, B2 and C. It is also known for its cooling effect. Being an appetizer, it aides digestion thereby helps to prevent constipation. It also protects against cancers. Cabbage can grow easily under wide range of environmental condition in both temperate and tropical, but cool moist climate is most suitable (Kibar et al., 2014). Optimum growth occurs at a mean daily temperature of about 17°C with daily mean maximum of 24°C and minimum of 10°C. Mean relative humidity should be in the range of 60 and 90% (FAO, 2012).

Cabbage is grown for its head in more than ninety countries throughout the world (Meena et al., 2010). The major cabbage growing countries of the world are China, India, South Korea, Germany, Japan and South Africa (Sarker, 2002). Cabbage ranks fifth among the vegetable crops of the world. The area planted with headed cabbage worldwide in 2011 was estimated at about 68.584 million hectare (FAO, 2008). In 2008, area planted by cabbage was about 2.5 million hectare in Asia, 0.5 million hectares in Europe, 80,000 hectare in America and 120,000 hectare in Africa. In Africa, a total of 2 million tons were produced in 2008 and it has shown an increase by 20% over the 10-year period between 1998 and 2008. The five cabbage producers in African countries are Kenya, Egypt, Ethiopia, Niger and South Africa and these five countries have maintained the dominance of the sector throughout this period. Ethiopia accounted for 12% of the total production in Africa (Nicolas et al., 2012). Area, production and yield of head cabbage in Ethiopia 2007/2008 were 1989 hectares, 11,765 tons and 5.9 t/ha, respectively. In 2008/2009 it grew to 3,399 hectares, 24,133.4 tons and 7 t/ha, respectively. Within these two years, the area has increased by 70% whereas the production has increased by 105%.

Ethiopia production of cabbage in 2005 is about 15,208 tones in an area of 2120 hectare with 7.2 tone/hectare (CSA, 2005). An overview of export data for 2013 showed that Ethiopia exported about 7.00 mt of cabbage, cucumber and eggplant to Djibouti and Somalia. Amhara region has a strong potential for production of cabbage. The region produced over 20.00 mt in 2013. The world average yield is 10-40 t/ha (Ogbodo, 2009).

Production of cabbage could be enhanced through efficient use of applied nitrogen through adoption of good management of strategies capable of promoting effective and efficient use of applied water as insured by drip irrigation technology (Asare et al., 2010).

Different cultural practices and growing environments are known to influence growth and yield of the cabbage. So far, research in the country was mainly focused on the identification of superior cultivars of onions and adopting improved management practices. Mineral nutrition is a main factor that affects growth and yield of cabbage. Nitrogen is often referred to as the primary macronutrients because of the probability of plants being deficient in this nutrient and the large quantities taken up by plants from the soil relative to other essential nutrients. Nitrogen comprises 7% of total dry matter of plants and is a constituent of many fundamental cell components (Brady and Weil, 2002). It is one of the most complexes in behavior, occurring in soil, air and water in organic and inorganic forms. For this reason, it poses the most difficult problem in making fertilizer recommendations. Plant demand for nitrogen can be satisfied from a combination of soil and fertilizer to ensure optimum growth and yield of cabbage.

The major essential plant nutrient nitrogen was found increasing in short supply in the soils of Eastern, Western and Southern Africa (Rao et al., 1998). Nitrogen is required in much greater quantities than most other nutrients. It is an important component of proteins, enzymes and vitamins in plants, and is a central part of the essential photosynthetic molecules; chlorophyll (Marschner, 1995). Plant demand for nitrogen can be satisfied from a combination of soil and fertilizer nitrogen to ensure optimum growth.

Thus, it requires applying of appropriate rate of fertilizer for the enhanced cabbage productivity and sustainable yield. Many experiments show that nitrogen application increases the total yield of cabbage (Patrick et al., 2012). But this is possible as long as it is managed properly in terms of rate and time of application.

Nitrogen levels have to be regulated to obtain high yield from cabbage. Thus, knowledge on this factor is helpful to optimize cabbage yield through efficient use of rate of fertilizer. In Western highlands of Ethiopia, particularly around Debre Markos, there is a need by farmers to increase productivity of cash crops like cabbage to maximize their profit from the small plot of farm they have. However, farmers of this area who grow cabbage frequently give less attention to nitrogen fertilizer rate. Moreover, information on cabbage nitrogen application rate for optimum yield and other agronomic practices are limited; since most of the farmers in this area has small plot of land and the yield obtained from this is very low. Due to this, their income is less and they unable to improve their livelihood. So it is important to increase the yield of cabbage from this small plot of land to improve the income of farmers around this area. The main objective of this present study was to evaluate the effect of different concentrations of nitrogen and to determine its optimum level for cabbage yield and growth.

MATERIALS AND METHODS

Description of experimental site

The experiment was conducted at Debre Markos University College

of Agriculture and Natural Resources in 2015 cropping season using furrow irrigation. Debre Markos University is geographically located at 300 Km North West of Addis Ababa at about 10^0 18'10"north latitude and 37^0 44'53" East longitudes at an altitude of about 2450 meter above sea level (m.a.s.l). The minimum and maximum temperature were 10.6 and 22.30°C, respectively. The mean annual rain fall of the area is about 1100 mm (Planning and Economic Development of East Gojjam, 2004).

Experimental materials

Cabbage seed variety Copenhagen market was used; Nitrogen fertilizer was used as experimental material during the study. There were four levels of nitrogen rates (0 kg/ha, 50 kg/ha, 100 kg/ha and 150 kg/ha). Urea was used as a source of nitrogen fertilizer.

Experimental design

The experiment was conducted in a Randomized complete block design with three replications. Cabbage seeds were sown in the nursery. The seeds were sown at the first week of February 2015 in the seed bed. After sowing, the seeds were covered by straw mulch. Irrigation was conducted frequently until the seedlings fully emerged. The permanent bed was prepared and the layout was arranged appropriately. The beds were irrigated sufficiently before seedlings were transplanted to make the soil suitable for them. After these, the seedlings were transplanted to the bed when they were two to three pairs of leaves. In the bed, plants were transplanted 40 cm between rows and 30 cm between plants. The total number of plant in each plot was 24 plants with 4 row and 6 plants with each row. Seedlings were transplanted in the permanent field at April, 2015 and fertilizer (UREA) was applied two times per growing season. The first application was 10 days after transplanting and the second application was one month after the first application.

Method of data collection

Plant height (cm)

This was measured by using ruler starting from ground level to the tip of the outer longest leaf of individual plant. The mean of five selected plants from a plot was recorded.

Number of leaves/ plant

Total numbers of fully developed outer leave from each sample plant was counted at time of harvesting.

Leaf length (cm)

This was measured by placing a ruler from leaf base to the tip of the leaf of an individual plant and then recorded. The average of the selected five plants per plot was recorded.

Leaf width (cm)

This refers to the maximum diameter of the longest leaf measured using ruler at the widest point of the leaf.

Head diameter (cm)

At harvest, randomly taken samples of cabbage heads from the center were taken and the head diameter (HD) was measured using caliper (model LEG ilex- 250 mm, US patent) and was expressed in centimeter.

Fresh weight (kg/P)

This was recorded from eight plants per plot (two central rows) resulting total yields per net plot. The whole plant parts were measured using the beam balance (Model WA310 rev-B aeadam equipment made in China).

Dry weight (g)

A homogenate (100 g) was prepared for determination of percent dry weight from each plot of head samples and oven (DP 203A: P/N 2123LST (A24) China) dried at a temperature of 120°C for 48 h. Then the weight was measured using digital balance.

Data analysis

The parameters considered in this study were subjected to Analysis of Variance (ANOVA) by using SAS Computer Software version 9.2 (SAS Institute Inc., 2008). When ANOVA showed significant differences, mean separation was carried out using LSD (Least Significant difference) test at 5% level of significance. Interpretations were made according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Plant height (cm)

The level of nitrogen had revealed non - significant (P> 0.05) effect on mean plant height (Table 1). This is because of the fact that nitrogen is responsible for vegetative growth of plants and laterit increases the head diameter through increasing head diameter rather than plant length.

This experiment was in contrary with that of Easmine et al. (2009) which states that increased nitrogen from 0 to 250 kg/ha increases plant height from 47.72 to 36.16 cm, respectively.

Outer leaf number

Mean number of leaves per plant at physiological maturity was not significantly (P>0.05) affected by Nitrogen fertilizer (Table 1). This is due to the fact that at maturity, cabbage leaves were folded and number of unfolded leaves was decreased. This result is in agreement with the findings of Pankaj (2006).

Table 1. Effect of nitrogen fertilizer on plant height and leaf number of cabbage.

N (kg ha^{-1})	Plant height (cm)	Leaf number (cm)
0	20.067[a]	8.93[a]
50	20.833[a]	9.33[a]
100	21.60[a]	9.8[a]
150	23.40[a]	10.39[a]
	NS	NS
LSD (5%)	3.86	2.09
CV%	9.0	10.9

N=nitrogen and Ns = Non significant at 5% level. Means followed by the same letter(s) in the same column are not statistically significantly at 5% level of significancy.

Table 2. Effect of nitrogen fertilizer on leaf length and leaf width of cabbage.

N (kg ha^{-1})	Leaf length (cm)	Leaf width (cm)
0	16.16[b]	16.93[b]
50	18.26[ab]	18.66[ab]
100	18.7[a]	19.56[ab]
150	20.1[a]	21.86[a]
	*	*
LSD (5%)	2.24	4.21
CV%	6.13	10.9

Leaf length (cm)

Application of nitrogen fertilizer significantly (*P<0.05*) influenced leaf length of cabbage (Table 2). The longest leaf length (20.10 cm) was obtained from the plot that received 150 kg of nitrogen per hectare when compared to the control (0 kg nitrogen per hectare) which was 16.16 cm. The positive effect of nitrogen (N) on leaf length might be due to its key role in the synthesis of chlorophyll, enzymes and proteins. The result was in agreement with that of Hadfield (1995) who reported that adequate application of nitrogen promotes vigorous growth and dark green color of cabbage and also, nitrogen is important in formation of chlorophyll and is also a component of protein. Similarly, Souza et al. (2008) reported that application of 200 kg N ha^{-1} significantly enhanced the length of cabbage leaves. Singh and Chaure (1999) also indicated that application of N at 150 kg ha^{-1} gave the best result with regards to cabbage leaf length.

Leaf width

The level of nitrogen had reveald significant *(p<0.05)* effect on the leaf width of cabbage (Table 2). The widest leaf was 21.81 cm and was obtained from 150 kg N/ha. However, it was not significantly different from that of 100 kg N/ha (18.7) while the narrowest leaf was (16.93 cm) obtained from 0 kg of nitrogen per hactare.

Head diameter

Head diameter was significantlly (*p<0.05*) affected with the application of different rate of nitrogen fertilizer (Table 3). The highest head diameter (11.04 cm) was obtained at 150 kg N/ha while the lowest head diameter (8.69 cm) was obtined with application of 0 kg/ha of nitrogen.This finding is in agreement with those of Keteseeman (2006) who reported, head diameter increased from 98 to 218 mm when the nitrogen level increased from 0 to 120 kg/ha, respectively. This was possibly due to higher synthesis of carbohydrate and their translocation to the sink, that is; cabbage head which subsequently helped in the formation of larger and comparatively broader head of the cabbage.

Fresh head weight /plant

Application of nitrogen fertilizer at different rate also showed a very highly significant (*P <0.01)* effect on fresh

Table 3. Effect of nitrogen fertilizer on Head diameter and fresh head weight /plant of cabbage.

N (kg ha^{-1})	Head diameter (cm)	Fresh head weight (Kg/P)
0	8.69^{c}	0.442^{c}
50	9.85^{bc}	0.631^{b}
100	10.47^{a}	0.616^{ab}
150	11.04^{a}	0.771^{a}
	*	**
LSD (5%)	1.17	0.134
CV	5.86	11.02

Means followed by the same letter(s) in the same column are not statistically significantly at 5% level of significancy.

Table 4. Effect of nitrogen fertilizer on head dry weight of cabbage.

N (kg ha^{-1})	Dry weight
0	0.043^{c}
50	0.082^{b}
100	0.083^{b}
150	0.114^{a}
	**
CV	17.82
LSD	28.9

Means followed by the same letter(s) in the same column are not statistically significantly at 5% level of significancy.

head weight per plant (Table 3). Increasing nitrogen level from 0 to 150 kg ha^{-1} resulted in progressive increase in head weight of cabbage. Cabbage grown at 150 kg ha^{-1} of nitrogen rate had the highest head weight per plant (0.771 kg /plant), however the result is simillar with 100 kg/ha while cabbage grown without nitrogen fertilizer had the lowest (0.442 kg/plant) fresh head weight. This is due to nitrogen that increases the vegetative growth and produces good quality foliage and promotes carbohydrate synthesis through photosynthesis and ultimately increased yield of plants (Mengel and Kirkby, 1987).

Dry weight

Regarding to the dry matter contents, nitrogen fertilizer highly significantly (P<0.01) influenced the mean head dry weight (Table 4). The increasing levels of nitrogen encouraged head with a significantly higher dry weigh as compared to the control plot. The maximum dry weight of cabbage head (0.114 kg per plant) were recorded with application of 150 kg ha^{-1}of N whereas, the minimum dry weight (0.043 kg per plant), were detected in the controls plot. There were no significant difference between plots that received 100 and 50 kg N/ha.

Conclusion

Nitrogen rate has a significant effect on leaf length of cabbage. The highest leaf length was recorded at plot receiving 150 kg /ha which was 20.1 cm while the lowest leaf length was recorded at plot recieving 0kg/ha of nitrogen which was 16.166 cm due to the role of nitrogen on photosyntsis and protein formation. Similarly, leaf width of cabbage had siginficant difference between different level of nitrogen. The largest leaf width was obtained from 21.86 cm while the lowest was 16.93 cm)from 150 and 0 kg/ha, respectively.

Nitrogen rate had significant effect on yield of cabbage. The highest head diameter (11.04 cm) was recorded at 150 kg/ha, while the lowest head diameter (8.69 cm) with 0kg/ha. Fresh head weight (0.771 kg/plant) was registerd at 150 kg/ha. On the other hand, the lowest fresh head wieght (0.442 kg per plant) 0kg N/ha. Similar result was obtained for dry head weight, that is; (0.114 kg/plant) with 150 kg N/ha and 0.043kg with 0 kg N/ha. In general, nitrogen level had significant effect on cabbage leaf length, leaf width, head diameter, head fresh weight and dry weight. Thus, we suggest to the growers and stakeholders in the study area to use 150 kg of N per hectare because of the yield and its components and definitely will become profitable.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Vol. 11(7), pp. 282-293, July 2017
DOI: 10.5897/AJPS2016.1441
Article Number: 389EAED64750
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Genotype × environment interactions for grain yield in rice under no drought and drought conditions

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Received 7 July, 2016; Accepted 4 August, 2016

Environments in sub-Saharan Africa fluctuate considerably across sites and seasons. This suggests the importance of assessing genotype x environment interaction (GEI) in cultivar development. The objective of this study was to estimate the magnitude of GEI for rice grain yield and identify high yielding and stable rice genotypes. Fifty six genotypes including 45 F_3 rice populations, their 10 parents and one check were evaluated in 7 x 8 alpha lattice design with two replications under three no drought and one random managed drought stress condition at reproductive growth stage at three sites in coast region of Kenya. The additive main effects and multiplicative interaction (AMMI) analysis and genotype plus genotype x environment interaction (GGE) biplot analysis were used to measure grain yield stability of the 45 F_3 populations and their 10 parents. Ranking of the genotypes changed in each environment and three mega environments were identified revealing a crossover type of GEI. The genotypes G39 (Luyin 46 x IR74371-54-1-1) and G40 (NERICA-L-25 x IR55423-01) were the most stable high yielding genotypes. These were identified as candidates with general adaption for advancement to homozygozity simultaneously selecting within each population good performing pure lines for release in the region.

Key words: Additive main effects and multiplicative interaction (AMMI), genotype x environment interactions, genotype plus genotype x environment interaction (GGE) biplot, rice, yield stability.

INTRODUCTION

Genotype x environment interaction (GEI) is the differential genotypic response to environmental changes (Fox et al., 1997). With significant GEI, differences between genotypes vary widely among environments. A significant GEI is manifested either as changes in the absolute differences between genotypes without affecting the rank order (non-crossover) or as rank order changes of the genotypes between environments (crossover GEI) (Crossa et al., 1995; Yan and Hunt, 2001). The crossover type of GEI is the most important to plant breeders (Fox

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et al., 1997). It reduces the association between phenotypic and genotypic values, complicating selection of superior cultivars and best testing sites for identifying superior and stable genotypes (Flores et al., 1998). Consequently, progress in providing farmers with high yielding cultivars is slowed down (Ceccarelli et al., 1994).

With occurrence of a large GEI, plant breeders tend to identify and recommend high yielding and stable genotypes that show little interaction with the environment or genotypes specifically adapted to certain environments (Fan et al., 2007). Several statistical methods which include regression (Finlay and Wilkison, 1963; Eberhart and Russell, 1966), principal component analysis (PCA) (Hill and Goodchild, 1981), additive main effects and multiplicative interaction (AMMI) (Gauch and Zobel, 1988) and genotype plus genotype by environment (GGE) analysis (Yan, 2001) have been developed to assess stability of a set of genotypes and patterns of GE. Of these, AMMI and GGE biplot are widely used. The AMMI model combines analysis of variance with PCA analysis generating a family of models (Yan and Hunt, 2001; Carlos et al., 2003). However, it is only the AMMI1 and AMMI2 models that may be used to visualise GEI patterns (Yan and Hunt, 2001). In AMMI1, a biplot of main effects with interaction PCA1 (IPCA1) facilitates visualisation of correlation among environments and the response patterns of the genotypes and their interactions with the environments by using sign and magnitude of IPCA1 values (Yan and Hunt, 2001). In AMMI2, a biplot of IPCA1 and IPCA2 is constructed which visualises magnitude of interaction for each genotype and environment (Yan and Hunt, 2001).

The GGE biplot analysis on the other hand puts together genotypic main effects (G) and genotype x environment interaction (GE) to facilitate graphical visualisation of cultivar evaluation and mega environment identification (Yan et al., 2000; Yan, 2002). The GGE biplot is constructed by the first two symmetrically scaled principal components (PC1 and PC2) derived from singular value decomposition (SVD) of environment centred data (Yan et al., 2000; Yan, 2002). This biplot is useful for visualisation and identification of the mega environments, specific and wide cultivar adaptations, high yielding and stable cultivars and interrelationship among environments (Yan and Tinker, 2006).

In sub-Saharan Africa, significant GEI for grain yield and other agronomic traits has clearly been demonstrated in studies involving evaluation of major field crops of economic importance (Badu-Apraku et al., 2011; 2012; Sanni et al., 2012; Nassir, 2013). For example, in a study involving rice germplasm evaluated in five environments in south West Africa, the AMMI analysis revealed significant GEI for grain yield and panicle attributes (Nassir, 2013). On grain yield, the first PCA axis of the interaction captured 52% of the interaction sum of squares while the GGE biplot captured 64% of the interaction component (Nassir, 2013). In another study evaluating 22 NERICA cultivars in three environments in two years again in West Africa, the AMMI analysis reported the existence of a significant GEI with the first four IPCA's contributing 98.5% of the total interaction sum of squares (Sanni et al., 2009). Significant GEI estimated using AMMI and GGE biplot statistical methods has also been reported in studies involving multi-location trials of maize germplasm across years in West Africa (Badu-Apraku et al., 2011, 2012) and in East Africa (Beyene et al., 2012). These studies clearly indicate that in sub-Saharan Africa, environmental conditions fluctuate considerably across years and locations and suggest the importance of considering GE effects in cultivar development and release.

At the beginning of this decade, a rice breeding programme was started at the Kenya Agricultural and Livestock Research Institute (KALRO) - Mtwapa to develop high yielding drought tolerant rice cultivars for the lowland and upland rice ecologies in the coastal lowlands of Kenya. Selected interspecific and *Oryza sativa* L. pure lines were hybridized and the breeding materials advanced to the third generation (F_3). At this stage, there is a need to identify and select promising populations so as to reduce the numbers to manageable levels. The objectives of this study were therefore to; a) estimate the magnitude of GEI for grain yield; b) identify high yielding and stable genotypes across the test environments and c) identify the most discriminating and representative environments as future multi-locational rice testing sites in the coastal lowlands of Kenya. This study is not meant for cultivar recommendation *per se* but to undertake early generation selections in F_3 rice populations.

MATERIALS AND METHODS

Germplasm

Forty five F_3 populations and their 10 parents were used in this study. The parents included five *O. sativa* L. and five interspecific rice pure lines drawn from the African Rice Centre (ARC), the International Centre for Tropical Agriculture (CIAT) and the International Rice Research Institute (IRRI). These parents were crossed in a 10 x 10 half diallel mating design and the resulting 45 F_1s advanced to F_3 populations using the bulk population method.

Study sites

The study was conducted on-station at Kenya Agricultural and Livestock Research Organisation (KALRO)-Mtwapa and KALRO-Matuga and on farm at Msambweni sub-county of Kwale county. KALRO-Mtwapa is located 20 km north of Mombasa in Kilifi south county, along Mombasa-Malindi road. It lies on latitude 3°50'S and longitude 39°44'E at an elevation of 15 m above sea level (masl). Annual mean temperatures are between 22 and 26°C. The area receives bimodal mean rainfall of about 1200 mm with reliable long rains of 600 mm falling mid-March to August and the variable short rains of 250 mm falling in mid-October to December. The soils are dominated by orthicacrisols (80% sand) with low inherent fertility (Jaetzold and Schmidt, 1983). KALRO Matuga is situated 15 km

Table 1. Features of the four environments used in this study.

Study site	Season	Ecology	Type of Environment	Code
Matuga	Short rain season (2014/15)	Upland	No drought stress	E1
Mtwapa	Short rain season (2014/15)	Upland	No drought stress	E3
Msambweni	Short rain season (2014/15)	Lowland	Random drought stress	E2
Msambweni	Long rain season (2015)	Lowland	No drought stress	E4

south of Mombasa from the Likoni ferry in Kwale county. The site is at Latitude 4°9'S and longitude 39°34'E at an elevation of 132 masl. Annual mean temperatures are between 24 and 26°C. The area receives bimodal mean annual rainfall of about 1200 mm with the long rain season of 750 mm and short rain season of 350 mm. The soils are derived from Pliocene sandstones and are commonly referred to as Magarini sands (Jaetzold and Schmidt, 1983). They are low in C, N, P, K and are moderately acidic (Jaetzold and Schmidt, 1983). The typical agro-ecological zonation for KARLO-Mtwapa and Matuga is coastal lowland 3 (CL3-coconut cassava zone). The Msambweni on-farm site is 50 km south of Mombasa from Likoni ferry. The site is at latitude 4°28'S and longitude 39°29'E at an elevation of about 19 masl and lies in coastal lowlands 2, (CL2), classified as the coastal lowlands sugarcane zone and occurs as a pocket in Ramisi area in Kwale county and is the wettest zone. The annual average temperatures range from 19 to 24°C. Rainfall in this zone is bimodal ranging from 1200 to 1400 mm annually. The long rain season of 800 mm falls between March and August and short rain season of 400 mm falls between mid-October and December (Jaetzold and Schmidt, 1983).

Experiments

The experimental materials consisted of 56 treatments (entries) including 45 F_3 populations, their 10 parents and 1 check. These were evaluated in 7 x 8 alpha lattice design with two replications under four environments; one random managed drought stress and three no drought stress conditions. The random drought stress environment was planted on farm at Msambweni during the short rain season. It was planted in mid-October 2014 and the last rainfall of 44 mm was received 65 days after planting. Random drought stress occurred during the reproductive stage from the panicle initiation stage to harvesting. The no drought stress experiments included two experiments established on station at KALRO-Matuga and KALRO-Mtwapa during the short rain season and one established on farm at Msambweni during the long rain season. The KALRO-Matuga experiment was planted mid-October 2014 and received supplemental irrigation water since rainfall during the short rain season was not adequate. At Mtwapa, the experiment was established in December 2014 and was irrigated. The Msambweni site was planted in April 2015 and was purely rainfed since the rainfall was adequate. Features of the four environments are summarised in Table 1.

Management of experiments

At KALRO-Matuga and Msambweni, the fields were un-flooded and aerobic conditions. The experimental plot were 3.2 m^2 with inter- and intra-row spacing of 20 cm to give a total of 80 plants per plot. Seed for each entry was first planted in plastic containers and transplanted to the field on the 12th day. Two seedlings were transplanted and later thinned to one seedling per hill. At KALRO-Mtwapa, plants were planted in an open field in black polyethylene pots with 25 cm internal diameter and 30 cm height. Each pot was filled with 20 kg of upland soil. Pots were watered to field capacity before planting. Five seedlings per pot were transplanted and there were five plants per pot spaced at 10 cm each. Each entry was assigned eight pots to give a total of 40 plants per entry. From transplanting to dough stage, each pot received one and half liters of water each in the morning hours on daily basis and by the end of the day, there was no standing water in each pot. Thereafter, watering was done after every two days to allow the plants to dry up for harvesting. The overall management was application of basal inorganic fertilizers; calcium ammonium nitrate (CAN) as a source of N and diamonium phosphate (DAP) as a source of P. The P was applied during planting at recommended rate of 60kg P ha^{-1}. The N was top dressed at the rate of 120 kg N ha^{-1} applied in three splits of 40 kg ha^{-1} at 21 days after transplanting, tillering stage and at panicle initiation stage. Source of micro nutrients was foliar feed which was sprayed once during the tillering stage. Rice stem borer was effectively controlled using a synthetic pyrethroid. Weeds were controlled by hand picking. Harvesting was carried out manually.

Data collection

Grain yield data was taken as the weight of unhulled grains harvested from an area of 2 m^2 for the experiments planted under field conditions and from 40 plants for the experiment planted in pots. This was then converted to tons ha^{-1} at 14% moisture content.

Data analysis

Analysis of variance

A combined analysis of variance (ANOVA) was performed to determine the effects of environment, genotype and GEI on grain yield of the 55 entries (45 F_3 populations and 10 parents) across four environments using PROC GLM in SAS (SAS Institute, 2012). The Genstat statistical package (14th Edition) (Payne et al., 2011) was used to estimate and graphically visualise grain yield stability of the entries using the AMMI (Additive Main Effects and Multiplicative Interaction) and the GGE (genotype and genotype x environment) biplot analyses.

AMMI model

The AMMI analyses were performed to clarify the presence of the GEI, summarize patterns and relationships of genotypes and environments and estimate the grain yield means that are adjusted for G x E using the model shown below (Crossa, 1990):

$$Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^{t} \lambda_k \xi_{ik} \eta_{jk} + \varepsilon_{ij}$$

Where, Y_{ij} is the mean yield (t ha^{-1}) of the ith genotype in the jth

Table 2. Analysis of variance of the grain yield (t ha^{-1}) of 45 F_3 rice populations and their 10 parents evaluated in four environments in coast region of Kenya.

Source	df	Sum of squares	Mean squares	*F* test
Rep (Env)	3	2.07	0.69	2.02^{NS}
Environment (E)	3	25.74	8.58	25.23***
Genotype (G)	54	112.15	2.08	6.11***
Interactions (GxE)	162	235.90	1.46	4.28***
Error	216	73.47	0.34	
Total	439	449.43		

Table 3. AMMI analysis of variance of grain yield (t ha^{-1}) of 45 F_3 populations and their 10 parents evaluated in four environments in coast region of Kenya.

Source of variation	df	Sum of squares	Mean squares	*F* test
Block	4	2.20	0.54	1.60^{NS}
Treatments	219	374.20	1.71	5.02***
Genotype	54	112.30	2.08	6.11***
Environments (E)	3	25.80	8.60	15.81***
Interactions (GxE)	162	236.10	1.46	4.28***
IPCA 1	56	114.10	2.04	5.99***
IPCA 2	54	63.80	1.18	3.48***
Residuals	52	58.20	1.12	3.29***
Error	216	73.50	0.34	
Total	439	449.80	1.03	

environment, μ is the overall mean, g_i and e_j are the main effects of the genotype and environment, respectively, *t* is the number of PCA axes considered, k is the singular value of kth PCA axis, λ_k Eigenvalues for kth PCA axis, ξ_{ik} *and* η_{jk} are scores for the ith genotype and jth environment on the kth PCA axis, and ε_{ij} is the residual term which includes experimental error. The AMMI biplot showing the main effects (genotype and environment) and the first interaction principal components axis (IPCA 1) was also presented to assess the relationships among entries, test environments and GEI for grain yield.

GGE Biplot

The GGE mathematical model based on PCA of environment-centred data (which contains G and GE as the main sources of variation) subjected to singular value decomposition (SVD) was used to visualize the relationship among genotypes and the environments. The basic model for a GGE biplot as described by Yan (2002) is:

$$Y_{ij} - \mu - \beta_j = \sum_{l=1}^{k} \lambda_l \xi_{il} \eta_{lj} + \varepsilon_{ij}$$

Where: Y_{ij} = Mean grain yield (t ha^{-1}) of the ith genotype in the jth environment; μ = Overall mean; β_j = main effect of the environment; λ_l= Eigen value associated with IPCA *l*; ξ_{il}= the Eigenvector of genotype *I* for PC *l*; η_{lj}= the eigenvector of environment *j* for PC *l*; ε_{ij}= error term associated with rice genotype *i* in environment *j*. The GGE biplot graphs were used to visualize interrelationships among the test environments, discriminating ability and representativeness of test environments, which-won-where-pattern polygon view and mean yield and stability among genotypes (Yan and Tinker, 2006; Yan et al., 2007).

RESULTS

Analysis of variance and AMMI analysis

The check was found to be late maturing and therefore was eliminated from the analysis. The combined analysis of variance for grain yield showed highly significant (*P*<0.001) genotype (G), environment (E) and genotype × environment (G×E) interaction explaining 25, 6 and 53% of the total sum of squares, respectively (Table 2). The G×E interaction effect was approximately nine times that of environmental effect and twice that of the genotype effect. The AMMI analysis of variance showed that grain yield of 55 genotypes at four environments was significantly (*P*<0.001) affected by the genotype, environment and genotype x environment interaction, explaining 30, 7 and 63% of the total treatment sum of squares, respectively (Table 3). The first and the second PCA axis (IPCA1 and IPCA2) of the interaction were highly significant (*P*<0.001). The IPCA1 explained 31% of the treatment sum of squares which is 48% of the G x E

Table 4. AMMI average grain yield (t ha-^{1}) of 45 F_3 rice populations and their 10 parents evaluated in four environments in coast region of Kenya.

Code	Genotypes	Environment				Mean
		Matuga	Mtwapa	Msambweni	Msambweni	
		No drought			Drought	
F_3 Populations						
G1	NERICA 1 x NERICA 2	4.43	4.19	3.94	4.05	4.15
G2	NERICA 1 x *Dourado*	3.13	3.73	3.19	4.20	3.56
G3	NERICA 1 x CT16333(1)-CA-22-M	4.17	3.49	3.10	3.11	3.47
G4	NERICA 1 x CT16323-CA-25-M	3.82	3.31	2.60	3.12	3.22
G5	NERICA 1 x Luyin 46	2.84	2.31	3.19	1.73	2.52
G6	NERICA 1 x NERICA -L- 25	3.45	2.91	3.86	2.31	3.13
G7	NERICA 1 x IR55423-01	3.38	3.32	2.41	3.47	3.15
G8	NERICA 1 x Vandana	3.77	2.99	4.84	2.02	3.40
G9	NERICA 1 x IR74371-54-1-1	2.28	3.10	4.00	3.36	3.18
G10	NERICA 2 x *Dourado*	5.12	3.38	3.22	2.29	3.50
G11	NERICA 2 x CT16333(1)-CA-22-M	3.47	3.94	4.09	4.15	3.91
G12	NERICA 2 x CT16323-CA-25-M	3.19	2.63	3.57	2.02	2.85
G13	NERICA 2 x Luyin 46	3.07	3.07	4.84	2.60	3.39
G14	NERICA 2 x NERICA-L-25	2.15	3.31	4.47	3.72	3.41
G15	NERICA 2 x IR55423-01	3.56	3.38	3.66	3.16	3.44
G16	NERICA 2 x Vandana	3.11	2.86	4.13	2.36	3.12
G17	NERICA 2 x IR74371-54-1-1	2.99	2.82	3.22	2.57	2.90
G18	*Duorado* x CT16333(1)-CA-22-M	4.89	3.64	2.07	3.20	3.45
G19	*Duorado* x CT16323-CA-25-M	3.82	3.59	2.39	3.69	3.37
G20	*Duorado* x Luyin 46	2.49	2.67	3.48	2.55	2.80
G21	*Duorado* x NERICA -L- 25	2.57	2.75	2.83	2.81	2.74
G22	*Duorado* x IR55423-01	3.06	2.23	2.53	1.60	2.35
G23	*Duorado* x Vandana	3.12	2.72	2.81	2.40	2.76
G24	*Duorado* x IR74371-54-1-1	2.62	2.58	2.45	2.55	2.55
G25	CT16333(1)-CA-22-M x CT16323-CA-25-M	3.85	3.08	2.64	2.66	3.06
G26	CT16333(1)-CA-22-M x Luyin 46	2.63	2.45	2.79	2.21	2.52
G27	CT16333(1)-CA-22-M x NERICA-L-25	2.55	3.14	2.30	3.68	2.92
G28	CT16333(1)-CA-22-M x IR55423-01	2.62	1.96	3.38	1.15	2.28
G29	CT16333(1)-CA-22-M x Vandana	3.04	2.82	3.38	2.51	2.94
G30	CT16333(1)-CA-22-M x IR74371-54-1-1	3.81	3.03	3.30	2.44	3.14

interaction sum of squares in 35% of the interaction degrees of freedom. The IPCA2 explained 17% of the treatment sum of squares which is 27% of the G x E interaction sum of squares in the remaining 33% of the interaction degrees of freedom.

Performance and ranking of the best four AMMI selections

Across environments, the AMMI average genotype grain yield ranged from 4.53 t ha^{-1} in G37 to 2.28 t ha^{-1} in G28 (Table 4). Grain yield for environments was highest at environment E1 (3.7 t ha^{-1}) and lowest at environment E2 (3.0 t ha^{-1}). Inconsistencies in genotype performance were observed across the four test environments (Table 5). The genotypes G37 (Luyin 46xIR55423-01) and G42 (NERICA-L-25 x IR74371-54-1-1), were ranked among the best four high yielding genotypes in more than one environment.

AMMI and IPCA scores biplot

The complete AMMI (combined main effects and IPCA1) explained 67% of the total treatment variation, while AMMI2 (IPCA 1+ IPCA 2) explained 48% of the total treatment variation. AMMI2 was dropped in favour of AMMI1 because the noise in the treatment sum of squares in AMMI1 was less, 31% as compared to 48% in

Table 4. Contd.

G31	CT16323-CA-25-M x Luyin 46	2.56	2.57	3.35	2.34	2.70
G32	CT16323-CA-25-M x NERICA-L-25	2.20	3.19	2.94	3.84	3.04
G33	CT16323-CA-25-M x IR55423-01	2.90	2.85	2.56	2.84	2.79
G34	CT16323-CA-25-M x Vandana	4.24	3.96	4.12	3.71	4.01
G35	CT16323-CA-25-M x IR74371-54-1-1	3.03	2.86	3.01	2.68	2.90
G36	Luyin 46 x NERICA -L- 25	2.54	3.10	5.33	2.88	3.46
G37	Luyin 46 x IR55423-01	3.96	4.15	6.31	3.70	4.53
G38	Luyin 46 x Vandana	4.35	3.56	6.75	2.24	4.23
G39	Luyin 46 x IR74371-54-1-1	2.05	3.77	4.65	4.61	3.77
G40	NERICA-L-25 x IR55423-01	3.90	3.89	4.27	3.75	3.95
G41	NERICA-L-25 x Vandana	4.06	3.78	6.40	2.93	4.29
G42	NERICA-L-25 x IR74371-54-1-1	3.38	3.94	3.56	4.35	3.81
G43	IR55423-01 x Vandana	3.43	3.83	3.87	4.03	3.79
G44	IR55423-01 x IR74371-54-1-1	3.21	3.73	3.43	4.10	3.62
G45	Vandana x IR74371-54-1-1	5.17	3.82	3.70	2.97	3.91
Parents						
P1	NERICA 1	3.84	3.50	3.60	3.23	3.54
P2	NERICA 2	3.55	3.48	3.60	3.36	3.50
P3	*Dourado precoce*	3.64	3.84	3.65	3.97	3.77
P4	CT16333(1)-CA-22-M	3.94	3.14	3.10	2.60	3.20
P5	CT16323-CA-25-M	3.80	3.38	2.25	3.35	3.20
P6	LUYIN 46	2.49	3.13	6.09	2.78	3.62
P7	NERICA-L-25	3.06	2.38	5.26	1.22	2.98
P8	IR55423-01	4.71	3.43	3.76	2.51	3.60
P9	Vandana	4.31	3.62	4.74	2.86	3.88
P10	IR74371-54-1-1	2.89	3.62	2.60	4.30	3.35
Mean		3.39	3.24	3.67	3.00	3.32

Table 5. The best F_3 populations from AMMI analysis at each environment.

Environment	Mean GY (tha-1)	PCA Score	Rank			
			1	2	3	4
E2	3.00	1.28	G39	G42	G55	G2
E3	3.24	0.55	G1	G37	G34	G42
E1	3.39	0.48	G45	G10	G18	G53
E4	3.67	-2.32	G38	G41	G37	G51

See Table 1 for environment and Table 4 for genotype codes.

AMMI2. Thus, AMMI1 was more effective because it had less predictive errors. Therefore, a biplot of main effects against IPCA1 was used to graphically visualise average productivity of the genotypes and environments and GE interaction for all possible genotype x environment combinations (Figure 1). The four environments fell into three groups: Environment E1 had large positive IPCA1 score strongly interacting positively with genotypes that had positive IPCA scores and negatively with genotypes that had negative IPCA scores. Environment E2 had large negative IPCA1 score strongly interacting with genotypes but in the opposite direction to that of E1. Environments E3 and E4 formed the third group with small IPCA1 scores, suggesting that they had little interaction with the genotypes. The genotypes showed variability in mean yield and in interaction scores. Genotype G37 was the highest yielding followed by G41, G38, G1 and G34. The most stable high yielding genotypes were G41, G1 and G34 in that order. The most unstable but high yielding genotypes demonstrating

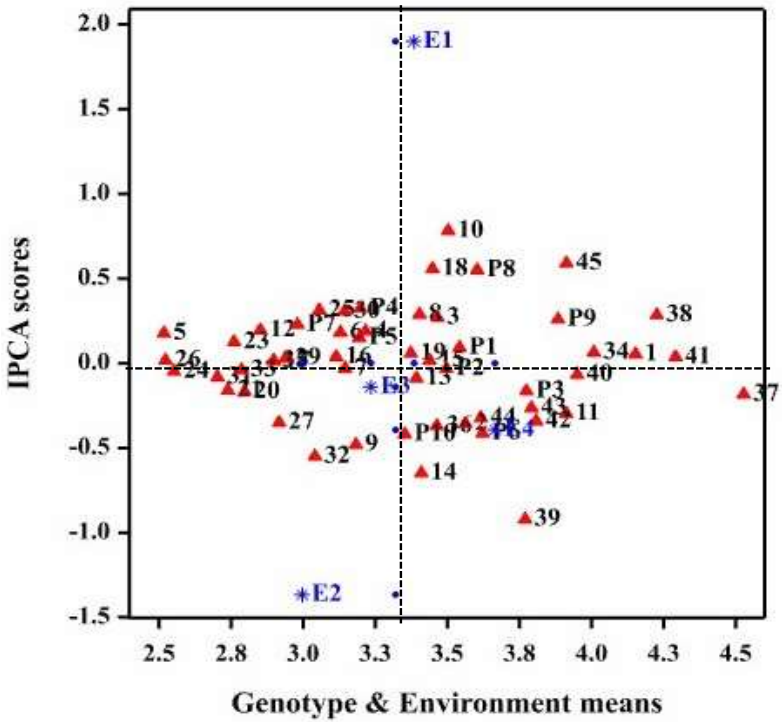

Figure 1. AMMI1 biplot on the grain yield of 45 F_3 rice populations and their 10 parents evaluated in four environments in coast region of Kenya. See Table 1 for environment and Table 4 for genotype codes.

a strong GEI were G10 and G39. G10 was specifically suitable for environment E1, while G39 was suitable for environment E4.

GGE biplot analysis

The goodness of fit of the GGE biplot was 67.94%; PC1 contributed 39.01% while PC2 accounted for 28.93% of the total variation (Figure 2). The cosine of the angle between vectors of environments E4 and E2 was a right angle. The acute angle between vectors of E2 and E3 was the smallest and largest between vectors of E3 and E4. The distance between E2 and E3 was the shortest followed by the distance between E3 and E1. The distance between E3 and E1 from E2 was shorter than the distance between these two sites from E4. Environments were ranked based on discriminating ability and representativeness of the 'ideal" (average) environment (Figure 3). Environments E1 and E2 were found to be close to the average environment and therefore the most representative of the target region. However, E2 had a longer vector than E1 and therefore was both discriminating and representative of the whole region. Environments E4 and E3 were further away from the average environment and therefore the least representative of the whole region. Environment E4 had a long vector and therefore classified as discriminating and non-representative. Environment E3 was both non-discriminating and non-representative of the target region since it had a short vector and was farther away from the average environment.

The polygon view of the GGE biplot displayed which-won-where-pattern of genotype by environment dataset of the four environments (Figure 4). The radial lines originating from the centre of the biplot divided the polygon into eight sectors. The four environments fell into three sectors and there were three mega environments. The first mega environment consisted of E3 and E2 and the winning genotype was G2. The second mega environment was represented by E1 and the winning genotype was G37. The third was represented by E4 and here the winning genotype was G38. Among the F_3 populations, genotype G37 had the highest grain yield followed by G38 and G41 in that order (Figure 5). Genotype G28 was the lowest yielding genotype. Among the parents, P9 was the highest yielding parent followed by P3, P6 and P8. The lowest yielding parent was P7. Grain yield of seven F_3 populations namely G37, G38, G41, G1, G34, G11, G2 and G39, was higher than the highest yielding parent P9. The most stable F_3 population with above average mean performance was G39 as it was located almost on the AEC abscissa and had a near zero projection onto the AEC ordinate. This was followed by G40. In contrast, G38 although high yielding, was the least stable followed by G41. Parent P8 (close to G14)

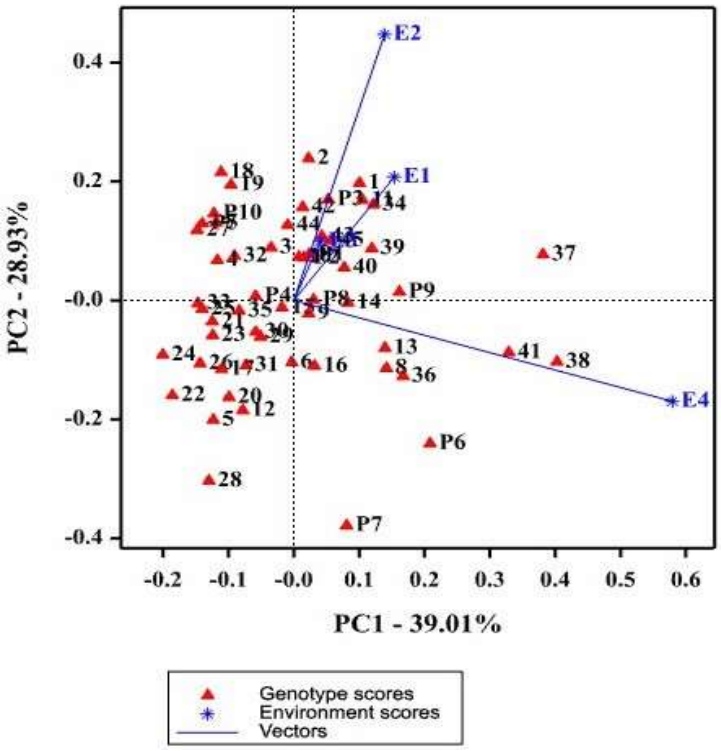

Figure 2. Relationship among test environments. See Table 1 for environment and Table 4 for genotype codes.

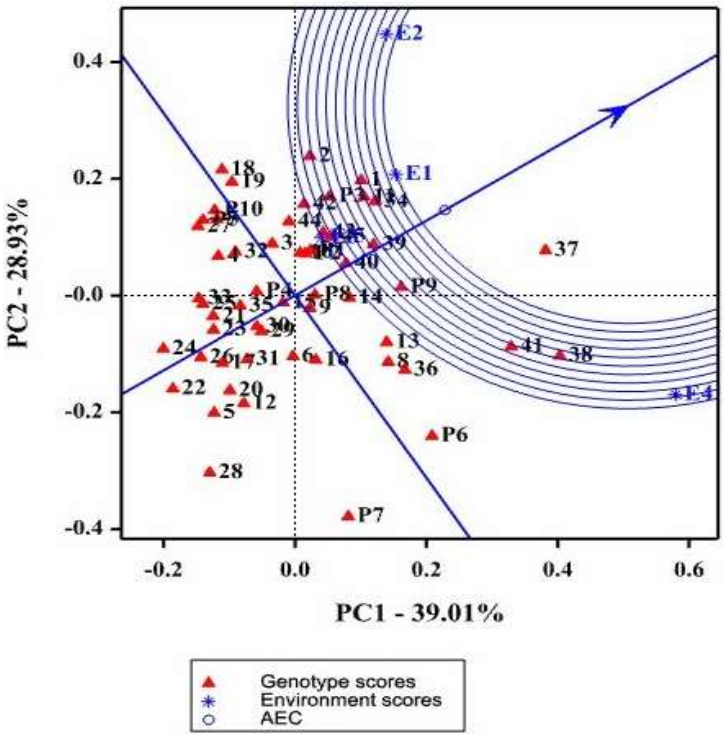

Figure 3. The discriminating and representative view showing the discriminating ability and representativeness of the test environments. See Table 1 for environment and Table 4 for genotype codes.

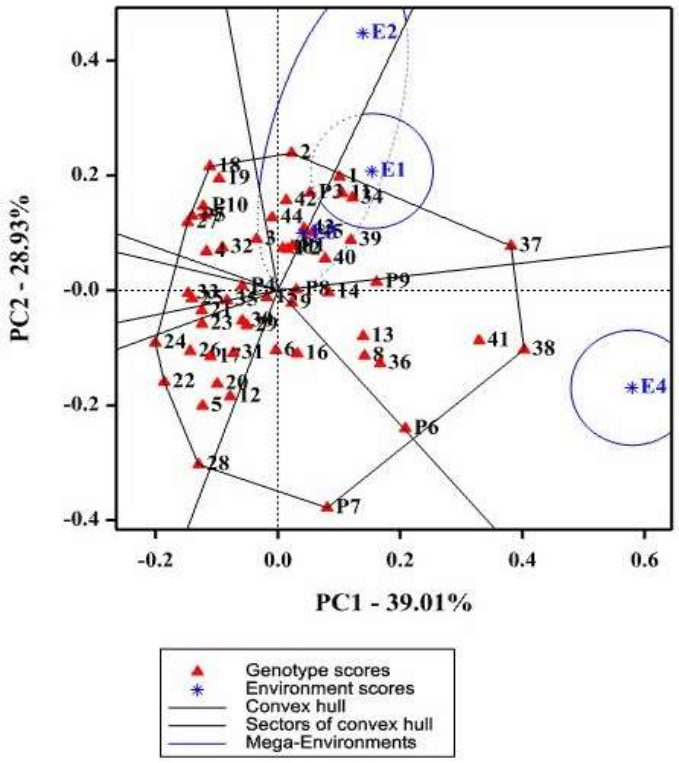

Figure 4. Polygon view of the GGE biplot based on symmetrical scaling. See Table 1 for environment and Table 4 for genotype codes.

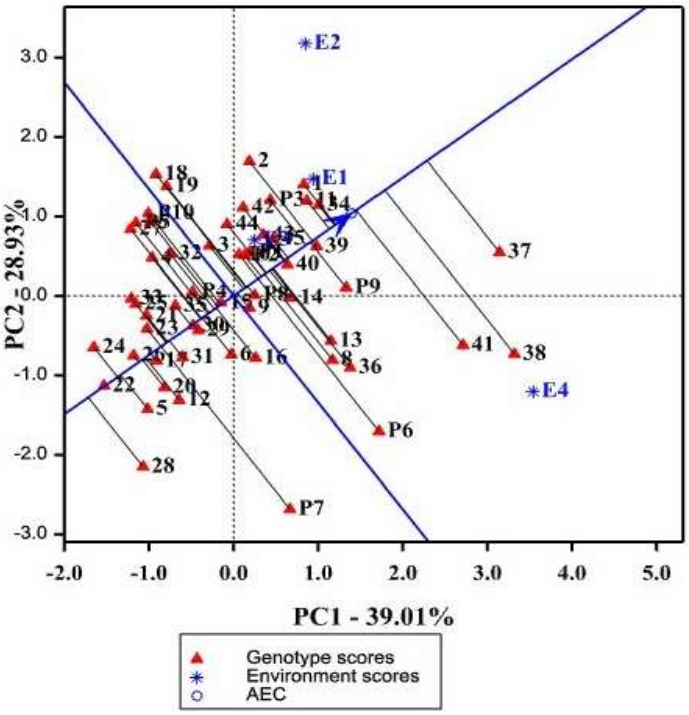

Figure 5. GGE-biplot based on genotype-focused singular value partitioning for comparison of the genotypes with the ideal genotype. See Table 1 for environment and Table 4 for genotype codes.

was found to be the most stable parent although it was located slightly away from the AEC abscissa. Parents P6 and P7 were found to be the most unstable among parents with almost similar level of poor stability with G41.

DISCUSSION

ANOVA and AMMI analysis

The ANOVA and AMMI analysis revealed that the environment and genotypic main effects and their interactions were highly variable. The genotype x environment interaction (GEI) for grain yield contributed approximately 50% of the total sum of squares. These effects were greater than what has been obtained in other studies (Sanni et al., 2009; Nassir, 2013). The high interaction effects observed could partly be explained by the wide variation among the genotypes and among the environments. The genotypes included in this study varied considerably since the parents were pure lines and their progenies were heterozygous in their third filial generation. In addition, the parents varied in species and maturity. Thus, the materials showed a wide genetic base in phenological, physio-morphological characters, grain yield and its contributing characters. Variability in environments could be attributed to differences in terms of levels of organic matter, soil nitrogen and other soil nutrients, water regimes and management conditions among others. The AMMI biplot classification of genotypes and environments revealed three mega environments: first, E1 (Matuga) with a large positive IPCA1 scores; second, E2 (Msambweni drought) with a large negative IPCA1 score and third, E3 (Mtwapa) and E4 (Msambweni no drought) with small IPCA scores. Environments E1 and E2 had the highest discriminating power and were therefore good for selecting genotypes with specific adaptation while E3 and E4 were good for selecting genotypes that perform well across the test environment. The most high yielding and stable genotypes across the test environments were G41 followed by G1 and G34. The most unstable but high yielding genotypes demonstrating a strong GEI were G10 and G39. G10 was specifically suitable for E1 while G39 was suitable for E2.

GGE biplot analysis

Although, the environment main effect may contribute upto 80% or more of the total yield variation, it is usually the genotype main effect and the genotype x environment interaction (GEI) that are relevant to cultivar evaluation (Yan, 2002). The use of GGE biplots has been appreciated by many researchers in rice and other crops (Hagos and Abay, 2013; Kivuva et al., 2014; Lakew et al., 2014; Muthoni et al., 2015) as it graphically displays general pattern of genotype responses across environments in multi-environmental trials data usually concealed in the general ANOVA. In this study, the GGE biplot results revealed that there was no correlation between environments E2 and E4, indicating that these two environments discriminated the genotypes differently. This was expected because although the two environments were established on the same location, (Msambweni site), differences in water regimes and rainfall seasons contributed to lack of correlation. The random drought environment (E2) was set up during the short rain season and drought developed from flowering to harvesting. In contrast, no drought environment (E4) was set up during the long rain season and rainfall was adequate for growth and development of rainfed rice. This also implies that there is a need for separate breeding programmes for the short and long rain seasons. The distance between E3 and E1 from E2 was shorter than the distance between these two sites from E4. This indicated that E3 and E1 were more positively correlated to E2 than E4. Thus, environments E1, E2 and E3 may have discriminated the genotypes similarly but different from environment E4. Environments E3 and E1 were set up under upland aerobic conditions indicating that during growth and development of the rice genotypes under study, some level of stress similar to that observed in environment E2 may have developed. The environment E2 was close to the average environment and had the second longest vector after E4, indicating that it discriminated among the genotypes and was representative of the whole target region. Based on the observation that E2 was positively correlated to E3 and E1 upland ecologies, this environment may be a good site for selecting genotypes with general adaptation to the upland ecology and drought tolerant genotypes for the lowland ecology. On the other hand, E4 was discriminating but non-representative. This site is therefore good for selecting specifically adapted genotypes if the target environment can be divided into mega environments and/or for culling unstable genotypes if the target environment is a single mega environment.

The polygon view of GGE biplot is very useful for visualising the best genotypes in each environments and grouping environments for visualisation of possible crossover GEI and mega environments (Yan and Tinker, 2006). Different environments fall into different sectors, which imply that there are different high yielding cultivars for those sectors and it shows crossover GEI, suggesting that the test environments could be divided into mega-environments (Yan et al., 2007). In this study, the environments fell into three sectors revealing the possibility of three mega environments and the presence of crossover type of GEI. The environments E2 and E3 fell into one sector and genotype G2 as the best performing genotype in this sector. Environment E1 fell into the second sector and the winning genotype was G37, while E4 fell into the third sector with genotype G38

winning in this environment. Other researchers in sub-Saharan Africa have also appreciated the use of the polygon view of GGE biplot in identification of the best genotypes in different environments and revealing possible mega environments among the test environments (Kivuva et al., 2014; Lakew et al., 2014; Muthoni et al., 2015). The biplot view of mean yield and stability revealed that the average grain yield of G37, G41 and G38 was higher than that of the average (ideal) genotype across the test environments. However, they had poor stability and were therefore good for specific adaptation. Genotype G37 was specifically adapted to environment E1 while G38 and G41 were specifically adapted to environment E4. Advancing different F_3 populations for each mega environment would be more time and resource consuming than selection of the best one or a few populations for the whole target region. Therefore, genotype G39 followed by G40 combined high yield and stability across the test environments. These genotypes were therefore identified as candidates with general adaption.

Conclusions

There were inconsistencies in the ranking of the genotypes in each environment while the four environments fell into three mega environments. This is a clear indication of crossover GEI. The environments E2 (Msambweni random drought) and E4 (Msambweni no drought) were shown to be two independent environments. The two environments were established on the same site but in different rain seasons and suggests the need for separate breeding programmes for the short and long rain seasons in the coast region of Kenya. For genotype evaluation, the GGE was more superior to the AMMI1 biplot since it explained more of the G + GE variation. Thus, based on GGE biplot, G37 was the highest yielding genotype followed by G38 and G41. However, these three genotypes were unstable across environments. The genotype G39 combined high yield and stability across the test environments. This was followed by G40. Therefore, these two genotypes were identified as candidates with general adaption for advancement to homozygozity simultaneously selecting within each population good performing pure lines for release in the region. The results of this study are based on a single year data, and therefore may not be decisive; more temporal and spatial environments will be needed to give meaningful recommendations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This research was funded by the Alliance for Green Revolution in Africa (AGRA) through the African Centre for Crop Improvement (ACCI), University of KwaZulu Natal, Republic of South Africa. The authors thank the International Foundation of Science for the grant that supported part of this work and Kenya Agricultural and Livestock Research Organization (KALRO) management for granting study leave to the first author.

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Vol. 11(7), pp. 294-297, July 2017
DOI: 10.5897/AJPS2017.1573
Article Number: 82B02DD64753
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Flowers of the intertidal seagrass *Halophila stipulacea* (Forsskål) Ascherson: A new record from tropical coast of Tanzania, Indo-Pacific

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Received 17 May, 2017; Accepted 7 June, 2017

Flowers of the seagrass *Halophila stipulacea* (Forsskål) Ascherson in Tanzania are currently unreported. The present study was conducted along the coast of Tanzania, Indo-Pacific, Kunduchi intertidal mudflats. Transplanted cuttings from Kunduchi intertidal mudflats were successfully grown in sand-mud substrate in the growth chamber in a 12 h photoperiod (1,250 µmol photons $m^{-2}s^{-1}$) and an inductive temperature, salinity, and pH range of 24 to 28°C, 34 to 38‰, and 7 to 8, respectively. Plants began to flower after four months of culturing. No flowers were observed in the first three months; 0.229±0.50 staminate and 0.123±0.45 pistillate were recorded between April and June; 0.440±0.65 staminate and 0.221±0.03 pistillate between July and September, and 0.282±0.36 staminate and 0.105±0.78 pistillate between October and December. Although, further research is required to fully assess the pollination success and sexual reproduction including fruiting of the species, our study is the first to report the presence of flowers *ex situ* in Tanzania.

Key words: Mudflats, laboratory culture, sexual reproduction.

INTRODUCTION

Flowering has been rarely reported for tropical seagrasses along the East African coast with the exception of Kenya where one flower of *Cymodocea serrulata* was collected in a beach drift in January 1967, at Diani Beach (Isaac and Isaac, 1968). The recorded collections are over fifty years; August 1965, from plants of *Halophila ovalis, Halophila stipulacea* (cited as *Halophila balfourii* Solered.), *Thalassodendron ciliatum,* and *Thalassia hemprichii* (den Hartog, 1970); pistillate flowers of *Syringodium isoetifolium*, *Halodule uninervis,* and *H. stipulacea* collected in August of 1968 (Isaac, 1968). These records suggest that observing of *H. stipulacea* flowers *in situ* is not common occurrence.

Studies of seagrass reproduction and phenology are therefore important in determining the contribution of reproduction to the population dynamics of different

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seagrasses (Clores and Agoo, 2013). Knowledge of reproductive biology may also be critical in the re-establishment of declining seagrass populations and in targeting the best species for use in revegetation (Orth et al., 1994). Due to their small sizes and inconspicuous nature, flowers of seagrasses are usually overlooked and not collected (Clores and Agoo, 2013; Sidik et al., 2010). The extent and timing of flowering in seagrasses worldwide is variable, between species, and between locations, making generalizations difficult. In tropical regions, seagrass flowering is a year-round phenomenon but with variations in intensity related to location, while in temperate regions, flowering often occurs in spring, but the timing of the whole reproductive cycle varies with both species and location (Walker et al., 2001).

Culturing of seagrasses in the laboratory had been attempted by many marine biologists. The attempt had been conducted as early as 1922 (Setchell, 1922). Different systems and diverse methods had been deployed on various seagrasses species in order to create a small-scale marine system that simulates the environmental conditions of the natural habitats according to species and locality. Among the successful culture systems are: Setchell (1922, 1924, 1929), Wood (1959), McMillan (1976, 1978, 1980a, b), and Bujang et al. (2008), where representatives of 9 out of 12 genera of seagrasses (*Thalassia, Halodule, Halophila, Posidonia, Zostera, Cymodocea, Syringodium, Enhalus,* and *Thalassodendron*) have been successfully cultured in synthetic seawater and under controlled environmental conditions. In these culture systems, studies focused on the biological, ecological, and phenological aspects of seagrasses (McMillan, 1980a, b; McMillan et al., 1981).

Despite the fact that culture systems have been developed, they were mostly established to suit particular seagrasses species in relevant environmental conditions (Hillman et al., 1995; Longstaff et al., 1999; Ralph, 1998; Short, 1985). And thus, these techniques may not apply to all seagrass species.

In the coastal waters of Tanzania, ten seagrasses species have been reported to occur (Lugendo et al., 1999; Oliveira et al., 2005; Richmond, 1997), of which two species belong to the genus *Halophila*, that is, *H. stipulacea* and *H. ovalis. H. stipulacea* is the most common species in the intertidal mudflats, sand-mud substrates and along the shallow intertidal coast and subtidal areas along Dar es Salaam coast. Although, common, information on its biology and phenology is scanty. This study attempts to develop a culture system to assess the reproductive biology (flowering) of *H. stipulacea* under favorable environmental conditions in order to obtain critical information about the species.

MATERIALS AND METHODS

This experiment was carried out at the Department of Aquatic Science and Fisheries of the University of Dar es Salaam, Kunduchi Station, Tanzania, from January to December, 2013. In the growth chamber (air condition controlled room), a small-scale culture system for growing *H. stipulacea* was comprised of 30 cm × 30 cm × 40 cm glass aquarium, which was provided with 6 cm thick of substrate/sediment and flooded with 20 L of 35‰ ocean water. The culture system was fitted with an external filter system and a submersible pump to provide filtration and circulation of water inside the aquarium. To keep the amount of water constant, water levels were monitored daily, and evaporation was compensated for by adding distilled water. To maintain water clarity, the external filter was cleaned with distilled water twice monthly. Samples of *H. stipulacea* collected from Kunduchi intertidal mudflats (6°39'- 6°41' S and 39°12' - 39°13' E) were randomly planted in the 6 cm substrate. Algal growths covering the substrate were removed manually when necessary. Experiments were performed in *H. stipulacea* native substrate of sand-mud, under Gro-Lux fluorescent lamps (1,250 µmol photons $m^{-2}s^{-1}$) under a daily 12 h photoperiod; and an inductive temperature, salinity and pH of 24 to 28°C, 34 to 38‰ and 7 to 8, respectively. No artificial nutrients were added, and water was changed every month, with the assumptions that seawater was of uniform physicochemical composition and that other physicochemical parameters do not influence the flowering of *H. stipulacea*. The culture ran for twelve months monitored at three month intervals: January to March, April to June, July to September and October to December; this coincided with the species flowering pattern (McMillan, 1976). Flowering observations were done twice monthly and monitored as per Short and Coles (2001). The floral density was calculated as per standard method also described by Short and Coles (2001).

RESULTS AND DISCUSSION

Sods of *H. stipulacea* were successfully grown in the laboratory using native sand-mud substrate under controlled temperature and salinity with minimum aeration. After initial planting in January, plants in culture system began to multiply through vegetative propagation by producing new shoots; a new shoot was produced after every seven to nine days. By the end of the first three months of culture, continuous propagation of *H. stipulacea* inside the aquarium area had densely populated the plugs.

H. stipulacea under the controlled conditions produced flowers. *H. stipulacea* is a dioecious plant, male and female parts are separated (den Hartog, 1970). Plants began to flower after four months of culturing; that is from April. Flowers (Figure 1) were recorded from April to December. No flowers were observed in the first three months; 0.229±0.50 staminate and 0.123±0.45 pistillate were recorded between April and June; 0.440±0.65 staminate and 0.221±0.03 pistillate between July and September, and 0.282±0.36 staminate and 0.105±0.78 pistillate between October and December (Figure 2).

No flowering was observed in *H. stipulacea* during the first three-months of culturing. Although there was insufficient flowering to determine inductive conditions with any accuracy, the recorded floral density was produced under day lengths of 12 hours, temperature of 24 to 28°C, salinity of 34 to 38% and pH range of 7 to 8. It seems likely that these conditions may represent the inductive ones, but a nutrient effect of the water column

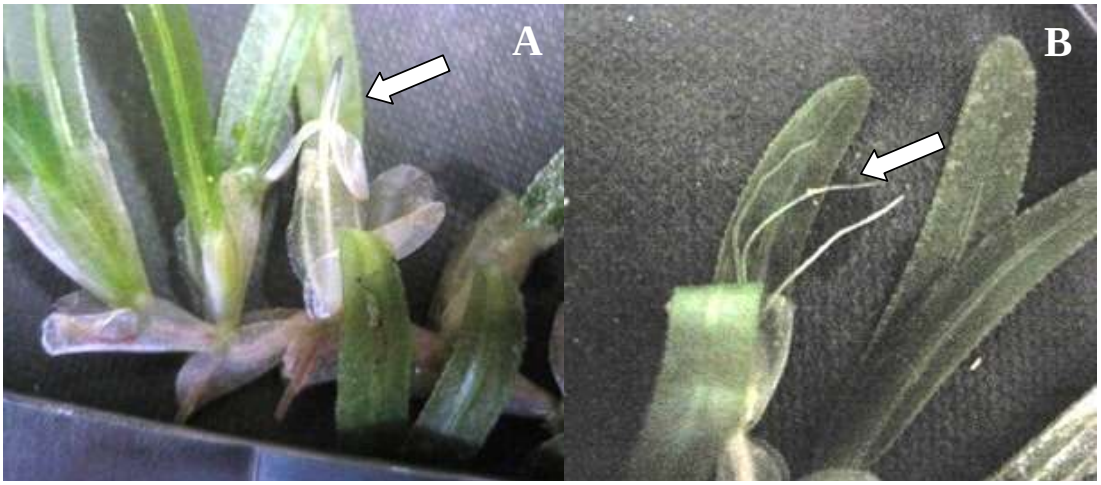

Figure 1. *Halophila stipulacea* flowers produced during experimental culture. (A) Staminate Flower (arrow), (B) Pistillate Flower (note the three pistils indicated by the arrow).

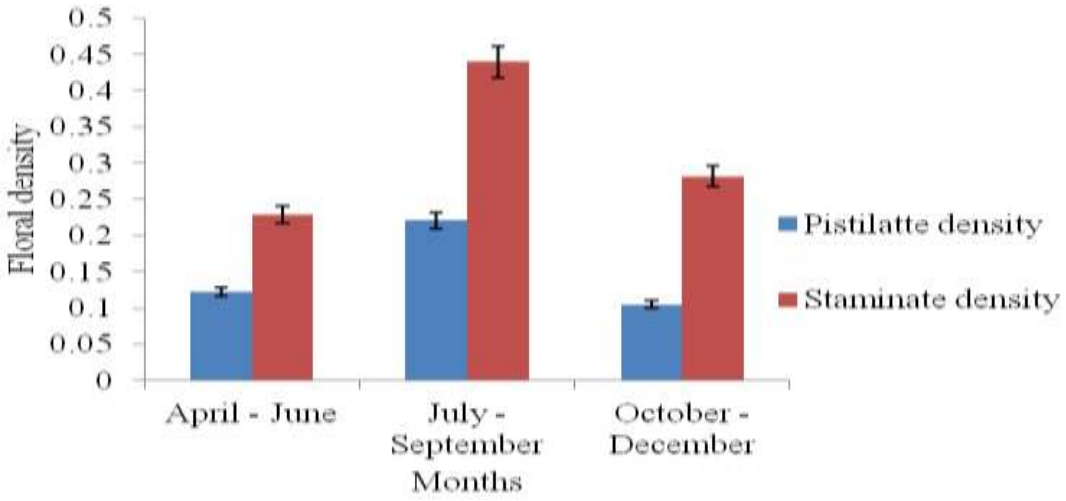

Figure 2. Floral density of *Halophila stipulacea* recorded throughout the experimental culture from January to December, 2013.

and/or sediments may also be involved.

Flowering in other seagrass species of the genus *Halophila* has primarily been a consequence of temperature. For example, in *Halophila engelmannii* Aschers., flowers were induced at 22 to 24°C under day lengths ranging from 14 to 24 h while *H. stipulacea* showed floral induction at 23.5 and 27.5°C (McMillan, 1976). However, in this study, *H. stipulacea* showed inhibition of flowering under 12 h day lengths at an inductive temperature. Plants that were kept at 28°C discontinued flower production after a brief flowering period and became vegetative, but plants that were at the slightly lower temperature, 25°C, continued to produce new flowering shoots during a nine-month observation period between April and December.

In the studies of *H. stipulacea*, the non-flowering materials under inductive temperature-salinity-pH-photoperiod conditions suggested that nutrient conditions may also play a role in flowering. Because flowering in laboratory culture has involved natural seawater and marine substrate planting medium, it may be possible to determine the role of nutrient conditions with more exactness.

This study allowed the identification of both male and female *H. stipulacea*. During field sampling of several seagrass species such as *H. ovalis*, often due to the short span of flowering in male flowers in particular and tiny flowers of female (Sidik et al., 2010), flowers and fruits are often overlooked. In culture systems, such as the one used in this study, it is easy to identify, separate

and follow the phenological development of both male and female plants. *H. stipulacea* produces flowers and propagates by vegetative and reproductive means.

Conclusion

A small-scale marine system had been successfully set up to simulate the natural environmental conditions of the habitat of *H. stipulacea.* The culture system established permitted observations on the phenological cycle. Future research could probably more critically identify the temperatures or salinities involved in flowering. The use of fixed temperatures, salinities and pH within the suspected inductive range, 24 to 28°C, 34 to 38‰ and 7 to 8, respectively, might permit the identification of possible optimal temperature, salinity and pH ranges for flowering. Because floral development may proceed slowly at the inductive temperature, salinity and pH, the use of a progressively increased temperature, salinity, and pH patterns might aid the determination of inductive differences. Phenophases should be studied for stages that precede the initial appearance of macroscopically visible floral buds. The results of the present investigation suggest that flowering in *H. stipulacea* is related primarily to temperature, salinity and pH; and that differences in flowering in responses to temperature, salinity or pH account for the nearly synchronous phenological timing in natural *H. stipulacea* meadows at different locations along tropical coast of East Africa. Our study is the first to document the presence of flowers in the tropical coast of Tanzania, Indo-Pacific through laboratory experiments. Future investigations should consider the pollination success and sexual reproduction (fruiting) of the species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Aquatic Sciences and Fisheries, University of Dar es Salaam for allowing them to conduct the experimental studies at Kunduchi Station. They wish to thank Mr. Ismail Abdallah who assisted in the field and laboratory work. They gratefully acknowledge the financial support of the Swedish International Development Agency Department for Research Cooperation (SIDA-SAREC) Tanzania Marine Bilateral Project, 2012.

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Vol. 11(7), pp. 298-307, July 2017
DOI: 10.5897/AJPS2017.1552
Article Number: C7E985664756
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

The suppression of *Arabidopsis* succinic semialdehyde dehydrogenase (SSADH) phenotype by using ethyl methane-sulfonate mutagenesis (EMS)

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Received 29 March, 2017; Accepted 12 May, 2017

The disruption of the succinic semialdehyde dehydrogenase (SSADH) function in Arabidopsis leads to aberrant growth phenotype. In human, the SSADH malfunction leads to a condition known as γ-hydroxybutyrate (GHB) aciduria. It is characterized by the accumulation of GHB as well as abnormal growth. GHB aciduria is treated pharmacologically with compounds analogous to gamma-aminubutyrate (GABA). One of these compounds called vagabatrin effectively suppressed the *ssadh* phenotype in Arabidopsis. Furthermore, the Arabidopsis *ssadh* phenotype has been suppressed genetically by simultaneous mutation in the *POP2* gene. In the present study, the presence of alternative solutions for suppression of the *ssadh* phenotype was investigated. For that, four ethyl methanesulfonate treated *ssadh* lines namely 7D, 13J, 17J and 21H were characterized phenotypically and chemotypically. Three- week-old plants of the suppressor lines grew much better than the parent *ssadh* line and resembled the wild type. The suppressor lines also accumulated reduced amounts of GABA and GHB. PCR based mapping using Sequence Characterized Amplified Region (SCAR) markers located the mutations to chromosome 2 and chromosome 5, suggesting the involvement of at least two mutations in suppression of the *ssadh* phenotype. Deep whole-genome sequencing of line 17J identified several mutations that induced amino acid change, frame shift and a stop codon. The findings showed that there are more possibilities to suppress the *ssadh* phenotype; furthermore, it provides an opportunity to identify genes that interact with the GABA shunt.

Key words: Gamma-aminubutyrate (GABA), shunt; *succinic semialdehyde dehydrogenase (SSADH)* mapping, *Arabidopsis*, succinic semialdehyde (SSA), γ-hydroxybutyrate (GHB).

INTRODUCTION

Succinic semialdehyde dehydrogenase (SSADH) is a Gamma-aminubutyrate (GABA)-shunt enzyme that catalyzes the oxidation of succinic semialdehyde (SSA) to succinate in the presence of NAD^+. *Arabidopsis* is an annual plant with short life cycle. In plant genetics study, *Arabidopsis* is often used as a model organism due to its small genome size and short seed to seed cycle. Its genome contains a single copy of SSADH encoding gene

(Busch and Fromm, 1999). Knockout of this single gene leads to an accumulation of reactive oxygen species (ROS), a stunted growth (dwarfism), a necrosis on leaves and hypersensitivity to environmental stresses (Fait et al., 2004; Bouche et al., 2003). Furthermore, this abnormal growth phenotype was associated with an accumulation of gamma hydroxybutyric acid (GHB) and hydrogen peroxide in tissues (Fait et al., 2004). In mammals, deficiency in SSADH activity causes a disorder called GHB aciduria, which is manifested by a high level of GHB in the physiological fluids (Jakobs et al., 1981; Hogema et al., 2001).

GHB aciduria in mammals is treated with several drugs. The most commonly used one is γ-vinyl-γ-aminobutyrate (Vagabatrin), a GABA analogue which binds to GABA transaminase irreversibly and prevents the degradation of GABA (Knerr et al., 2007). A similar rescue of the *ssadh* phenotype has been reported in *Arabidopsis*. *Arabidopsis ssadh* mutant treated with vagabatrin showed a reduced accumulation of ROS, reduced cell death and an improved growth compared to the *ssadh* mutant (Fait et al., 2004). In 2008, Ludewig et al. (2008) reported a complete suppression of the *ssadh* phenotype by simultaneous knockout of the *POP-2* gene, whose encoded protein functions upstream of the SSADH. This observation suggested that blocking the degradation of GABA is sufficient to suppress the *ssadh* phenotype.

Functional characterizations of genes are usually done using either forward or reverse genetics approaches. With the reverse genetics approach, one way of mutating genes is by insertion of T-DNA. However, this approach is random and lack specificity. Recent advancements in biotechnological tools allowed targeted edition of genes. CRISPR-Cas technology is one of those technologies that allow targeted modifications of genes (Eid et al., 2016; Schimel et al., 2014). Hyun et al. (2014) used CRISPR-Cas technology to mutagenize FLOWERING LOCUS T and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE genes. Site directed mutagenesis is another method that allows modification of specific amino acids thereby altering the functional properties of proteins. Majeed et al. (2015) and Fontenot et al. (2015) employed site directed mutagenesis to alter single amino acid in UGT76E1 and PhT1 proteins, respectively. The substitution of amino acid threonine (T) by alanine (A) at the position 134 of the UGT76E1 protein abolished the glycosyltransferase activity of the protein (Majeed et al., 2015). Furthermore, the replecement of tyrosine (Y) by Aspartic acid (D) at the 312 position of the PhT1 protein enhanced its Pi transport activity (Fontenot et al., 2015).

Forward genetics approach, on the other hand, involves the induction of random mutation, assessment of phenotypic changes and identification of the mutations responsible for phenotypic change. Several mutagens such as chemicals and rays are used to induce random mutation in the genome of an organism. Ethyl methanesulfonate (EMS) is one of those chemicals which are used as a mutagen in biological studies. EMS induces single nucleotide polymorphisms (SNPs) usually by substitution of bases (Kim et al., 2006). Therefore, EMS mutagenesis has been used widely in forward genetics to identify functions of genes. Previously, Ludewig et al. (2008) isolated two point mutations within the *pop2* ORF that suppressed the *ssadh* phenotype. In the same work, the authors reported the isolation of several EMS suppressor lines. Despite the improved phenotype of the EMS lines, the mode of suppression and genes involved in the process are unknown.

Here, it was hypothesized that mutations in genes other than the *POP2* are responsible for suppression of the *ssadh* phenotype. First, the phenotype and chemotype of four EMS suppressor lines was characterized and named hereafter as 7D, 13J, 17J and 21H. The growth of all four EMS suppressor lines is much better than the parent *ssadh* plant. The point mutations that suppressed the *ssadh* phenotype of line 17J were mapped to chromosome 2 and chromosome 5, confirming that mutations in genes other than in *POP2* were responsible for suppression of the *ssadh* phenotype. Deep sequencing of the entire genome of line 17J identified several candidate SNPs that led to amino acid substitution, frame shift and a premature stop codon. Our findings provide a basis for further studies to identify new GABA-shunt interacting genes.

METHODOLOGY

Generation of EMS-suppressor lines

The generation of the EMS suppressor lines was reported previously (Ludewig et al., 2008). Briefly, seeds of *ssadh-2* mutant were incubated in water containing 0.23% ethyl methanesulfonate (EMS) for 12 h at room temperature. Then, the seeds were washed several times with water and finally dried. The suppressor lines were isolated by visual observation after sowing the seeds on soil.

Phenotypic analysis

The phenotype lines 7D, 13J, 17J and 21H was compared to wild type and *ssadh* homozygous plants. For that, seeds of EMS suppressor lines were germinated and grown on soil. After four weeks of growth pictures were taken. Furthermore, the growth of EMS suppressor lines was analyzed on agar plate containing 0.3 mM SSA. For that, seeds of Wt, 7D, 13J, 17J, 21H and *pop2 x ssadh* mutant (*gs*) were germinated and grown on ½ MS plate without and with 0.3 mM SSA. Two weeks after germination the

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fresh shoot weight and root length of plantlets was determined.

Metabolite analysis

The chemotype of lines 7D, 13J, 17J and 21H was compared with the wild type and the *ssadh* mutant. For that, leaf material (100-200 mg) was collected from four-week-old wild type, *gad1/2-ssadh* and EMS suppressor lines, and was snap-frozen in liquid nitrogen. To get sufficient material from *ssadh* mutant, the entire shoots of six-week-old plants excluding flowers were used. Metabolite extraction and measurement was carried out as described previously (Renault et al., 2010; Mekonnen et al., 2016).

Isolation of mapping population

First, line 17J was crossed to *Landsberg erecta* (L*er*) wild type. F1 seeds were sown on soil and the resulting F1 plants were selfed. Next, F2 plants were screened for homozygousity of the T-DNA insertion in the *SSADH* gene by PCR and also for an improved growth visually. Those lines with homozygous *ssadh* mutation and improved growth were selected as mapping population. However, this procedure was very laborious, since only few plants tested belonged to the mapping population. Hence, preliminary selection was adopted on agar plates. For that, sterilized F2 seeds were germinated and grown on ½MS plates. A week after germination, only yellowish plantlets were transferred to fresh ½MS plates and after another week of growth the plantlets were transferred to soil. Approximately two weeks after transferring to soil, plants belonging to the mapping population were screened by PCR and visual observation.

Mapping of EMS-induced mutations

To determine the position of SNPs that suppressed the *ssadh* phenotype, genomic DNA was extracted from the mapping populations as described previously (Sholz et al., 2015). Markers discriminating between the Columbia (Col-0) and Landsberg erecta (L*er*) fragments were designed on all five chromosomes (Figure S1 and Table S1). PCR was performed on genomic DNA extracts of the mapping population. For genomic DNA extraction the procedure described by Scholz et al. (2016) was used. The abundance of Col-0 and L*er* fragments was counted as follows. A single band per lane corresponding to Col-0 or L*er* is counted as two and double bands per lane counted one for each genotype. The number of bands was summed up and the percent of Col fragment was calculated as shown by the following formula:

$$\%\, Col = no.of\ Col-0\ fragments \div total\ number\ of\ fragments \times 100$$

Genomic DNA extraction for whole genome sequencing

High quality genomic DNA was extracted from three-week-old 17J line germinated and grown on soil. For that ~100 mg of leaf material was collected from line 17J and immediately frozen in liquid nitrogen. The extraction of the genomic DNA was carried out according to the DNeasy Plant Mini Kit protocol (Cat. No. 69104). The concentration of the DNA was determined using a Nano Drop.

RESULTS AND DISCUSSION

The EMS suppressor lines grow better than the *ssadh* mutant

The growth of *Arabidopsis ssadh* mutants is severely inhibited (Ludewig et al., 2008; Fait et al., 2004). However, the simultaneous knockout of the *POP2* gene completely suppressed the *ssadh* phenotype (Ludwig et al., 2008). In the present study, the *ssadh* phenotype was suppressed by random mutations induced by EMS. The growth of EMS suppressor lines (7D, 13J, 17J and 21H) was much better than the *ssadh* mutant (Figure 1A). Three-week-old plants of line 17J and 21H resemble the wild type and *pop2 x ssadh* mutant, confirming the suppression of the *ssadh* phenotype (Figure 1A). To ensure that the T-DNA insertion in the *SSADH* gene was stable, the EMS suppressor lines were genotyped using gene- and T-DNA-specific primer combinations. Indeed, all lines were homozygous for T-DNA insertion; since, the PCR using gene specific forward and reverse primers did not yield a product (Figure 1B).

Furthermore, if EMS suppressor lines would be sensitive to SSA treatments differently compared to the controls was tested. Similar to soil grown plants, there was no difference in shoot growth between the EMS suppressor lines and the controls (Wt and *gs*) on ½ MS plates (Figure 2A). However, a shorter root was measured in all EMS lines when compared to the wild type on ½ MS plates (Figure 2B). This might be due to the accumulation of GABA after the disruption of the SSADH function. The accumulation of GABA in SSADH deficient Arabidopsis plants has previously been reported (Ludwig et al., 2008; Fait et al., 2004). A high GABA level is associated with reduced root growth. Ramesh et al. (2015) showed a reduced growth of wheat roots after 10 mM exogenous GABA application. Treatment of 0.3 mM SSA reduced the growth of all lines including the wild type (Figure 2C), an observation in line with the previous report (Mekonnen and Ludwig, 2016). The suppression of the *ssadh* phenotype by EMS induced mutation could be in two ways; first by preventing the production of the toxic intermediates; second, by promoting the degradation of the toxic intermediate(s). The phenotype of EMS lines following SSA treatment probably suggests the first mode of suppression of the *ssadh* phenotype. The phenotypic difference among EMS lines might indicate the presence of alternative ways to rescue the *ssadh* phenotype.

EMS induced mutations reduced the GABA and GHB levels

Some of the features of the *ssadh* chemotype include the accumulation of high GABA and GHB in tissues (Ludwig et al., 2008). In the present report, the abundance of GHB and its correlation with the suppression an *ssadh* phenotype was investigated. Interestingly, the GHB content was reduced by more than 30-fold in shoots of EMS suppressor lines compared to the *ssadh* mutant (Figure 3B). Similarly, the GABA content in EMS suppressor lines was reduced by more than 50% compared to the *ssadh* mutant (Figure 3A).The reduction in levels of GABA and GHB suggests that prevention of

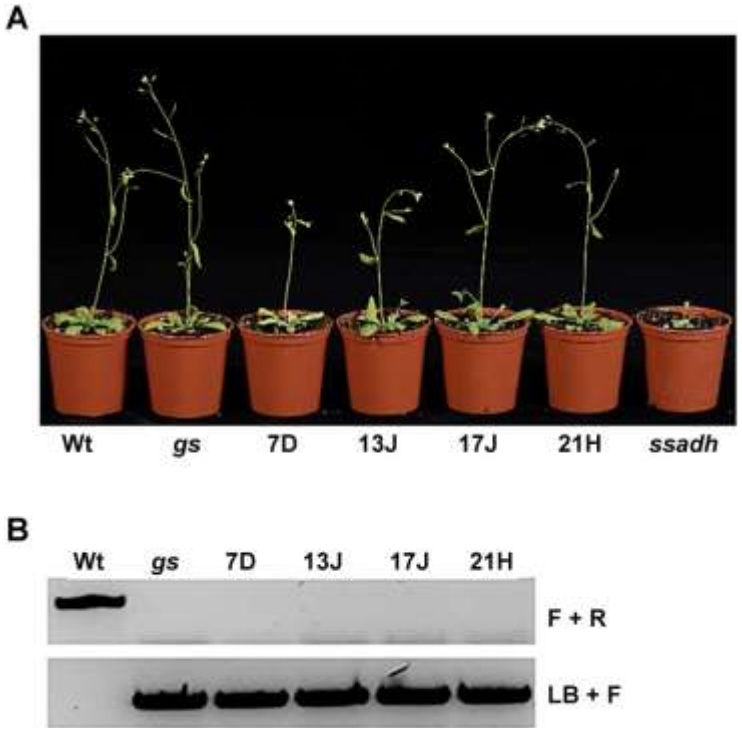

Figure 1. Phenotype of Wt, *pop2 x ssadh, ssadh* and four EMS lines (A), and genotyping of EMS lines (B); seeds of Wt, *pop2 x ssadh* and four EMS lines (7D, 13J, 17J and 21H) were germinated and grown on soil; pictures were taken from three-week-old plants; phenotype are representatives of at least 10 plants of similar phenotype; screening of EMS suppressor lines for T-DNA insertion by using gene specific primers (F + R) and a combination of gene specific and T-DNA specific primers (LB); *gs*-represents the *pop2 x ssadh* double mutant.

toxic intermediates production was probably the mode of *ssadh* phenotype suppression. Intriguingly, lines 7D and 13J exhibited a relatively reduced shoot growth phenotype compared to 17J and 21H (Figure 1A) despite accumulating several folds less GHB in the tissue, suggesting that the severity of the *ssadh* phenotype is not correlated to the GHB level. Akaboshi et al. (2003) reported the absence of correlation between GHB levels and clinical features in mammals. Our recent report also showed that GHB induced damage in Arabidopsis is modest and zero in yeast at high concentrations (Mekonnen and Ludwig et al., 2016). This observation adds further evidence to a previous report that GHB is not the main cause for *ssadh* phenotype.

SNPs in at least two genes suppressed the *ssadh* phenotype

The PCR based mapping system using Sequence Characterized Amplified Region (SCAR) markers has been used widely to locate SNP's. The presence of natural polymorphisms between two Arabidopsis ecotypes (Col and *Ler*) lays a good platform for designing SCAR markers. For example, Konieczny and Ausubel (1993) identified an average of one nucleotide change in 261 base pairs between Col and *Ler* ecotypes that comprised recognition cites for *CAPS* marker. Based on the nucleotide polymorphisms between these two ecotypes, several SCAR markers were designed spanning the shorter and longer arms of all five chromosomes (Figure 4 and Table S1). Using a combination of PCR genotyping and visual analysis, more than 90 lines belonging to the mapping populations was isolated for line 17J. Interestingly, PCR analysis using markers 1.8, 2.6 and 5.9 identified three regions on chromosome1, 2 and 5, respectively, which is rich in Col-0 fragments (Table 1A). The lower arm of chromosome 1 is a position where the *ssadh* T-DNA insertion was located. Therefore, it was not surprising to see dominance of Col-0 fragments. To confirm the position of the SNPs, additional PCRs was performed by using more lines from the mapping population and found that the mutations were, indeed, in chromosome 2 and 5 (Table 1B). Those findings show that the *ssadh* phenotype was suppressed by at least two mutations in two different

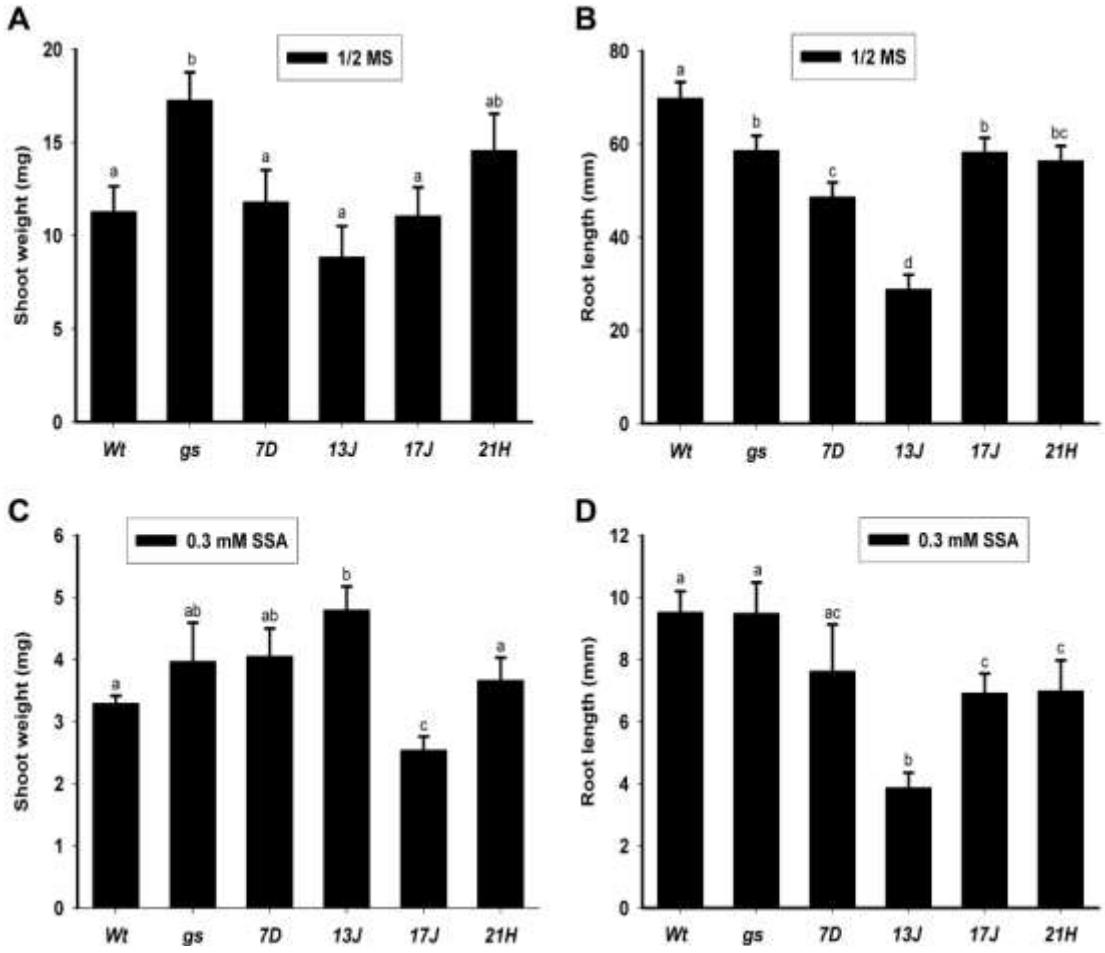

Figure 2. Phenotypic characterization of Wt, *pop2 x ssadh* and four EMS suppressor lines (7D, 13J, 17J and 21H); seeds of Wt and mutant lines were germinated and grown on ½ MS plate without (A&B) and with 0.3 mM SSA (C&D); shoot fresh weight (A&C) and root length (B&D) were measured after two weeks of growth; bars represent the satandard error of means; different letters indicate statstical significances after students *t*-test; *gs*- represent the *pop2 x ssadh* mutants.

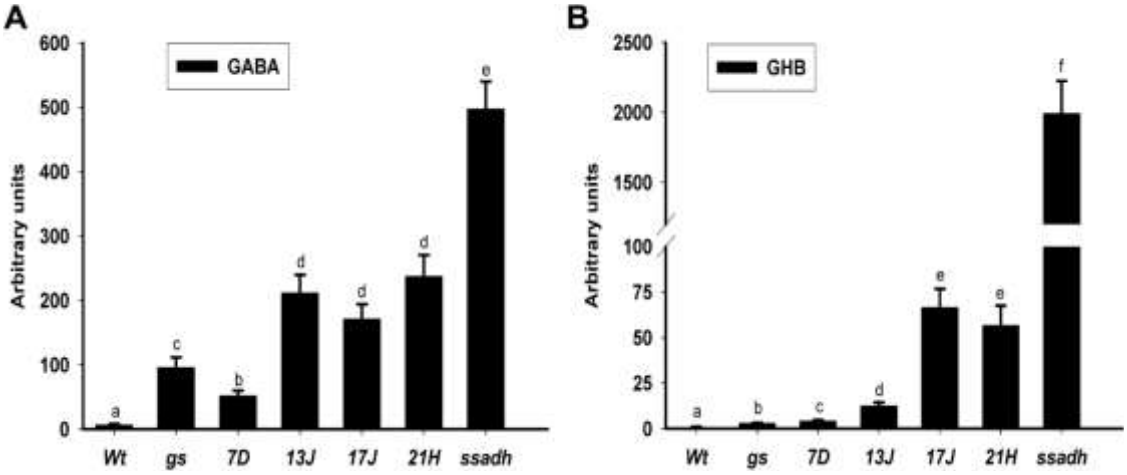

Figure 3. Analysis of GABA (A) and GHB (B) in four-week-old wild type, *pop2 x ssadh (gs),* four EMS lines (7D, 13J, 17J and 21H) and six-week-old *ssadh* plants values are means of al least five independent plants; bars represent the standard error of means; different letters indicate statstical significances after students *t*-test; *gs*- represent the *pop2 x ssadh* mutants.

Table 1. Preliminary (A) and detailed (B) analysis of EMS induced SNPs in line 17J; to determine the position of SNPs, primers that span all five chromosomes were used (A); to verify the positions additional plants and primers were used (B).

Chromosomes	Marker name	% Col	No. of independent lines
A. Rough SNP mapping			
1	1.1	38.8	20
	1.8	88.8	20
2	2.1	47.2	20
	2.6	94.1	20
	2.7	33.0	20
3	3.1	41.6	20
	3.7	52.7	20
4	4.2	22.2	20
	4.7	44.4	20
5	5.3	66.6	20
	5.9	100.0	20
B. Detailed SNP mapping			
2	2.6	95.2	72
5	5.8	89.5	72
	5.9	91.5	72
	VG	95.0	72

chromosomes. Despite the absence of a segregation analysis data, the proportion of plants belonging to the mapping population in the F2 generation was close to 1/16.

Deep sequencing of line 17J genome uncovered several candidate mutations

The introduction and accessibility of full genome sequencing facilities greatly eased the identification of SNP's. The whole genome sequencing of line 17J identified hundreds of point mutations compared to the reference allele. To determine the regions of interest on chromosome 2 and 5, the following calculations were made. Markers 2.6 and VG on chromosome 2 and 5, respectively, yielded a 95% Col-0 fragment and 5% *Ler* fragment (Table 1B). Centimorgan (cM) is a unit of measuring the linkage between genes on the same chromosome. The 5% *Ler* fragments in chromosomes 2 and 5 (Table 1B) indicate that five crossing over has occurred between the markers and EMS induced SNP's. From the above definition, it can be deduced that the markers and EMS induced SNPs are 5 cM apart. Although the conversion of 1 cM to base pair depends on the position on the chromosome, the average number was calculated to be 208950 and 203800 base pairs in chromosome 2 is 5, respectively (http://www.arabidopsis.org/ servlets/mapper). The region of interest was obtained by multiplying the estimated base pairs with 5. To ensure the selection of the right SNPs, a 5% cut off value was set for the ratio of reference calls to alternative calls, that is, any ratio value higher than 0.05 was discarded.

Sequence analysis revealed hundreds of SNPs within the desired region of both chromosomes. However, only 47 and 22 SNPs were true in chromosome 2 and 5, respectively, since more than 95% of the calls favored against the reference allele. The SNPs were located within the exon, intron, 3' UTR or 5' UTR of the gene. Among all SNPs, 14 and 10 point mutations in chromosome 2 and 5, respectively, were located within the exon of genes (Table 2). The point mutations induced amino acid changes, premature stop codon and a frame shift (Table 2). Furthermore, there were some notable changes in terms of the amino acid polarity and charge in both chromosomes. For example, the substitution of Gly by Asp, Arg by Glu, Gly by Arg, and Gly by Glu were the notable ones in genes of both chromosomes (Table 2). Such substitution could change the function of the protein. Single amino acid substitutions have previously been shown to alter the functional property of proteins. Lee et al. (2015) reported the enhanced peroxidase and chaperone activity of 2-Cys Peroxiredoxin (2-Cys Prx A) protein following the substation of Cys150 for Ser150. Similarly, the replacement of Thr134 by Ala134 of Arabidopsis UGT76E1 protein abolished its glycosyltransferase activity (Majeed et al., 2015). Such functional alteration of proteins will likely have consequences on the phenotype (Ng and Henikoff,

Table 2. SNPs identified in exon of genes in chromosome 2 (A) and chromosome 5 (B) of line 17J.

S/N	Gene ID	Reference/Alternative alleles	Amino acid change	Annotation
		A. Chromosome 2		
1	At2g25340	0.050	Ser to Asn	Vesicle associated membrane proteins
2	At2g25640	0.000	frame shift	Transcription elongation factor S-II protein
3	At2g25660	0.032	Arg to Glu	Embryo defective 2410
4	At2g25790	0.045	Pro to Leu	Protein serine/threonine kinase activity
5	At2g28930	0.033	Pro to Ser	Product protein kinase 1B
6	At2g29065	0.000	Leu to Phen	GRAS family transcription factor
7	At2g29660	0.026	Asp to Asp	ZINC finger (C2H2 type) family protein
8	At2g30300	0.023	Ser to Ser	Major facilitator superfamily protein
9	At2g30500	0.030	Ala to Threo	Kinase interacting-like proteins
10	At2g31080	0.000	frame shift	Non-LTR retrotransposone family
11	At2g31370	0.000	Pro to Ser	bZIP transcription factor family protein
12	At2g31560	0.050	Gln to stop	Protein of unknown function
13	At2g32310	0.016	Phe to Phe	CCT motif family protein
14	At2g32730	0.022	Leu to Phen	26S proteasome regulatory complex, non-ATPase sub complex, Rpn2/Psmd1 subunit
		B. Chromosome 5		
1	At5g51340	0.00	Ileu to Ileu	Tetratricopeptide repeat like super family protein
2	At5g51630	0.04	Asp to Asp	Disease resistance protein family
3	At5g51660	0.04	Pro to Leu	Cleavage and polyadenylation specificity factor
4	At5g52115	0.00	Frame shift	CDS ribonuclease H superfamily
5	At5g52400	0.00	Pro to Ser	Member of CYP715A
6	At5g53150	0.03	Gly to Arg	DNAJ heat shock protein
7	At5g53660	0.05	Glu to Glu	Growth Regulating Factor 7
9	At5g53850	0.04	Gln to His	Dehydratase enolase phosphatase complex 1
10	At5g53970	0.01	Gly to Glu	Tyrosine aminotransferase 7

2006). The suppression of the *ssadh* phenotype was, indeed, the manifestation of the amino acid change. To our knowledge no report has been published indicating interaction between these candidate genes and the GABA shunt.

Conclusion

The only known way of suppressing of the *ssadh* phenotype in *Arabidopsis* is through mutations in *POP2* gene. The finding here provided potential candidate genes that have never been reported to interact with the GABA shunt before. The candidate genes can be used to study their role in suppression of *ssadh* phenotype as well as their mode of interaction with the GABA shunt. Considering the diverse roles of the GABA shunt in plant growth and development, there is a promise of identifying new enzymatic pathways. Furthermore, in humans the disorder resulting from *ssadh* malfunction is treated only with chemical drugs (GABA-analogues) that competetively inhibit the function of GABA-T (*POP2*). The identification of additional pathways that suppress the *ssadh* phenotype is helpful; since, the information could be used as an input for the discovery of drugs in GHB aciduria treatment of mammals. Taken together, the result of the study lays a good basis to identify new genes and possibly pathways that interact with the GABA shunt.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors are very grateful for IGSDHD program for supporting this work financially.

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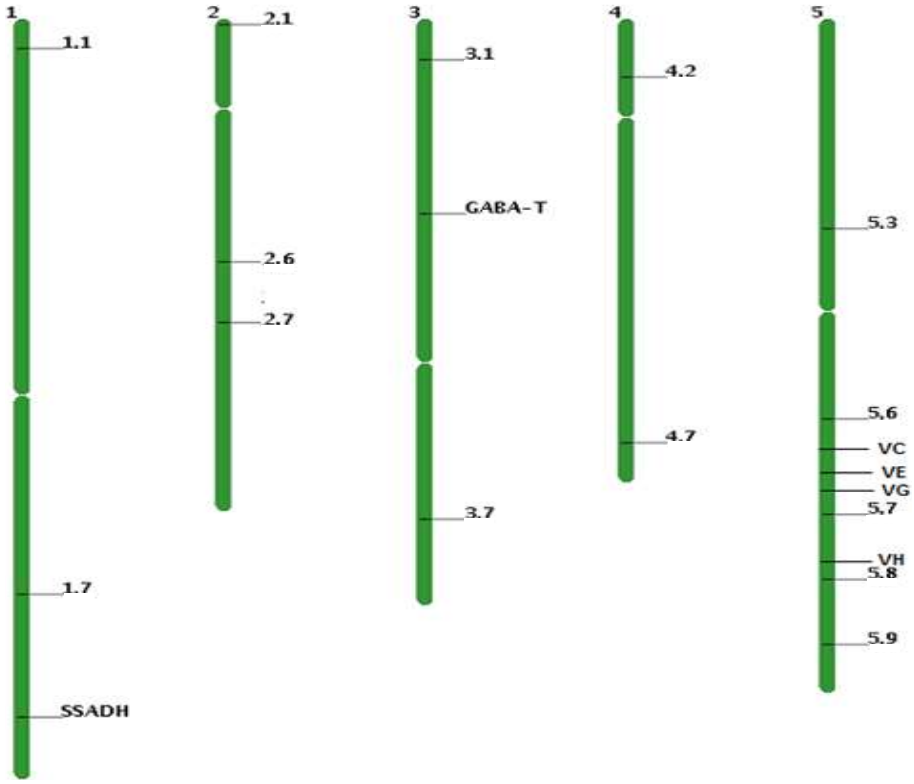

Figure S1. Schematic representation of the position of SCAR markers on Arabidopsis chromosomes.

Table S1. List of SCAR markers used in mapping of the EMS induced SNPs.

1.1-F	AACATCCAACGGTCTGAACC	Mapping
1.1-R	CGTGTCCGGTGAAAAAGAGT	Mapping
1.7-F	AAAACCCTGTTGTTTCTGAGC	Mapping
1.7-R	GGTTGAGACTGGTAACAAGG	Mapping
2.1-F	CCCATGCTTCCTCCTATTGC	Mapping
2.1-R	GGAGGCTCTTGAACTCACAC	Mapping
2.6-F	TGTCACTGAAGAACCCTAGC	Mapping
2.6-R	GCAGCTTCGAGTGGATTCTA	Mapping
2.7-F	CTTCTTTCAAGGATCTCTTGC	Mapping
2.7-R	CCATGAATTCACCTCTCTATTC	Mapping
3.1-F	CACATTTTCAGTTATCTTAATGC	Mapping
3.1-R	GGAATGAGACATTGACTTC	Mapping
3.7-F	TGGGAACAAAGGTGTCATCC	Mapping
3.7-R	GCAAGTTAAAACCTGAAACTAAG	Mapping
4.2-F	GCTTTTAACCAGCTAACTTAGG	Mapping
4.2-R	GGTCTCTCACCTAAGGAGAT	Mapping
4.7-F	CCAATGATTGGTCACTACTGC	Mapping
4.7-R	GGTCATCAATTCATTTCTTAAGC	Mapping
5.3-F	CAGCTGCCTTCAAGTATTCC	Mapping
5.3-R	GCTGTGTTTTTGTAGAGAGCG	Mapping
5.6-F	CGTCAAAGACGACACATGG	Mapping
5.6-R	GCTCATGCTTCCTCCCATTG	Mapping
5.7-F	ATAAGATAGGTTTGGCAAATGG	Mapping
5.7-R	CCTACTATTCAAATTGTTTAAGAA	Mapping
5.8-F	CAAAAATTGATCGATCGATAGG	Mapping
5.8-R	CGTTATTGAGTCCGGTTGAG	Mapping
5.9-F	CTCTCGCTAACGCTCTTTGG	Mapping
5.9-R	GCACGAGTTAACGTTATTGAG	Mapping
VC-F	TCGGAAAAAGTATGTTGGGAGT	Mapping
VC-R	TGTTCAACAATAGCTGCCAAA	Mapping
VE-F	CCGATATAACTAAAGGTGCAGAGA	Mapping
VE-R	AAAATTCCTACCAAACGAAGCA	Mapping
VG-F	GATTTCGCTCTCTGCCAAAA	Mapping
VG-R	AAAAATGACGGGACGAAAGT	Mapping
VH-F	TCACCTTACTTAATTCAACTGCAAA	Mapping
VH-R	CCAGATTCGATGTACTTCACTTTC	Mapping

academicJournals

Vol. 11(7), pp. 308-319, July 2017
DOI: 10.5897/AJPS2017.1538
Article Number: AD5C78664758
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

GGE biplot analysis of genotypes by environment interaction on *Sorghum bicolor* L. (Moench) in Zimbabwe

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Received 28 February, 2017; Accepted 21 April, 2017

The genotype by environment interaction (GEI) reduces the success of genotype selection and recommendations by breeders, thus slowing down the progress of plant breeding. The understanding of genotype by environment interaction (GEI) multi-locational yield trials (MLYT) enables researchers to identify locations which are efficient in distinguishing tested genotypes, which are ideal across the test-locations as well as environments which are good representatives of the target regions of interest. The main objective of the study was to assess the genotype by environment interaction on grain yield stability of promising sorghum genotypes across five diverse environments of Zimbabwe. Sorghum yield data of twenty-seven cultivars was obtained from the replicated trials. After performing a pooled analysis of variance for grain yield across five diverse environments during the 2013/14 rainy season, the GxE interaction was significant (P<0.001), and this justified need for testing for GEI components using the GGE biplot analysis to enhance the understanding the effects of components. The results revealed that three mega-environments were identifiable which are Matopos, Save-Valley and Kadoma falling in one mega-environment, then Makoholi was identified as a second mega-environment and then Gwebi was identified as the third mega-environment. Gwebi had the most discriminating ability and good representativeness whereby Save Valley had a poor discriminating ability as well as least representativeness.

Key words: Sorghum, genotype x environment interaction, GGE, adaptation and yield stability, mega-environment, discriminating ability, representativeness.

INTRODUCTION

Sorghum bicolor L. (Moench) is an important cereal crop which is ranked 5th in the world based on its use and production after maize, wheat, rice and pearl millet. The crop is predominantly grown in dry and hot regions due to its tolerance to drought. Sorghum is versatile and can be grown as grain, forage and sweet crop and it thrives well under temperatures and humidity which are as high as 40 to 43°C and 15 to 30%, respectively as long as soil moisture is available. The crop carries natural characteristics which make it adaptable to drought

conditions. Sorghum characteristics such as dense and deep roots, ability to reduce transpiration through leaf rolling and stomatal closure among others make the crop able to survive dry periods. Hence sorghum has become a strategic crop in Zimbabwe's driest regions in the face of climate variability. Despite all the crop's advantages over other cereals under dry condition, the crop production is still very low and very low yields are being obtained. Research through the national breeding programmes has been done for years but with little progress due to limited knowledge on the relationship and effects of genotype and environment and their interaction on the crop yield performance.

It is important to show the relationship between genotypes and environments for selected traits graphically by use of a genotype by genotype by environment (GGE) biplot that allows visual assessment of genotype by environment interaction (GEI) pattern of multi-locational or multi-environment data (Yan et al., 2000; Yan and Hunt, 2001). GGE is the most recent approach for analysis of GEI and increasingly being used in GEI studies in plant breeding research (Butran et al., 2004). The model was proposed by Yan et al. (2000), and has shown extensive usefulness and a more comprehensive tool in quantitative genetics and plant breeding (Yan et al., 2001; Yan and Rajcan, 2002). The model covers very critical areas in the study of stability of multi-locational trials, like the which-won-where pattern, mean performance and stability of genotypes, discriminating ability, mega-environment investigation, and representativeness of environments.

The GGE method emphasizes on two concepts, whereby in the first concept, it clearly points out that even though the measured yield is a result of combination effect by Genotype (G), Environment (E) and genotype x environment interaction (GEI), only G and GEI are relevant and must be considered simultaneously when evaluating genotypes, thus the name GGE. The second concept is based on the biplot technique which was developed by Gabriel (1972) which is used to estimate and show the GGE of MEYT, hence the name GGE biplot. The GGE biplot is made by the first two principal components (PC), PC1 and PC2 also known as the primary and secondary effects, respectively. This is derived from subjecting the environment centred yield data (due to GGE) to singular value decomposition.

This now makes it very easy for one to see which genotype won in which environments, thus facilitating mega-environment (ME) identification (Yan et al., 2000; Yan, 2001). This is facilitated in the form of a polygon to visualize the interaction patterns between genotypes and environments (Yan and Kang, 2003), whereby furthest genotypes are connected from the biplot origin such that all genotypes are contained in the polygon (Kaya et al., 2006). Some genotypes will be located on the vertices of the polygon and they are either the best or the poorest in one or more environments (Yan et al., 2000; Yan and Rajcan, 2002; Yan and Tinker, 2006). The rays are drawn perpendicular to the sides of the polygon dividing it into sectors, such that the vertex genotypes in each sector is also the best genotype for sites whose markers fall into respective sector so that sites within the same sector share the same winning genotype (Yan, 2002; Yan et al., 2000). GGE biplot is a visual display of the G + GE of multi-environmental data where groups of locations with similar cultivar responses are presented and it identifies the highest yielding varieties for each group. PC1 tend to correlate highly with the genotype means, the ideal cultivar is the one which possess large scores for PC1, thus indicating high average yield and small PC2 scores indicating less GEI and greater stability.

The study was however done to analyse the multi-locational yield data from across five diverse locations. The objectives of this study were to (i) to identify the genotype and environmental components that are associated with the GxE interaction across the diverse environments so as to aid better management of sorghum crop in Zimbabwe, (ii) to measure the correlation among the five test locations, (iii) to determine whether the test-locations belong to a single mega-environment or not and (iv) to rank locations based on discriminating ability and representativeness by using the genotype, genotype by environment interaction (GGE biplot analysis).

MATERIALS AND METHODS

Study sites

The multi-locational yield trials (MLYT) were carried out at five different locations in Zimbabwe, Matopos Research Station, Makoholi, Gwebi, Save Valley and Kadoma to assess and confirm the effects of genotype, environment and genotype by environment interaction. The locations have different agro-climatic conditions with Gwebi representing the high-potential area with good rains and soils, Kadoma representing the intermediate potential area with average rainfall, Makoholi, Save Valley and Matopos representing the low potential area with erratic and low rainfall (Table 1). According to the 2013/14 season weather data collected at study sites, the low potential areas had an average of 300 mm annual rainfall and temperatures were 34°C, whilst the high potential areas received an average of 550 mm and temperatures of 29°C. Generally, such rainfall averages depict poor season as compared to good seasons where low potential areas and high potential areas receive an average of 450 and 800 mm, respectively. The sites are also characterised by different soil types, which range from the Red-clay soil at Gwebi, Black sandy-loam soils at Kadoma and Black clay at Matopos and Sandy soils at Makoholi.

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Table 1. Description of sites used in the multi-locational trials.

Code	Location	Altitude (m)	Longitude/latitude	Natural region
E1	Gwebi	1448	30°32E/17°41S	IIb
E2	Kadoma	1149	29°53E/18°19S	III
E3	Makoholi	1204	38047'E/19050'S	IV
E4	Matopos	1328	28028'E/20024'S	IV
E5	Save Valley	450	30°E/20°48S	V

Table 2. Description of sorghum genotypes used in the multi-locational trials.

Variety/line code	Code	Type and breeding status	Origin
NL9411	(G1)	Grain/Advanced	CBI-Zimbabwe
NL9803	(G2)	Grain/Advanced/CBI	CBI-Zimbabwe
NL9923	(G3)	Grain/Advanced/CBI	CBI-Zimbabwe
NL9907	(G4)	Grain/Advanced/CBI	CBI-Zimbabwe
NL9921	(G5)	Grain/Advanced/CBI	CBI-Zimbabwe
NL2014	(G6)	Grain/Advanced/CBI	CBI-Zimbabwe
ICSV93046	(G7)	Dual/Advanced/ICRISAT	ICRISAT-India
NL2015	(G8)	Grain/Advanced/CBI	CBI-Zimbabwe
NL2020	(G9)	Grain/Advanced/CBI	CBI-Zimbabwe
NL2009	(G10)	Grain/Advanced/CBI	CBI-Zimbabwe
SV4	(G11)	Grain/Released/CBI	CBI-Zimbabwe
SV2	(G12)	Grain/Released/CBI	CBI-Zimbabwe
MACIA	(G13)	Grain/Released/ICRISAT	ICRISAT-India
NL2012	(G14)	Grain/Advanced/CBI	CBI-Zimbabwe
NL9412	(G15)	Grain/Advanced/CBI	CBI-Zimbabwe
SDS6013	(G16)	Dual/Advanced/ICRISAT	ICRISAT-India
ICSR93034	(G17)	Sweet sorghum/Advanced/ICRISAT	ICRISAT-India
S35	(G18)	Sweet sorghum/Advanced/ICRISAT	ICRISAT-India
SPV1022	(G19)	Dual/Advanced/ICRISAT	ICRISAT-India
CSV15	(G20)	Dual/Advanced/ICRISAT	ICRISAT-India
NTJ2	(G21)	Sweet sorghum/Advanced/ICRISAT	ICRISAT-India
SPV422	(G22)	Sweet sorghum/Advanced/ICRISAT	ICRISAT-India
E36-1	(G23)	Dual/Advanced/ICRISAT	ICRISAT-India
PVK801	(G24)	Dual/Advanced/ICRISAT	ICRISAT-India
JJ1041	(G25)	Dual/Advanced/ICRISAT	ICRISAT-India
SEREDO	(G26)	Dual/Advanced/ICRISAT	ICRISAT-India
MATEBELE	(G27)	Sweet sorghum/Landrace	Farmers-Zimbabwe

Experiment design and measurements

Twenty-seven genotypes of sorghum (*Sorghum bicolor*) were evaluated during the 2013/14 season in five diverse locations across Zimbabwe. The twenty-seven genotypes included three Zimbabwean released varieties, one farmer variety and twenty-three advanced lines (Table 2).

The trials were planted in a two-factor randomised complete block design (RCBD) replicated three times. Each plot comprised of 4 rows which are 5 m long with inter-row and intra-row spacing of 75 and 20 cm, respectively. Basal fertilizer of compound D with ratio nitrogen : phosphorus : potassium (N:P:K) of 7:14:7 was applied at planting at a rate of 200 kg/ha. The trials were raised under rainfed across all the sites. Topdressing with ammonium nitrate (34.5% N) was applied at a rate of 150 kg/ha after six weeks from germination. Weeding was done using hoes at all trial locations. The data considered for analysis was from the candidates of the net plot, thus the two centre rows. The harvested panicles were sundried for two days before being tested for moisture content where 12% was the preferred average content. Grain yield data was then obtained by weighing the dried grain using a digital scale. The data was then statistically analysed by analysis of variance (ANOVA) using GenStat version14 statistical package. The presence of significant genotype by environment interaction GEI ($P<0.001$) justified further testing for GEI components using the GGE biplot analysis to enhance the understanding the effects and components. The further partitioning of variance components was computed using the GGE model (Yan, 2001). The first two principal components (PC1 and PC2) derived from environment centred yield data were used to construct the GGE biplot (Yan et al., 2000). That enabled selection

Table 3. Summary of the general analysis of variance for grain yield (kg/ha) showing the level of significance for the genotype, environment and GEI of 27 sorghum varieties grown at 5 environments of Zimbabwe during 2013/14 season.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	Exp % ss
REP stratum	2	$1.645x10^7$	$8.224 x10^6$	2.74		
Genotype (G)	26	$1.841 x10^8$	$7.081 x10^6$	2.36	<0.001	15.2
Environment (E)	4	$5.049 x10^8$	$1.262 x10^8$	41.99	<0.001	41.8
Genotype x Environment	104	$5.190 x10^8$	$4.990 x10^6$	1.66	<0.001	43
Residual	268	$8.056 x10^8$	$3.006 x10^6$			
Total	404	$2.030 x10^9$				

*** DF= Degrees of freedom; SS= sums of square; MS= means square.

of best environments to be regarded and used as test-locations and genotypes which are high yielding and widely adapted. The GGE biplot was generated from the environment-centred yield data following the method described by Yan et al. (2001, 2007). This method enabled to determine the genotype with yields above average for a specific environment on "which-won-where "and the most discriminating environments as well as with good responsiveness as well as the correlation between environments. The best genotypes were also selected such that if the angle between the genotype and environment is less than 90°, it shows that the genotype performed above average on that particular environment, and angle above 90° depicts below average performance whilst that with equal to 90° is near average performance. In this study, comparison between two genotypes was done by connecting the two with a straight line, and followed by a perpendicular line that passes through the biplot origin (the equality line of the two genotypes).

RESULTS

Combined analysis of variance

A general combined analysis of variance (ANOVA) was performed and the results revealed that variances due to genotypes, environments and GxE interaction were highly significant (P<0.001) (Table 3). Environment mean yield ranged from 1752.26 kg/ha at E3 (Makoholi) to 4940.26 kg/kg at E1 (Gwebi) (Table 4). Genotype mean yield ranged from 1977.4 (G11) to 4706 kg/ha (G1) (Table 4). The GxE interaction component explained 43% of the total sum of squares and this indicates the need for further analysis for stability and adaptability to recommend genotypes which are high yielding and stable as well as genotypes which are promising and adaptable to an environment.

Discriminating ability, environmental correlation and genotype performance per environment (GGE biplot analysis)

The similarity (covariance) between two environments is determined by both the length of their vectors and the cosine of the angle between them (Figure 1). Location E1 had good discriminating ability as shown by a long environmental vector, followed by E3 and then E2 (KADOMA) and E4 (MATOPOS). However, E5 (Save-Valley) had poor discriminating ability, as was indicated by its short environmental vector. The study shows that E1 (GWEBI), E2 (KADOMA) and E4 (MATOPOS) were the most discriminating locations which means such sites gave more information on the performance of the varieties, while E5 (Save Valley) was the least discriminating environment. This means if the study is carried out for several seasons and same sites continue to be non-discriminating (less informative); it means the locations can be dropped and not to be used as test locations.

Information on relationships among the test environments was also given (Figure 1) as is indicated by the cosine of the angles; acute angle indicates a positive correlation, right angle and obtuse angles indicate no correlation and negative correlation, respectively. Angles between any of the three environments; E4 (Matopos), E2 (Kadoma) and E5 (Save Valley) were acute and hence showed positive correlations and the same environments E4 (Matopos), E2 (Kadoma) and E5 (Save Valley) had negative correlation with environments E1 (Gwebi) and E3 (Makoholi).

A wide negative correlation between three environments (E4 (Matopos), E2 (Kadoma) and E5 (Save Valley) and two environments E1 (Gwebi) and E3 (Makoholi) indicated a crossover GE interaction; thus, the changes in ranking order form one environment to another. Such close associations among most test environments suggests that same information in terms of performance can be obtained from fewer test locations and some may be dropped without losing any information about the cultivars under test, thus reducing experimental costs (Yan and Tinker, 2005).

The results from the study shows that genotypes G22, G12, G18, G26, G6, G19 and G27 performed above average in environments E4, E2 and E5 but below average in E3. However, G1, G2, G14, G24 and G8 performed below average in E4, E2 and E5 whilst G11 and G16 were near average in the same environments. Genotypes G14, G3 G6 and G5 performed above average in E3 whilst G13, G14, G21, and G25 performed

Table 4. Genotype mean and environment mean for 27 sorghum advanced genotypes yield performance evaluated across 5 environments.

Genotype	Environment	E1	E2	E3	E4	E5	Mean
G1		8327	3833	4963	3177	3230	4706
G10		6396	4072	845	3651	2316	3456
G11		2826	3127	-80	2493	1521	1977.4
G12		1911	5523	-103	4851	3393	3115
G13		7506	3741	512	3436	1905	3420
G14		6853	1737	5128	917	1760	3279
G15		2689	3729	2649	2835	2785	2937.4
G16		521	4214	1489	3262	2936	2484.4
G17		3867	2578	975	1921	1353	2138.8
G18		5586	3433	230	2996	1693	2787.6
G19		3435	3621	765	2963	2111	2579
G2		3644	2894	1124	2209	1650	2304.2
G20		5623	3526	1176	3004	2038	3073.4
G21		7239	3522	2887	2969	2446	3812.6
G22		5112	3935	638	3428	2221	3066.8
G23		7600	4958	2342	4505	3363	4553.6
G24		4208	2283	1540	1596	1274	2180.2
G25		6995	3800	3028	3218	2713	3950.8
G26		5822	3220	565	2767	1615	2797.8
G27		2218	3846	657	3103	2318	2428.4
G3		5603	1745	4074	924	1528	2774.8
G4		5883	3506	2210	2907	2310	3363.2
G5		2406	2564	2788	1619	1952	2265.8
G6		4641	3804	2032	3126	2555	3231.6
G7		6565	3194	1051	2755	1697	3052.4
G8		4369	2349	2553	1581	1612	2492.8
G9		5542	3858	1273	3325	2324	3264.4
Mean		4940.26	3430.07	1752.26	2797.7	2171.07	3018.27

above average in both E3 and E4. Genotypes G1, G23, G13, G10, G7 and G4 performed above average in E1 whilst G17 is near average in environment E3 and G3 near average in environment E1.

Environment representativeness

Figure 1 presents the representativeness of the test locations and a test location with a small angle to the average environmental axis (AEA) is more representative than other test locations. This means that E5 (SAVE VALLEY) is the most representative test location but with poor discriminating ability as indicated in Figure 1, whereas E1 indicated both good discriminating ability and representativeness, making it an ideal and best location for testing the sorghum genotypes. Environments E2 (KADOMA), E4 (MATOPOS) and E3 (MAKOHOLI) are the least representative. Test locations which are discriminating but non-representative like E2 (KADOMA) and E4 (MATOPOS) and E3 (SAVE VALLEY) are important under circumstances when selecting genotypes that are specifically adapted if the target locations can be divided into mega-environments. However, where the target locations cannot be divided into mega-environments such test environments like E2 (KADOMA) can be useful for culling unstable genotypes.

An ideal environment is the one which is on the intrinsic circle (Figure 2). So E1 (GWEBI) is found on the closer proximity or on the edge of the intrinsic circle (Figure 2). However, E3 (MAKOHOLI) and E5 (SAVE VALLEY) cannot be ideal test locations for selecting cultivars which can be adaptable for the whole region. Since this study was carried out for one season, it is of paramount importance to repeat the experiment in more seasons so as to confirm that a certain test location is ideal.

Ranking of genotypes based on environment E1 (good discriminating ability, representativeness and ideal)

Genotypes can be ranked based on their performance in

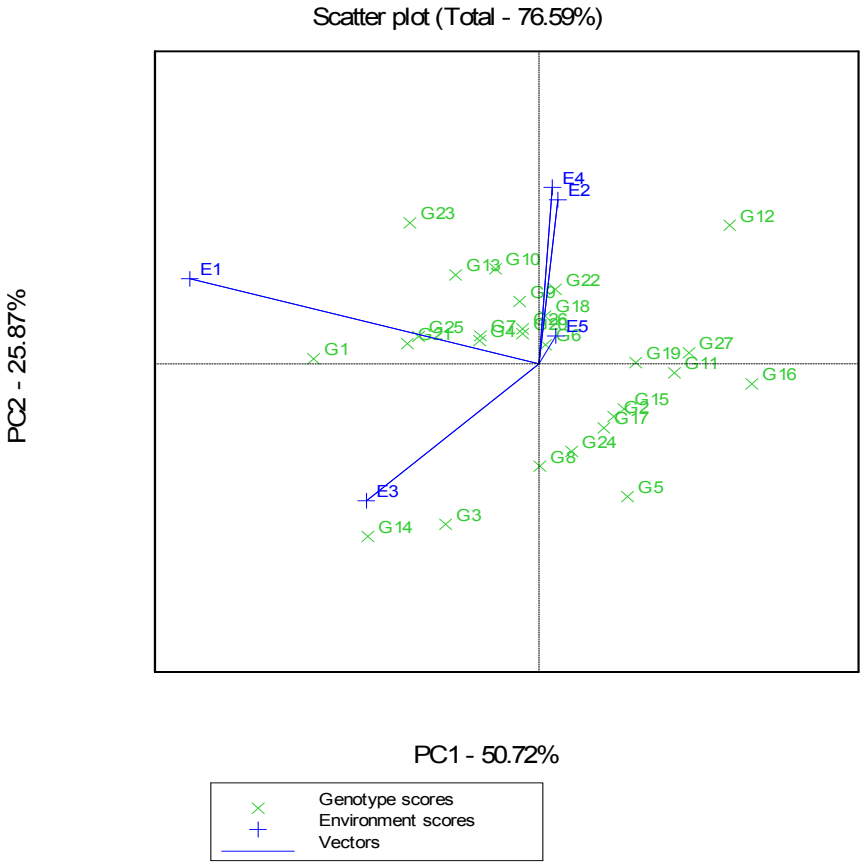

Figure 1. The environment vector view of the GGE drawn to show similarities among test-environments in discriminating environments.

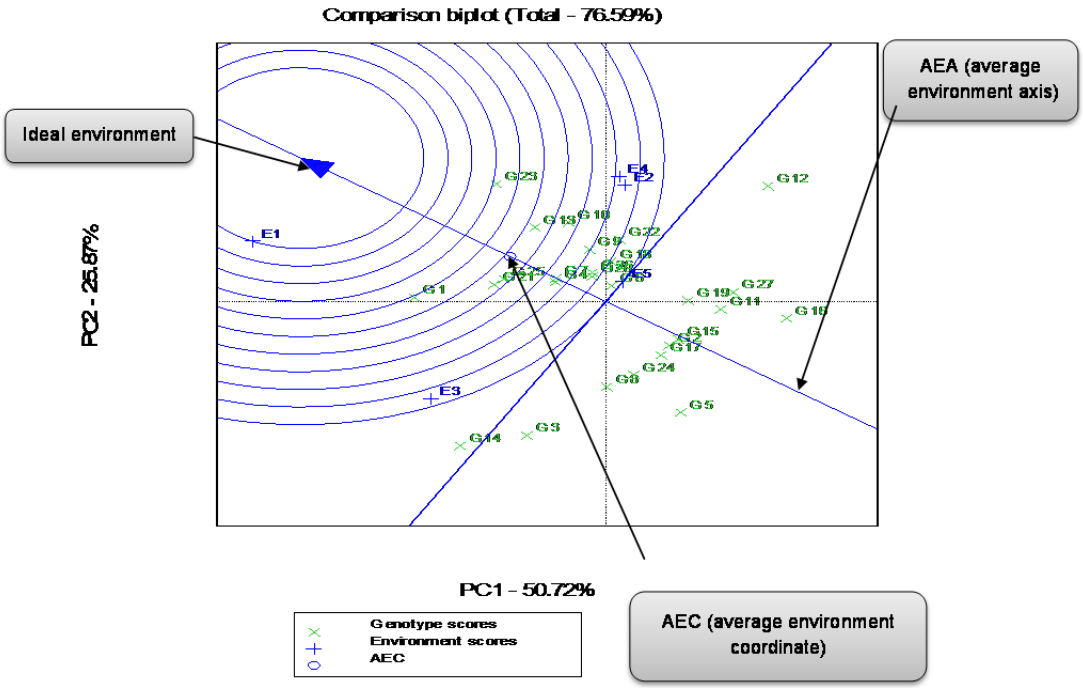

Figure 2. The average environment coordination (AEC) view to rank genotypes relative to the centre of the concentric circles.

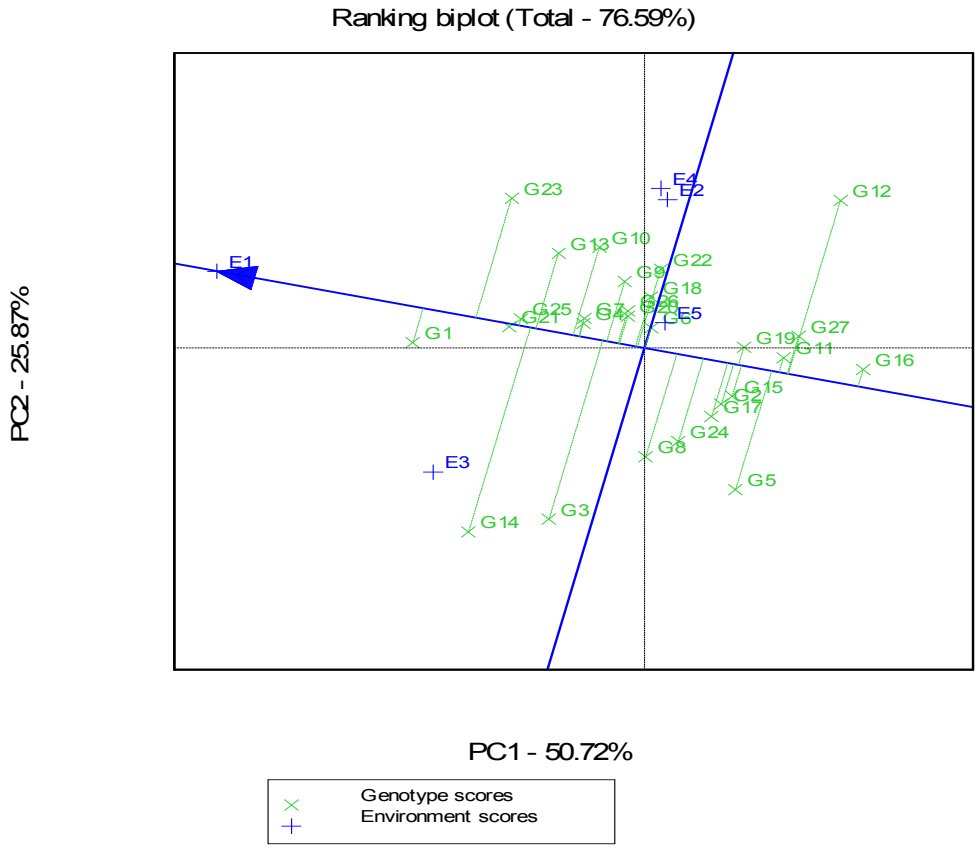

Figure 3. Ranking genotypes based on the performance of a specific environment (E1).

an environment by a line drawn that passes through the biplot origin and the environment called the axis of the environment. In Figure 3, genotypes performance is shown based on E1 (GWEBI), and the graph shows that genotype G16, G5 (NL9921) and G12 (NL9803) had a lower than average yield, whilst G6 (NL2014), G22 (SPV-422), G4 (NL9907) and G7 (ICSV93046) had a performance near the average yield and G1 (NL9411), G23 (E-36-1), G13 and G21 had performance above the average yield. So, the highest yielding genotype in E1 (GWEBI) is G1 (NL9411) whilst the lowest yield is G16 (SDS6013).

Ranking of environments based on the highest yielding genotype (G1 – NL9411)

Environments can be ranked based on the performance of a genotype. This is shown in Figure 4 where a line is drawn through the biplot origin and the genotype called the line of axis of genotype. The axis in Figure 4 was drawn based on G1 (NL9411), and this showed that G1 (NL9411) performed below average in E2 (KADOMA), E4 (MATOPOS) and E5 (SAVE VALLEY), whilst it performed above average in E3 (MAKOHOLI) and E1 (GWEBI).

Comparison plot for genotypes based on the concentric circle

Figure 5 shows the comparison plot for genotypes, and an ideal genotype is one which is near or at the centre of the concentric circle. Hence in the study, the plot reflected that G23 is the most ideal genotypes as shown by its position and followed by G1 and G25. This also reflects that the genotype has high mean and it is stable. Good genotypes are those which are closer to the ideal genotypes, thus G4, G7, G13, G10, G20, G26 and G21. They are positioned closer to the ideal genotypes. However, G5 (NL9921), G11, G17, G16 and G12 are the worst genotypes as their position in the plot are located far from the concentric circle.

Comparison between performances of two genotypes

In Figure 6, two genotypes, G1 (NL9411) and G12 (SV-2 released variety) were connected and the graph shows that G12 had high yield in E2 (KADOMA), E5 (SAVE VALLEY) and E4 (MATOPOS) whilst G1 (NL9411) produced high yields in E1 (GWEBI) and E3 (MAKOHOLI). This clearly shows that the genotypes

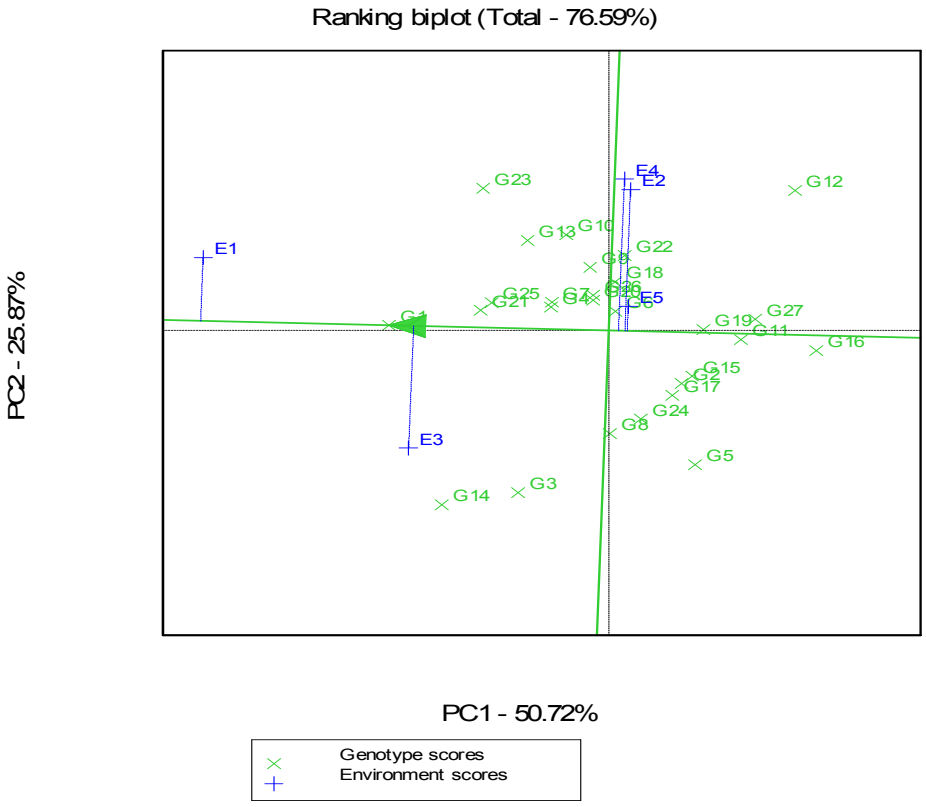

Figure 4. Ranking environments based on the performance of a genotype.

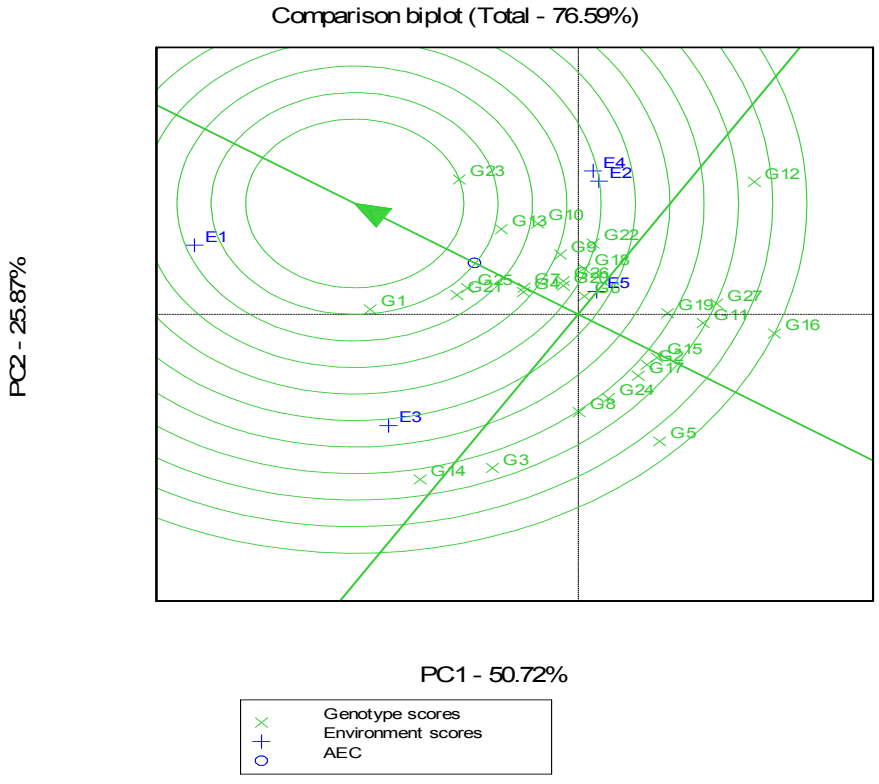

Figure 5. The average environment coordination (AEC) view to rank genotypes relative to the centre of the concentric circles.

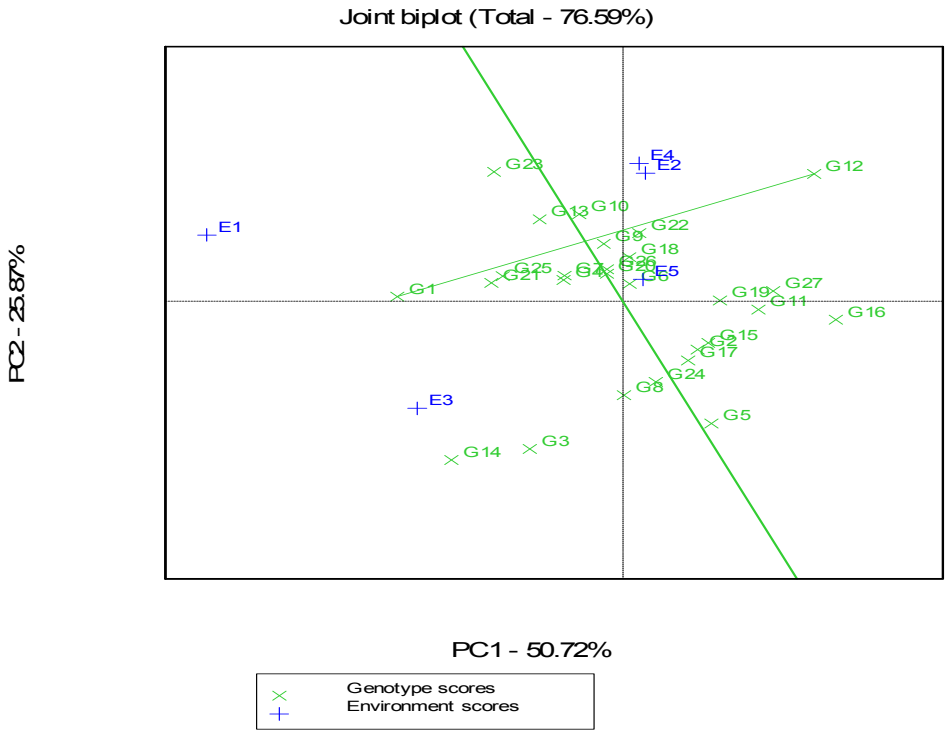

Figure 6. View of two genotypes in their performances in individual environments.

changed their rankings in those different environments thus explaining an example of crossover interaction. The same biplot was also used to measure the difference between the genotypes that varies by environment being proportional to the distance of the environment to the equality line. The biplot shows that the difference between G1 and G12 was relatively large in E4 (MATOPOS) (MATOPOS) and E1 (GWEBI) as well as in E2 (KADOMA) and E3 (MAKOHOLI) but very small in E5 (SAVE VALLEY).

Comparison of genotypes in two environments

The vertical and horizontal axis can be used to determine which variety performed above average or below average on two environments with one plotted on the horizontal axis and the other one plotted on the vertical axis. In Figure 7, E1 (Gwebi) was plotted on the x-axis and it shows that all the genotypes on the right of the vertical axis performed above average whilst those on the left side of the vertical axis performed below average. In this case, genotypes like G1, G13, G23, G14, G3, G7, G21 and G18 performed above average in that particular environment while G16, G5, G11, G15, G12, G27 and G19 performed below average. E4 (Matopos) was plotted on the y-axis and it shows that all genotypes which are above the horizontal axis performed above average and those below the horizontal axis performed below average in E4. The genotypes which performed above average include G1, G11, G15, G27, G12, G22, G10, G23 and G25 while genotypes like G16, G5, G19, G2, G24, G3, G14, G21 and G7 performed below average in E4. Genotypes lying on the equality or diagonal line performed equally in both environments for example G20. The perpendicular divided the environments into two groups meaning that each of these genotypes (G1 and G12) would yield better than the other at environments with markers on its side of the perpendicular (Yan et al., 2000).

Ranking plot based on mean performance and stability

Mean performance and stability of the genotypes can be predicted within a single mega environment when the genotype metric is preserved (SVP=1) (Figure 8). Predictions are mainly based on the average environment coordination (AEC) view of the biplot. In Figure 8, the single arrowed line is the AEA which shows the direction to higher mean yields across the tested environments. In this study, the graph shows that G23 and G1 had the highest mean yield, whereas G6 (NL2014) gave a mean yield almost similar to the grand mean and G16 and G5 gave the lowest mean yield. Stability and high performance make a candidate the best genotype. In this biplot (Figure 9), G17, G2 and G15 were most stable

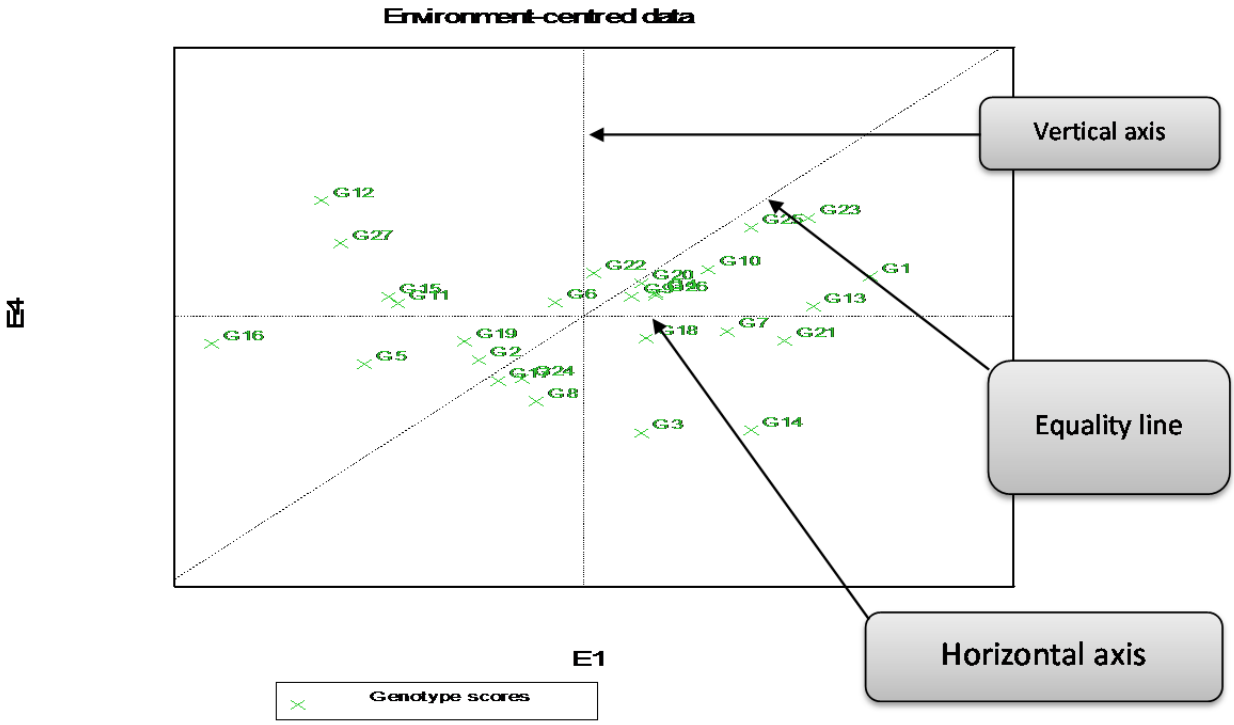

Figure 7. Centered scatter plot for genotype in two environments (E1 and E4).

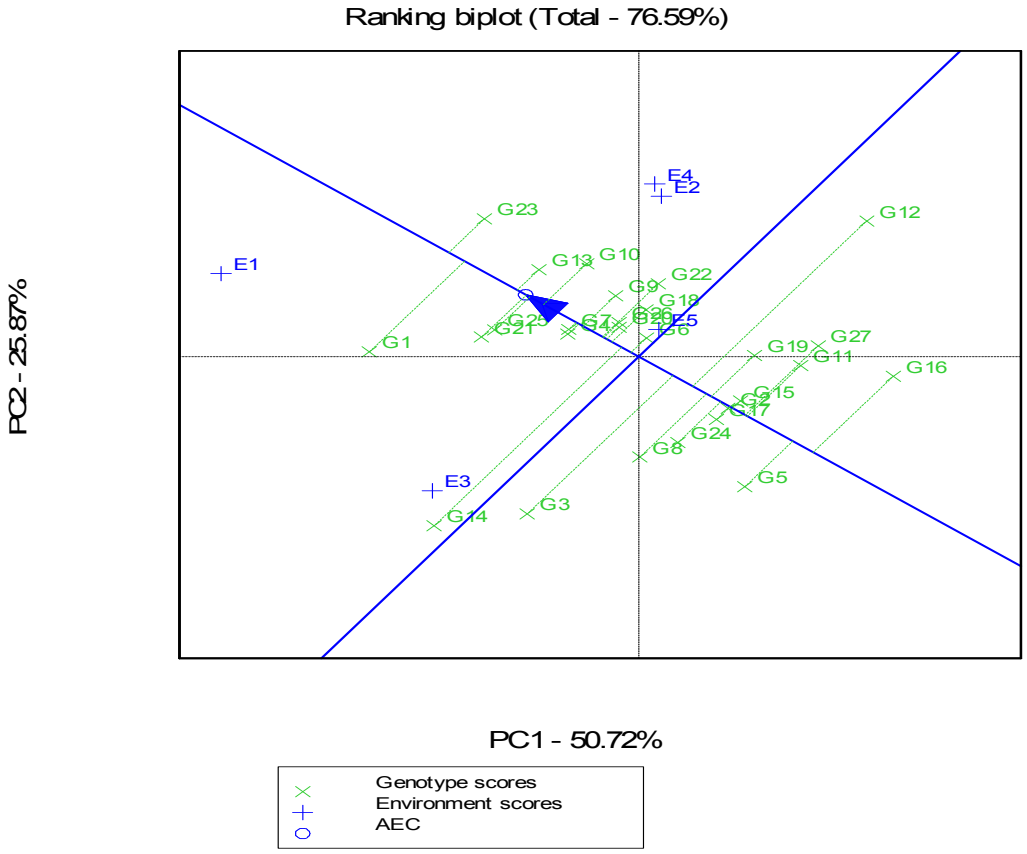

Figure 8. Ranking plot based on mean performance and stability.

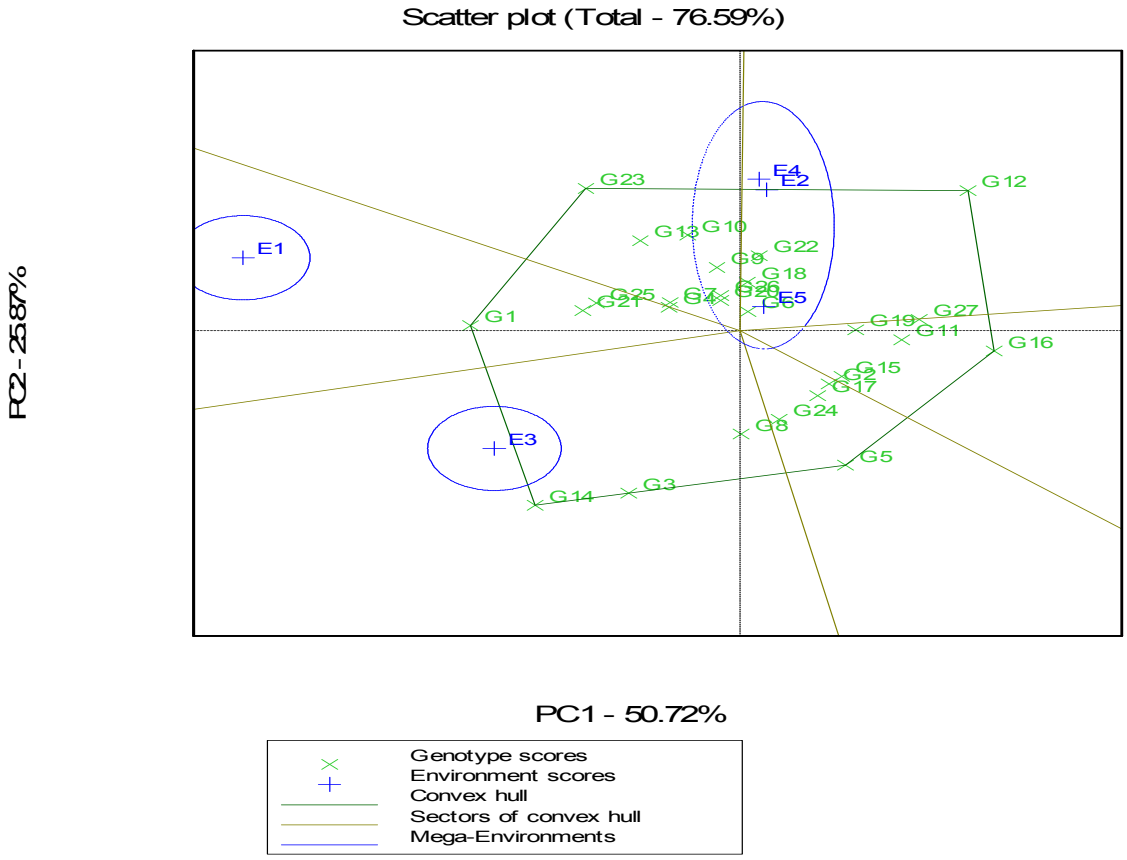

Figure 9. The which-won-where view of the GGE biplot to show which genotypes performed bets in which environments (mega-environment Identification).

but low yielding, whereas genotypes G4, G7, G26 and G20 are the most stable and high yielding; hence, can be selected as the most favourable genotypes. However, G12 and G14 are the most unstable even though G14 is above mean yield more than the low yielders but G17, G2 and G15 are stable.

Mega-environments (which-won-where)

An important feature of the GGE biplot (which-won-where) was also predicted. In mega-environment identification process, furthest genotypes are connected together to form a polygon, and perpendicular lines are drawn to form sectors which will make it easy to visualise the mega-environments. Mega-environment concept requires multi-year data, but in this study, mega environment study was carried out and the results (Figure 9) indicated three mega-environments thus three environments, E2 (KADOMA), E4 (MATOPOS) and E5 (SAVE VALLEY) formed one mega-environment, while E1 (GWEBI) and E3 (MAKOHOLI) formed two separate mega-environments, respectively. The winning genotypes for each sector are those positioned at the vertex. G12 is the winning genotype for the mega-environment which consists of E2 (KADOMA), E4 (MATOPOS), E5 (SAVE VALLEY), while G14 is the winning genotype for E3 (MAKOHOLI) mega-environment and G1 (NL9411) winning genotype for the E1 (GWEBI) mega-environment. The equality line between G14 and G5 shows that the G14 was better than G5 in all environments. On the line that connects the two is G3 which also means the three can be ranked G14, G3 and G5 in all the environments.

DISCUSSION

As the pooled ANOVA showed the presence of GEI for the sorghum grain yield, it means a breeder faces challenge of selection genotypes for advancement and or release, hence further testing for genotypes with wider and specific adaptation and locations with good discriminating ability and representativeness was done. This is similar to the study which was done by Gasura et al. (2015), where they tested 20 sorghum varieties and there was a large effect of GEI about seven times larger than the effect of genotypes. The GGE biplot analysis showed that IPCA1 accounted for 50.72% and IPCA2

accounted for 25.82%, both accounting for a sum of 76.59% (Figure 1) and this showed similarity with study of Gasura et al. (2015) where PC1 and 2 explained 36.8 and 29.5%, respectively. The biplot analysis identified the discriminating ability and representativeness as well as the correlation of environments (Sujay et al., 2014) and genotype average performance and the results showed the importance of testing and comparing genotypes so as to select the ones with specific and wide adaptation accordingly and environments which are representativeness to reduce experimenting costs by discarding unrepresentative locations and those with poor discriminating abilities. The greater IPCA1 shows greater discriminating ability of an environment. This gives the importance of determining the discriminating ability to enhance separation through differences in performances of different genotypes. The results revealed that E5 though low yielding but gave more information on the tested genotypes than the other environments as was also detected by Yan and Kang (2003) when they used the GGE vector view plot. So this study provides important information on selecting and releasing best and ideal genotypes which are good for production in specific and widely adapted environments as well as determine the most effective and necessary environments which gives more information on varieties in future breeding trials.

Identification of mega-environments (Figure 9) was studied also and very important information on which-won-where was unveiled in the results obtained. The mega-environment identification involved a situation whereby one or more environments with similar or homogenous characteristics were bunched into one big location, like in this study where E2, E4 and E5 were bunched into one environment meaning in the future, costs of raising multi-locational trials will be reduced by putting that effect into consideration. Which-won-where (Yan et al., 2007) identified best winners for the mega-environment or sector. This enables the researcher to have specific and valid justification to recommend genotypes which are good for that particular environment (Gasura et al., 2015). This also means the genotypes can be tested in those few mega-environments and still good yield data results can be obtained. The GGE biplot also gave information which is important if a researcher has to make decisions and conclusions about specific correlations among environments and genotypes. The study results gave a better understanding of how biased a researcher can be if there is GEI and fails to do further GEI biplot analysis. The GGE have a lot of information which validates appropriate environment for testing and appropriate genotypes for selection and recommendation (Sujay et al., 2014), there was effective evaluation of environments and genotypes and evaluation of genotypes based on the mean performance and stability across environments which is important required information for a researcher.

In conclusion, the results showed that the grain yield performance of the 27 genotypes was significantly influenced by environment, genotype and their interaction. A further analysis on the adaptability and stability across the 5 environments was done. G1, G23, G21 and G25 showed both high yielding and stability across the test environments. These have been identified as possible candidates for advancement, for release and for use as parents in future breeding programmes. Test environments E1-Gwebi, E2-Kadoma and E4-Matopos were the most discriminating locations which means they gave more information on the performance of the varieties. However, only E1-Gwebi showed good discriminating ability, and representativeness, making it the most ideal environment in this multilocational yield trials.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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