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Full Length Research Paper

Organic and mineral fertilizer effects on growth and yield of cocoyam in a tropical Ultisol in South Eastern Nigeria

Mary Oluchi Iwuagwu[1]*, Dominic Aja Okpara[2], Cosmas Osita Muoneke[2] and Adanma Augustina Ukaoma[3]

[1]Department of Plant Science and Biotechnology, Abia State University, P.M.B. 2000, Uturu, Abia State, Nigeria.
[2]Department of Agronomy, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria.
[3]Department of Biological Science, Federal University of Technology, Owerri. P.M.B. 1526, Owerri, Imo State, Nigeria.

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The performance of cocoyam (*Colocasia esculenta* L. Schott) to varying levels of cow dung and potassium fertilizer was investigated in 2012 and 2013 cropping seasons under field conditions at National Horticultural Research Institute (NIHORT), Imo State, Nigeria. The experiment was a 4 × 2 factorial arrangement in a randomized complete block design with three replications. The treatments were four levels of cow dung (0, 10, 20 and 30 t ha^{-1}) and potassium fertilizer (0, 20, 40 and 60 kg K_2O ha^{-1}). Application of cow dung at the highest rate of 30 t ha^{-1} significantly ($P < 0.05$) enhanced plant height, number of leaves, leaf area index, number of suckers and yield of cocoyam (23.6 t ha^{-1}) in 2012 cropping season relative to the lower rates and the control. However, in 2013 cropping season, cow dung at the lower rate of 20 t ha^{-1} gave optimum corm yield of 23.3 t ha^{-1}. In general, potassium fertilizer application did not exert much influence on growth and yield of cocoyam as significant effect was observed only in 2013 cropping season. Corm yield was significantly ($P < 0.05$) improved by the application of 60 kg K_2O ha^{-1} (22.3 t ha^{-1}) in 2013 cropping season relative to the control. The results of this study have shown the effectiveness of cow dung in improving the productivity of cocoyam, thereby enhancing food security in Nigeria.

Key words: *Colocasia esculenta*, corm yield, cowdung, food security, potassium fertilizer, soil fertility.

INTRODUCTION

Cocoyam (*Colocasia esculenta (*L) Schott) is a starchy tuber crop that has been widely cultivated and consumed in the Southeastern agricultural Zone of Nigeria for decades (Ndon et al., 2003). It is regarded as a poor man's food or a woman's crop and as such has lagged behind the preferred and highly valued staple root/tuber crops such as yam and cassava in research attention (Ikwelle et al., 2003). However, the high cost of yam and the increased awareness of the industrial and export potential of cassava has given way to high patronage

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

of the relatively obscure crops like cocoyam (Shiyam et al., 2007). The current level of cocoyam production in Nigeria estimated at 5 million metric tones in 2007 (FAO, 2007) is grossly inadequate to satisfy the increasing demand for the crop as alternative food crop (Shiyam et al., 2007).

This low level of cocoyam production is attributed to increasing decline in soil fertility levels and lack of soil management practices for continuous cocoyam cultivation (Agbede and Adekiya, 2016). The use of organic and mineral fertilizers are the two major and common ways in which soils are managed since the extinction of shifting cultivation as well as reduction in bush fallow periods (Makinde et al., 2011). The impact of increased use of mineral fertilizers on crops has been high but the resulting soil physical degradation, increased soil acidity and soil nutrient imbalance, resulting in reduced crop yield (Ojeniyi, 2000; Mbah and Mbagwu, 2006), escalating cost and unavailability of mineral fertilizers (Suge et al., 2011) have drawn the attention of researchers back to the use of organic manures. These organic manures are cheaper, readily available and affordable for soil fertility management and improvement in crop yield.

In recent years, however, the focus on soil fertility has shifted towards the combined application of organic and inorganic fertilizers for judicious management of resources and soil conservation under intensive cropping (Fening et al., 2011). Sole use of organic manures to sustain cropping has been reported inadequate especially in the year of application (Patel et al., 2000). They are required in rather large quantities to meet crops' nutrient supply because of their relatively low nutrient content (Palm et al., 1997). Supply of nutrients from organic materials can be complemented by enriching them with inorganic nutrients that will be released fast and utilized by crops to compensate for their late start in nutrient release (Ayoola and Makinde, 2009).

Cocoyam responds very well to input of fertilizer whether organic or inorganic as reported by several workers (Hota et al., 2014; Ogbonna and Nwaeze, 2012; Ojeniyi et al., 2013; Shiyam et al., 2007; Uwa et al., 2011). It has a high requirement for potassium like all other tuber crops (Obigbesan, 1980). In tuber crops, potassium plays a vital role in the movement of sugars produced in the leaf by photosynthesis to the tubers where the sugars are converted to starch (Abd El-Latif et al., 2011). Surveys in Nigeria revealed inconsistences in the amount of potassium for optimum performance of cocoyam due mainly to differences in soil types and soil potassium status (Obigbesan, 1980; Ohiri et al., 1988).

However, the Ultisols in south eastern Nigeria are low in potassium and thus require potassium fertilization for good crop yields (Unamba-Opara, 1985). The need to increase the production of cocoyam in a marginal soil using organic and inorganic sources of fertilizer necessitated this study. The objective was therefore to evaluate the effects of cowdung and potassium fertilizer on the performance of cocoyam in a tropical Ultisol in southeastern Nigeria.

MATERIALS AND METHODS

The experiment was conducted at National Horticultural Research Institute (NIHORT), Mbato sub-station, Okigwe, Imo State, Nigeria. NIHORT is located at latitude 5°33'N and longitude 7°23'E and 139 m above sea level. The area is characterized as a humid rainforest zone and the soil is sandy loam. The total annual rainfalls for 2012 and 2013 were 1902.8 and 2210.0 mm, respectively while the total rainfalls during the period of experimentation (April to December) for 2012 and 2013 were 1775.6 mm and 2009.0 mm, respectively.

The cocoyam (*Colocasia esculenta* var NCe001) cormels used in this study were sourced from National Root Crops Research Institute, Umudike, Abia State. The cow dung was obtained from the animal farm of Michael Okpara University of Agriculture, Umudike, while the potassium fertilizer (muriate of potash, K_2O) was obtained from the fertilizer unit of Abia State Ministry of Agriculture, Umuahia. Composite sample of the cowdung was air dried, crushed, sieved and then analyzed in the laboratory for its nutrient compositions. The analysis revealed that the cowdung used in this study is composed of 2.54, 1.34, 1.16, 1.56, 0.46 and 50.70%, N, P, K, Ca, Mg and organic matter, respectively in 2012 and 2.24, 1.67, 0.65, 2.80, 0.61 and 30.76%, N, P, K, Ca, Mg and organic matter, respectively in 2013.

The site was double-ploughed, ridged and marked out into three blocks, which represent the replicates. Each block was divided into sixteen experimental plots, thus a total of forty-eight plots were used. Each gross plot measured 4 m × 3 m (12 m^2) with a net plot of 2 m × 2 m. Soil samples were collected with soil auger at a depth of 0 to 20 cm from different locations of the site and bulked into composite sample. The composite soil sample was air dried, passed through 2 mm sieve, and then analyzed for its physico-chemical properties (Table 1).

The experiment was a 4 × 2 factorial arrangement in a randomized complete block design and replicated three times. The treatments comprised four rates each of application of cow dung (0, 10, 20 and 30 t ha^{-1}) and potassium fertilizer (0, 20, 40 and 60 kg K_2O ha^{-1}). A total of sixteen treatment combinations and three replications were used. The cow dung was incorporated into the soils of the experimental plots in a single application based on the treatment combinations, at two weeks before planting to allow decomposition while the Potassium fertilizer was applied to the cocoyam stands according to treatment allocation at 3 weeks after planting (WAP) using band placement method. One cormel was planted per hole at a depth of 15 cm and at a spacing of 0.5 m x 1.0 m resulting to about twenty-four plants per plot and a total of about 20,000 plants per hectare. All plots were kept weed free by manual weeding.

Five cocoyam plants were randomly selected from each of the net plots, tagged and then used for the determination of plant height (cm), number of leaves and leaf area index (LAI) at 1, 2 and 3 months after planting (MAP), number of suckers at 3 MAP, number of corms per plant, corm weight (kg/plant) and corm yield (t ha^{-1}) at physiological maturity.

The leaf area was determined using the formula of Biradar et al. (1978) as:

Leaf Area of Cocoyam = 0.917 (LW).

Where L and W are length and width of the cocoyam leaf. The leaf area index was then calculated by dividing the total leaf area by the area occupied by the plant (Biradar et al., 1978). Number of suckers and number of corms were counted and mean values

Table 1. Some physicochemical properties of soils of the experimental site in 2012 and 2013.

Property	2012	2013
Physical properties		
Sand (%)	67.80	65.80
Silt (%)	14.40	13.40
Clay (%)	17.80	20.80
Textural class	SL	SCL
Chemical properties		
pH (in H_2O)	4.6	5.5
P (mg/kg)	35.50	44.30
N (%)	0.02	0.04
OC (%)	0.19	0.81
OM (%)	0.32	1.40
Exchangeable bases (cmol kg^{-1})		
Ca	2.40	4.40
Mg	2.00	0.80
K	0.022	0.088
Na	0.31	0.497
EA (cmol kg^{-1})	0.80	0.96
ECEC (cmol kg^{-1})	5.532	6.745
BS (%)	85.53	85.76

SL = sandy soil; SCL = sandy clay loam; OC = organic carbon; OM = organic matter; EA = exchange acidity; ECEC = effective cation exchange capacity; BS = base saturation.

recorded. Corms and cormels were harvested, weighed and the total weight recorded. Data collected were subjected to analysis of variance using Genstat Discovery Edition 3 Package of 2007. Significant means were separated using Least Significant Difference (LSD) at probability level of 0.05.

RESULTS

In 2012 cropping season, differences in plant height due to application of cow dung were significant ($P < 0.05$) at 2 and 3 MAP (Table 2). All cases of cow dung application significantly increased plant height of cocoyam compared to the control at 2 MAP. However, at 3 MAP, incremental application of cow dung up to 20 t ha^{-1} significantly increased plant height. In 2013, cocoyam plant height increased with application of cow dung at 10 t ha^{-1}, above which significant reductions in height occurred at 1 and 2 MAP. At 3 MAP, plant height increased at the highest rate of 30 t ha^{-1} compared to the lower rate of 10 t ha^{-1} or no application in both seasons.

At 3 MAP in 2012 cropping season, application of potassium at 40 kg K_2O ha^{-1} increased significantly

Table 2. Main effects of cow dung and potassium fertilizer on plant height (cm) of cocoyam at 1, 2 and 3 MAP in 2012 and 2013.

Treatment	Months after planting (MAP)					
	1	2	3	1	2	3
	2012			2013		
Cow dung (t ha^{-1}						
0	14.6	33.8	50.1	19.3	37.3	46.9
10	14.5	40.8	61.5	22.6	43.3	56.2
20	13.1	40.0	67.6	20.1	40.3	58.9
30	13.1	40.2	69.9	19.4	39.7	60.6
Mean	13.8	38.7	62.3	20.4	40.2	55.7
LSD (0.05)	NS	5.1	4.0	2.2	2.5	4.3
Potassium (kg K_2O ha^{-1})						
0	14.0	38.3	60.0	18.9	34.5	51.3
20	14.0	38.8	62.6	20.1	40.4	55.7
40	14.0	40.0	65.4	20.5	41.7	56.2
60	13.7	37.8	61.1	21.8	42.0	59.4
Mean	13.9	38.7	62.3	20.3	39.7	55.7
LSD (0.05)	NS	NS	4.0	NS	2.5	4.3
C x K	NS	NS	NS	NS	NS	NS

Ns= not significant.

cocoyam plant height than no potassium application (Table 2). On the other hand, at 2 and 3 MAP in 2013, application of potassium at 20 kg K_2O ha^{-1} resulted in higher plant height than the control. All cases of applied potassium produced similar plant height values.

Application of cow dung caused significant ($P < 0.05$) increase in the number of leaves per cocoyam plant from 2 MAP in both years of cropping (Table 3). In 2012 and at 2 MAP, cow dung application irrespective of rate recorded statistically similar number of leaves that were significantly ($P < 0.05$) higher than the control. At 3 MAP, incremental application of cow dung significantly ($P < 0.05$) increased the number of leaves of cocoyam compared to no application. Effect of potassium on number of leaves of cocoyam was significant only in 2013 cropping season. Application of potassium fertilizer significantly ($P < 0.05$) increased number of leaves of cocoyam compared to the control while the different rates of applied potassium fertilizer recorded statistically similar values.

In both years, application of cow dung had no effect on LAI at 1 MAP but effect was more apparent at 2 and 3 MAP (Table 4). Application of cow dung at different rates resulted in significant ($P < 0.05$) increase in LAI relative to the control at 2 MAP in 2012 and at 2 and 3 MAP in 2013. LAI increased steadily with plant age. Application of potassium produced significant effects on cocoyam LAI at 3 MAP in 2013 (Table 4). Application of potassium at 20 or 40 kg K_2O ha^{-1} resulted in significantly ($P < 0.05$)

Table 3. Main effects of cow dung and potassium fertilizer on number of leaves per cocoyam plant at 1, 2 and 3 MAP in 2012 and 2013.

Treatment	Months after planting (MAP)					
	1	2	3	1	2	3
	2012			2013		
Cow dung (t ha^{-1})						
0	4.2	7.3	17.5	4.4	7.4	17.5
10	4.3	10.1	25.7	4.9	11.7	24.8
20	3.9	10.0	29.2	4.8	11.1	26.1
30	3.9	11.9	32.4	5.0	11.5	27.7
Mean	4.2	9.8	26.2	4.8	10.4	24.0
LSD (0.05)	NS	2.1	2.9	NS	2.3	3.1
Potassium (kg K_2O ha^{-1})						
0	4.0	9.7	24.5	4.7	9.8	20.0
20	4.1	10.3	26.7	4.7	10.0	24.0
40	4.1	9.9	27.2	5.0	11.1	25.3
60	4.1	9.0	26.3	4.8	10.8	26.7
Mean	4.1	9.7	26.2	4.8	10.4	24.0
LSD (0.05)	NS	NS	NS	NS	NS	3.1
C x K	NS	NS	NS	NS	NS	NS

NS = not significant.

Table 4. Main effects of cow dung and potassium fertilizer on leaf area index (LAI) at 1, 2 and 3 MAP in 2012 and 2013.

Treatment	Months after planting (MAP)					
	1	2	3	1	2	3
	2012			2013		
Cow dung (t ha^{-1})						
0	0.15	0.77	1.87	0.25	0.79	2.02
10	0.19	1.19	3.07	0.37	1.24	3.02
20	0.13	1.22	3.60	0.30	1.21	3.28
30	0.15	1.23	3.80	0.30	1.21	3.20
Mean	0.16	1.10	3.09	0.31	1.11	2.88
LSD (0.05)	NS	0.26	0.45	NS	0.38	0.62
Potassium (kg K_2O ha^{-1})						
0	0.15	1.09	2.90	0.25	0.90	2.23
20	0.16	1.12	3.04	0.31	1.14	2.80
40	0.14	1.12	3.18	0.33	1.21	3.02
60	0.16	1.08	3.26	0.33	1.20	3.48
Mean	0.15	1.10	3.10	0.31	1.11	2.88
LSD (0.05)	NS	NS	NS	NS	NS	0.62
C x K	NS	NS	NS	NS	NS	NS

NS = not significant.

higher LAI than no application while application of the higher rate of 60 kg K_2O ha^{-1} recorded higher LAI value than the lower rate of 20 kg K_2O ha^{-1}.

Application of cow dung had significant effect on the number of suckers produced per cocoyam stand at 3 MAP in both years of cropping (Table 5). In 2012, incremental application of cow dung resulted in significant ($P < 0.05$) increase in the number of suckers produced over the control where as in 2013, application of cow dung above 10 t ha^{-1} did not result in any significant ($P < 0.05$) increase in the number of suckers. Application of potassium fertilizer did not significantly ($P < 0.05$) influence the number of suckers produced per cocoyam stand in 2012 but did influence it significantly in 2013. Potassium fertilizer applied at 40 or 60 kg K_2O ha^{-1} significantly ($P < 0.05$) increased the number of suckers compared to no fertilizer application. The different rates of applied potassium fertilizer recorded statistically similar values.

The number of corms produced per plant was significantly ($P < 0.05$) increased by the application of cow dung in both years (Table 5). In 2012, incremental application of cow dung up to the highest rate of 30 t ha^{-1} increased significantly ($P < 0.05$) the number of corms per plant. In 2013 however, increasing cow dung rate up to 30 t ha^{-1} increased significantly the number of corms per plant compared to the lower rates and the control. Application of 10 and 20 t ha^{-1} cow dung rates produced comparable number of corms. Potassium fertilizer application did not have any effect on number of corms per plant in 2012 but in 2013. All cases of applied potassium resulted in higher number of corms than no potassium application. There was no significant interaction effect between cow dung and potassium fertilizer on number of corms produced.

Similarly, application of cow dung significantly increased the weight of corms as the manure rate was raised to 30 t ha^{-1} in 2012 and 20 t ha^{-1} in 2013 (Table 5). Increasing cow dung rate above 20 t ha^{-1} in 2013 did not result in marked improvement in weight of corms. On the contrary, corm weight was not significantly influenced by potassium fertilizer in 2012 but in 2013 application of potassium fertilizer at the highest rate of 60 kg K_2O ha^{-1} increased corm weight over zero application. Interactions were not significant for corm weight in the two cropping seasons.

Corm yield response to application of cow dung followed the same trend as corm weight in both years (Table 5). There was a significant ($P < 0.05$) linear corm yield response to cow dung application in 2012. In 2013 however, application of cow dung above 20 t ha^{-1} rate did not significantly increase yield. When averaged over the two cropping seasons, cow dung application at the rates of 10, 20 and 30 t ha^{-1} gave corm yields of 17.7 t ha^{-1}, 21.3 t ha^{-1} and 24.81 t ha^{-1}, respectively while zero application gave an average yield of 11.5 t ha^{-1}. Increase in cow dung rate from 0 to 10 t ha^{-1}, increased corm yield by 54%, additional increase to 20 and 30 t ha^{-1} increased corm yield by 85 and 116%, respectively.

Table 5. Main effects of cow dung and potassium fertilizer on number of suckers per cocoyam stand at 3 MAP, number of corms per cocoyam plant, corm weight (kg plant^{-1}) and corm yield (t ha^{-1}) at harvest in 2012 and 2013.

Treatment	2012				2013			
	NSS	NC	CW	CY	NSS	NC	CW	CY
Cowdung (t ha^{-1})								
0	3.7	14.2	0.54	10.86	3.8	13.8	0.61	12.19
10	4.9	16.6	0.78	15.59	5.2	18.9	0.99	19.76
20	5.5	19.3	0.97	19.38	5.5	20.8	1.16	23.27
30	6.1	22.0	1.18	23.58	5.7	23.1	1.30	26.04
Mean	5.1	18.0	0.87	17.35	5.1	19.2	1.02	20.32
LSD (0.05)	0.5	1.9	0.12	2.35	0.5	2.1	0.16	3.50
Potassium (kg K_2O ha^{-1})								
0	4.7	17.7	0.85	17.09	4.5	17.2	0.91	18.15
20	5.2	18.3	0.88	17.64	4.9	19.5	1.02	20.33
40	5.1	18.4	0.85	17.00	5.3	19.5	1.02	20.50
60	5.1	17.8	0.88	17.67	5.4	20.4	1.11	22.27
Mean	5.0	18.1	0.87	17.35	5.0	19.2	1.02	20.31
LSD (0.05)	NS	NS	NS	NS	0.5	2.1	0.17	3.50
C x K	NS	NS	NS	NS	NS	NS	NS	NS

NSS = number of suckers; NC = number of corms; CW = corm weight; CY = corm yield; NS = not significant.

Table 6. Effect of cow dung and potassium fertilizer on mean corm yield (t ha^{-1}).

Potassium (kg K_2O ha^{-1})					
Cow dung (t ha^{-1})	**0**	**20**	**40**	**60**	**Mean**
0	8.84	12.38	11.63	18.75	11.52
10	16.00	19.46	17.17	18.04	17.67
20	21.21	20.75	21.50	21.84	21.32
30	24.42	23.37	24.71	26.75	24.81
Mean	17.62	18.99	18.75	19.97	-

$LSD_{(0.05)}$ for cow dung (C) mean =2.39 ; $LSD_{(0.05)}$ for potassium (K) mean=NS; $LSD_{(0.05)}$ for C x K mean=NS.

Increasing from 20 to 30 t ha^{-1} gave an increase of 16.5%. Potassium fertilizer effects on corm yield were not consistent in both years. Corm yield was not influenced by potassium in 2012 but in 2013. Application of 60 kg K_2O ha^{-1} resulted in significantly ($P < 0.05$) higher yield than the control. All cases of applied potassium fertilizer produced comparable corm yields. Application of potassium fertilizer at 60 kg K_2O ha^{-1} gave a yield of 22.27 t ha^{-1}, which was significantly greater than the control by 23%.

As mean across two years, corm yield increased significantly ($P < 0.05$) with incremental application of cow dung up to 30 t ha^{-1} while application of potassium had no effect on yield (Table 6). In both cropping seasons, there was no significant cow dung x potassium interaction effect on corm yield.

DISCUSSION

In general, application of cow dung resulted in increase in cocoyam plant height, the number of leaves produced per plant, LAI and number of suckers produced per cocoyam stand especially at 2 and 3 MAP. The higher growth following cow dung application would be attributed to the probable effects of the manure in improving soil physical,

chemical and biological properties (Balemi, 2012; Najm et al., 2012; Khalid et al., 2014), which are important for crop performance.

The effects of cow dung on cocoyam appeared more pronounced later in crop growth at 2 MAP, due to the slow release of nutrients by the manure. This result is consistent with the findings of Miyasaka et al. (2001) who attributed the enhanced growth and yield response of the crop to organic amendment to slow release of nutrients by the organic manures, which tied the crop over the long duration of its growth.

Cow dung at 30 t ha^{-1} produced the highest corm yield in the relatively more acidic and less fertile sandy loam soil. However, for the more fertile sandy clay loam soil with higher pH of 5.5, cow dung at the lower rate of 20 t ha^{-1} gave optimum corm yield. This implies that at these rates in these soils, cow dung released and made available adequate nutrients for optimum crop development. Cocoyam like any other root and tuber crop is a heavy feeder, exploiting a large volume of soil for nutrient and water (Osundare, 2004). This could explain the high rate of cowdung (20 to 30 t ha^{-1}) required for optimum production of this crop as reported in this study. Gyllapsy et al. (1993) reported that the availability of sufficient nutrient facilitates sink function as this plays a role in the control of carbohydrate accumulation and partitioning. Plants nourished with sufficient amount of nutrients in adequate proportion are expected to have higher number and size of cells (Akanbi et al., 2007) and hence more yield.

In this study application of cow dung at 30 t ha^{-1} produced average corm yield of 24.8 t ha^{-1} and this was higher than the yields of cow dung at 0, 10 and 20 t ha^{-1} by 116, 40 and 16%, respectively. Similarly, cow dung at 20 t ha^{-1} produced average corm yield of 21.3 t ha^{-1}, which was higher than the values at 0 and 10 t ha^{-1} cow dung rates by 85 and 20%, respectively. These yields recorded in these investigations were higher than the yields obtainable in Nigeria (5 to 7 t ha^{-1}), Ghana (4 to 8 t ha^{-1}) and China (17.5 to 19 t ha^{-1}) but compared favourably with the yields obtainable in Egypt (23.5 to 35.0 t ha^{-1}) (Onyeka, 2014).

Application of 20 or 40 kg K_2O ha^{-1} potassium fertilizer recorded significantly higher plant height and number of leaves compared to the control while application of 60 kg K_2O ha^{-1} potassium fertilizer recorded the highest LAI (3.5) and was optimum for fresh cocoyam yield (22.3 t ha^{-1}). This result agrees with the earlier report by Ohiri et al. (1988) that cocoyam requires high potassium levels with the best results obtained at rates between 50 and 80 kg K ha^{-1} depending on soil type.

Okpara et al. (2010) reported improvement in root yield of cassava following application of 50 kg K_2O ha^{-1} in south eastern Nigeria with a native soil potassium level of 0.19 to 0.25 cmol kg^{-1}, while FFD (2002) observed an improvement in cassava yield at 75 kg K_2O ha^{-1} of applied potassium fertilizer when the native soil potassium was between 0 and 0.15 cmol kg^{-1}. In this study, the native soil potassium level was 0.02 cmol kg^{-1} in 2012 and 0.09 cmol kg^{-1} in 2013. According to Murata and Akazawa (1968) the beneficial effects of potassium fertilizer have mostly been attributed to the fact that potassium increases the activities of starch synthetase, which results to high yield, especially if there are inadequate native supplies of the nutrient. Potassium plays a vital role in the movement of sugars produced in the leaf by photosynthesis to the tubers where the sugars are converted to starch (Abd El-Latif et al., 2011). Except for 3 MAP in 2013, potassium application had no significant effect on LAI at all sampling periods in both years and its effect on yield was only significant in 2013 cropping season. According to Brennan and Bolland (2009), yield response to potassium application depends to a great extent on the level of nitrogen supply.

In this study, the level of percentage soil nitrogen was higher in 2013 than in 2012 so also the organic matter content (Table 1), which upon decomposition releases nitrogen and other nutrients to the soil. This may perhaps explain the positive yield response of cocoyam found in 2013 cropping season. Potassium did not also exert as much influence as cow dung on cocoyam yield and only affected yield in 2013. This is expected since cow dung contains nutrients other than potassium, which are necessary for plants growth and development. In addition, cow dung being an organic manure releases nutrients slowly from decomposing organic manure, which are stored for a longer time in the soil thereby ensuring a long residual effect, improved root development and higher crop yields (Rashid et al., 2013; Agbede and Adekiya, 2016). They were lower values for organic matter, nitrogen and potassium in the cow dung but higher rainfall of 2009.0 mm in 2013, in which corm yield appeared higher by 17% compared to 2012. This supports the report by Onwueme (1987) that cocoyams require rainfall above 2000 mm per annum for optimum yields.

This study showed that both cowdung and potassium fertilizer improved growth and yield of cocoyam with cow dung having a better improvement than potassium fertilizer. Optimum yields were obtained at 20 to 30 t ha^{-1} cowdung rate and 60 kg K_2O ha^{-1} of potassium fertilizer.

Conflict of interests

The authors have not declared any conflict of interests.

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Evaluation of large seeded faba bean genotypes for agronomic performance in vertisol areas of Southern Tigray, Ethiopia

Birhanu Amare Gidey

Alamata Agricultural Research Center, Pulse and Oil Cros Case Team, P. O. Box 56, Tigray, Ethiopia.

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Thirty-two faba bean genotypes were evaluated for their yield and yield components in verstisol areas of Hashenge and Aiba in 2015. Alpha lattice design with two replications was used for the experiment. The Analysis of Variance showed highly significant differences (P<0.01) for thousand seed weight over locations. Based on this, genotype EH 06007-2 scored highest 1000 seed weight (1111 g) followed by EH 06088-6 (971.5) and EH 06007-4 (938.5 g) over locations. Least thousand seed weight was obtained from the local genotype (505.5 gm) followed by the standard check Walki (587.3) and ET 07017-bulk (648.0 g). Grain yield showed significant differences (p<0.05) at Aiba location but no significant differences in Hashenge. At Aiba, genotype ET 07013-1 gave the highest grain yield (59.31 qt/ha) followed by genotype ET 07005-1 (57.85) and EH 06088-1 (54.77 qt/ha). Significant positive correlations were recorded between TSW with DM (0.624), number of seeds per pod (0.567) and NTPP (0.427) but it was negatively correlated with NPPP (-0.487). Grain yield was significantly and positively correlated with plant height (0.49) and NPPP (0.369). The highest distance (0.692) was between cluster V and IV, which suggested that the members of these clusters diverge on most of the studied traits and could be used in breeding programs. Principal component (PC) analysis revealed that the first four PCs explained 83.7% of the total variation. The variance explained by PC1 was mostly due to traits related to DM, TSW, NSPP and NTPP, whereas PC II was mostly related to grain yield, plant height, NPPP and thousand seed weight traits. The PC analysis ultimately showed the amount of variability for the traits that could be used for the improvement of large seed sized faba bean genotypes.

Key words: Large seed size, genotypes, grain yield, faba bean *Vicia faba*, vertisols

INTRODUCTION

Faba bean (*Vicia faba*) has been grown in various parts of the world including Ethiopia, which is the 2nd largest producer after China (Biruk, 2009). According to the CSA (2013), faba bean grows in the highland areas of northern and central Ethiopia and the total cultivated area and average yield of the crop in 2008/2009 was 538, 820.5 ha and 12.92 qt/ha, respectively. Regional shares of faba bean production area are 4.11%, 48.05%, 37.46%, and 10% for Tigray, Amhara Oromia, and SNNPR regions, respectively.

E-mail: birhaamg@gmail.com.

Faba bean (*V. faba* L.) is one of the major pulse crops grown in the highlands (1800 – 3000 m asl) of Ethiopia (Temesgen and Aemiro, 2012). Faba bean is a valuable protein-rich leguminous crop cultivated and consumed as human food in the specified areas. In addition, its straw is used as animal feed. With a cheap protein source, it partly compensates for the large deficiency in animal protein sources. Faba bean plays a significant role in improving the productivity of soil by fixing atmospheric nitrogen and is a suitable rotation crop for cereals as well as in interrupting disease and insect pest cycles (Barri and Shtaya, 2013).

Vertisols cover 10.3% (about 12.7 million ha) of the Ethiopian land mass and are the fourth most abundant soils after Histosols, Cambisols and Nitosols. It is estimated that Vertisols comprise about 24% of the country's cropped highland soils (Tekalign et al., 2002). Vertisols are potentially among the most productive soils of sub-Saharan Africa, but they are agriculturally underutilised within the traditional farming practices due to water logging during heavy rains. High moisture level limits faba bean production on vertisol as the crop is highly sensitive to water logged conditions (Getachew et al., 2003). Moreover, the problem of black root rot (*Fusarium solani*) is widely present in the vertisols (Beniwal and Dereje, 1987).

The highland area of Southern Tigray is suitable for the production of not only faba bean but also other pulse crops. In spite of this, however, faba bean production and productivity is by far below the genetic potential of the crop (MoARD, 2008). Low access of improved varieties and susceptibility of the available varieties of faba bean to water logging becomes the most important constraint in this region. The problem of water logging has resulted in the outbreak of not only root rot but also to chocolate spot and ascochyta blight (Couchman and Hollaway, 2016). The objective of this study was to evaluate faba bean genotypes for agronomic performance in vertisol areas of southern Tigray.

METHODOLOGY

Description of study area

The experiment was conducted at Hashenge and Aiba, located in the southern zone of Tigray Region and 148 and 102 km south of Mekelle (capital city of Tigray), respectively. These locations have an altitude of 2420 and 2700 m above sea level, respectively, and the soils are vertisols. The study areas were selected based on their suitability for faba bean production and presence of water logging condition.

Experimental materials

The materials used in this experiment comprised of 32 large seed size Faba bean genotypes (New, EK 05024-2, EK 05023-1, EK 05014-3, EK 05027-5, EK 05002-3, EK 05005-4, ET 07002-1, ET 07002-2, ET 07002-bulk, ET 07005-1, ET 07005-2, ET 07005-3, ET 07005-bulk, ET 07013-1, ET 07017-bulk, ET 07019-bulk, EH 06007-2, EH 06007-4, EH 06088-1, EH 06031-3, EH 06023-4, EH 06022-4, EH 06022-1, EH 06028-1, EH 06070-3, EH 06007-6, EH 06022-3, EH 06088-6 as well as Hachalu. Walki and local). These materials were sourced from Holleta Agricultural Research Center in 2014. The experiment was conducted using Alpha Lattice Design with two replications and plots of 2 m long and 2.4 m wide and inter- and intra-row spacings of 40 and 10 cm, respectively. Four rows were harvested for yield and yield component evaluation. DAP fertilizer at the rate of 100 kg/ha was applied at planting. During growth, data were recorded on various agronomical traits including days to maturity, stand count at harvest, plant height, number of pods per plant, number of seeds per pod, grain yield, thousand seed weight, and diseases reaction.

Statistical analysis

Data collected from the experiment were analyzed using SAS (1999) for the analysis of variance and Minitab Version 14 (Minitab 1998) for Multivariate analysis (cluster analysis) statistical packages. Analysis of variance (ANOVA) and Pearson correlation analyses were performed according to the methods described by Gomez and Gomez (1984). All the quantitative and qualitative data were used for principal component analysis (PCA) and cluster analyses. The mean data were standardized prior to multivariate analysis to eliminate the effects resulting from using different scales. To separate the 32 genotypes into groups and to evaluate the patterns of similarity and dissimilarity, the data were subjected to cluster analysis according to Gower distance (Gower 1971), using PAST software version 2.15 (Hammer et al., 2001). Principal component analysis (PCA) of the correlation matrix was performed with the same software to determine the sources of variation among genotypes.

RESULTS AND DISCUSSION

Seed size and disease reaction of faba bean genotypes

Since seed size, grain yield and disease reaction are the most important traits for pulse crops in general and faba bean crop in particular, analysis was focused to these characters, Based on this, there was highly significant difference (*P<0.01*) for thousand seed weight at Hashenge and Aiba locations. Genotype EH 06007-2 scored highest 1000 seed weight (1111 g) followed by EH 06088-6 (971.5) and EH 06007-4 (938.5 g) over locations. On the other hand, least seed size was recorded from the local (505.5 gm) genotype followed by the standard check Walki (587.3) and ET 07017-bulk (648.0 g) genotypes. From the tested genotypes, about 25 genotypes scored more 1000 seed weight than the standard check (Hachalu) but the other standard checks (Walki and local) showed least seed weight (Table 1).

The most important diseases affecting faba bean at Aiba and Hashenge locations are Chocolate spot, Ascochyta blight and Faba bean gall (Teklay et al., 2014). In 2015, these diseases did not occur at Hashenge. For this, disease severity scoring was taken only at Aiba location for Chocolate Spot and Ascochyta Blight (Table 1).

As indicated in Table 1, the analysis of variance for

Table 1. Thousand Seed weight, Grain yield and disease severity score of Faba Bean Genotypes grown at Hashenge and Aiba in 2015.

Genotype	Thousand seed weight (g)			Grain yield (kg/ha)			Disease score at Aiba (0 – 9)	
	Hashenge	Aiba	Mean	Hashenge	Aiba	Mean	Chocolate spot	Ascochyta blight
EH 06007-2	1102	1120.	1111.3	3716	4774	4245	2.5	2
EH 06088-6	1012	931	971.5	3399	4236	3818	4	3
ET 07005-2	947	802.5	874.8	3301	4604	3953	2.5	2.5
EH 06070-3	946	857.5	901.8	3073	3587	3330	4	2
ET 07005-1	937.5	815	876.3	3808	5785	4797	4.5	2
EH 06007-6	923	862	892.5	3225	4680	3953	3	2.5
EH 06022-1	906.5	706	806.3	3251	2519	2885	3	2
EH 06088-1	900.5	843	871.8	3514	5477	4496	2.5	2
ET 07002-bulk	899.5	824	861.8	3487	3643	3565	4.5	2
EH 06028-1	872.5	918	895.3	2868	2778	2823	4	2.5
EH 06022-3	863.5	762	812.8	3733	4161	3947	2	1.5
EH 06007-4	837.5	1039.	938.5	3273	4994	4134	3	1.5
ET 07005-3	836	883.5	859.8	3770	4501	4136	2.5	1
EH 06022-4	835	792	813.5	3484	3555	3520	4	2
EK 05027-5	834	759	796.5	3364	2557	2961	4	2.5
ET 07002-2	834	776.5	805.3	3677	3685	3681	5	3
EH 06023-4	831.5	757.5	794.5	3513	5315	4414	2.5	2
EH 06031-3	815.5	720	767.8	3414	3852	3633	4	2.5
New	813	818.5	815.8	3318	4719	4019	3.5	2.5
EK 05024-2	810	833.5	821.8	3849	5279	4564	3	2.5
ET 07019-bulk	804.5	808	806.3	3535	4605	4070	3.5	3
ET 07002-1	769.5	735.5	752.5	3773	3610	3692	3.5	3
EK 05023-1	766	668.5	717.3	3898	4381	4140	2	1.5
Hachalu	736.5	655.5	696.0	3583	4041	3812	2.5	2
ET 07013-1	724.5	669	696.8	3627	5931	4779	2.5	1.5
EK 05014-3	721	687	704.0	3566	5069	4318	2	1.5
ET 07005-bulk	715.5	751	733.3	3484	4404	3944	3.5	3
EK 05005-4	696.5	651	673.8	3684	2720	3202	5	2.5
EK 05002-3	685	591	638.0	3575	2915	3245	3.5	2
Walki	647.5	527	587.3	4218	4367	4293	4.5	2.5
ET 07017-bulk	642	654	648.0	2918	3726	3322	4	3
Local	517	494	505.5	3838	3497	3668	4	2.5
S. E (m)	50.2	47.94	48.01	5.93	8.53		0.97	0.62
LSD (5%)	115.4***	110.2***	44.2 ***	NS	17.43 *		NS	NS
CV (%)	6.14	6.21	6.04	16.89	20.1		29	27.6

* implies significant difference at 0.05, Disease score of 0 = most resistant; Disease score of 9 = most susceptible.

disease reaction has shown non-significant difference. Accordingly, although the severity scores of chocolate spot for all the genotypes were slightly higher than those for ascochyta blight, the scores were in the range of 1 to 5 for both diseases thus indicating resistant to moderately resistant disease reaction.

Variations of faba bean genotypes for grain yield

The analysis of variance for grain yield has showed significant difference ($p<0.05$) among genotypes at Aiba location but no significant difference in Hashenge. At Aiba location, 15 genotypes gave statistically more grain yield than the first standard check of walki (43.66 qt/ha). Accordingly, genotype ET 07013-1 gave the highest grain yield (59.31 qt/ha) followed by genotype ET 07005-1 (57.85) and EH 06088-1 (54.77 qt/ha).

Even though no statistical significant difference has showed for grain yield at Hashenge, the above genotypes (ET 07013-1, ET 07005-1 and EH06088-1) scored higher grain yield as 36.27, 38.08 and 35.14 qt/ha in that order. In this location, the highest grain yielder gave 42 18 qt/ha, which indicated a very similar in yielding potential

Table 2. Pearson correlation coefficients for 8 quantitative traits of 32 faba bean genotypes grown in 2015 at Hashenge and Aiba.

Traits	DM	SCH	NTPP	PHT	NPPP	NSPP	GY	TSW
DM	**1**							
SCH	0.085	**1**						
NTPP	0.679***	-0.238	**1**					
PHT	0.079	0.100	0.091	**1**				
NPPP	-0.377*	-0.063	0.068	0.116	**1**			
NSPP	0.606***	0.107	0.434*	0.223	-0.220	**1**		
GY	0.072	0.087	0.272	0..493**	0.369*	0.249	**1**	
TSW	0.624***	-0.214	0.427*	-0.016	-0.487**	0.567***	0.100	**1**

DM: Days to mature; SCH: Stand Count at Harvest; NTPP: Number of Tillers per Plant: PHT: Plant height; NPPP: Number of pods per plant; NSPP: Number of seeds per pod; TSW: Thousand seed weight and GY for Grain yield. ***, ** and * significant at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, respectively.

with the highest yielder genotype. In general, the genotypes that showed significant yield difference at Aiba had also higher and reasonable grain yield at Hashenge location (Table 1).

Correlation analysis

Pearson correlation coefficients between different pairs of characters were computed and are shown in table 2. The correlation coefficients of the 1000 seed weight trait showed that it was positively and significantly correlated ($P<0.01$) with days to maturity (0.624), number of seeds per pod (0.567) and number of tillers per plant (0.427). On the other hand, thousand seed weight was highly and negatively correlated with number of pods per plant (-0.487). As pods of faba bean per plant increases, seed size decreased to accommodate more pod clusters in a node. Similarly, highest and positive significant correlations were found between number of tillers per plant and days to maturity (0.679), number of seeds per pod and days to maturity (0.606).and grain yield with plant height (0.49). These results reflected the importance of days to maturity, number of seeds per pod and number of pods per plant in the determination of thousand seed weight in faba bean. Al Barri and Shtaya (2013) reported the importance of number of seeds per pod and number of pods per plant on 100 seed weight determination.

Cluster analysis

Cluster analysis was used to further investigate the inter-relationships of the genotypes using eight agronomic traits (days to maturity; stand count at harvest; number of tillers per plant: plant height; number of pods per plant; number of seeds per pod; thousand seed weight and grain yield). The genotypes were grouped into two main clusters (A and B). Main cluster A was also divided into four sub clusters and cluster B was divided in to three sub clusters (Figure 1).

Among the seven clusters, cluster I comprised of 7 genotypes (ET 07005-1, EH 06023-4, ET 07005-3, ET 07019 - -bulk, New, ET 07005-bulk and ET 07005-2) that have similar maturity time and number of tillers per plant. From this cluster, genotypes ET 07005-3 and New had the lowest dendrogram distance (1.21). Cluster II comprised six genotypes, including two standard checks and they were characterized by relatively early maturation, high number of pods per plant and more grain yield but lower thousand seed weight. The third cluster that consisted of six genotypes had highest thousand seed weight, more number of seeds per pod and late matured varieties. The genotypes in the fourth cluster were characterized by lowest thousand seed weight and lowest number of seeds per pod. The local check with its lowest seed size was included in this cluster. Cluster V that consisted of only one genotype (ET 06007- 6) has showed late maturation, poor in stand establishment but highest in tillering capacity. This cluster has more dendrogram distance (24.18) than others. On the other hand, cluster VI, comprising of five genotypes has less number of pods per plant. Finally, the lowest grain yield was recorded from the genotype in the seventh cluster. This cluster which includes only two genotypes also had low stand count at harvest and short plant height. Even though these genotypes were moderately resistant to Ascochyta blight and Chocolate spot diseases, more scores were recorded from them.

To evaluate the genetic variability or similarity of the genotypes among the clusters, the inter-cluster Gower distance and relationships were calculated using PAST software (Table 3). The highest distance (0.692) was between cluster V and IV, which suggested that the members of these clusters diverge on most of the studied traits and could be used in breeding programs. The lowest distance (0.148) was between cluster III and cluster I, suggesting that their maternal origin may be

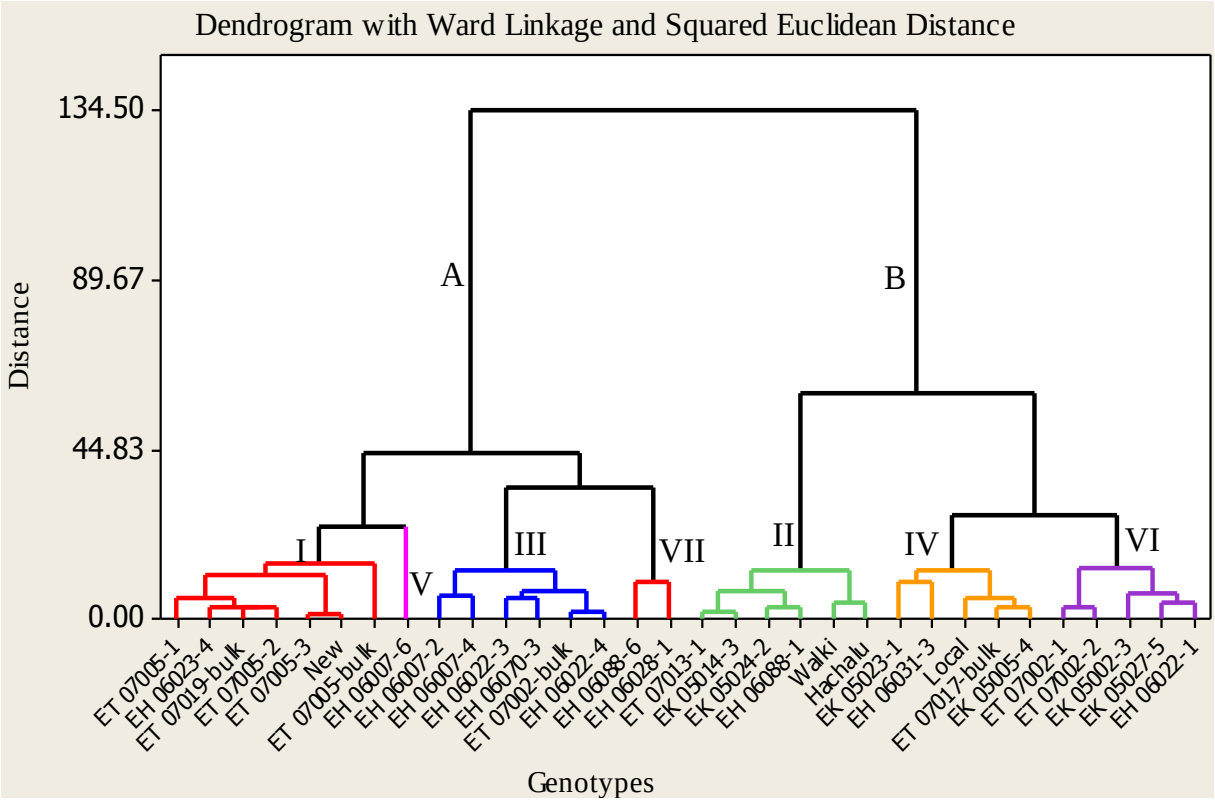

Figure 1. Cluster analysis showing relationships among faba bean genotypes determined on the basis of 8 yield traits.

Table 3. Gower distance and similarities among seven clusters of 32 genotypes of faba bean.

Cluster	I	II	III	IV	V	VI	VII
I	0						
II	0.387	0					
III	**0.148**	0.497	0				
IV	0.535	0.335	0.505	0			
V	0.285	0.601	0.321	**0.692**	0		
VI	0.393	0.327	0.365	0.272	0.544	0	
VII	0.370	0.580	0.346	0.465	0.444	0.378	0

very closely related (Kumar et al., 2013).

Principal component analysis

Principal component analysis (PCA) was used to identify hidden patterns in the data and was performed to obtain more reliable information on how to identify groups of genotypes that have desirable yield traits for breeding. Eight components were extracted from the 8 studied traits by PCA analysis. But based on Diana (1999 as cited from Kaiser, 1960), factors to be retained should have more than 1 eigenvalues, at least 5% variance explained for each component, and/or more than 75% cumulative proportion of variance explained.

The results (Table 4) indicated that the first four components accounted for 83.7% of the total variation, whereas, the remaining 4 components accounted for only 16.3% of the morpho-agronomic diversity. PC I explained the most variability (36.1%), followed by PC II (22.3%), PC III (15.4%) and PC IV (10.0%). In the first principal component, DM, TSW, NSPP and NTPP were more important traits contributing more to the variation and this component was more associated with the high values of the above traits negatively.

The sign indicates the direction of the relationship

Table 4. Eigenvalues, proportion of variance and cumulative variance for 8 quantitative characters in Faba bean genotypes.

Character	PC1	PC2	PC3	PC4
DM	-0.520	0.086	-0.074	-0.280
SCH	0.038	-0.104	-0.791	-0.471
NTPP	-0.429	-0.152	0.389	-0.385
PHT	-0.118	-0.515	-0.240	0.552
NPPP	0.229	-0.512	0.341	-0.402
NSPP	-0.470	-0.087	-0.191	-0.024
GY	-0.154	-0.620	-0.002	0.099
TSW	-0.482	0.200	0.080	0.274
Eigenvalue	2.8847	1.7873	1.2303	0.7966
% of total variance	36.1	22.3	15.4	10.0
Cumulative variance	36.1	58.4	73.8	83.7

* DM: Days to mature; SCH: Stand count at harvest; NTPP: Number of tillers per plant: PHT: Plant height; NPPP: Number of pods per plant; NSPP: Number of seeds per pod; TSW: Thousand seed weight and GY for grain yield.

between the components and the characters (Yemane and Fasil, 2002). Due to more variation explained by the PC 1 (Table 4), its scores could effectively represent the genotype effect (Ali et al., 2011). In the second principal component, the observed variation (22.3%) was caused mainly by GY, PHT, NPPP and TSW and of which, TSW had positive relationship with this PC. PC III was positively dominated by the effect of number of tillers per plant and number of pods per plant and negatively by stand count at harvest. On the other hand, Plant height, stand count at harvest and number of pods per plant in the fourth principal component constituted large part of the total variation. In this experiment, the PC analysis ultimately showed the amount of variability for the traits that could be used for the improvement of large seed sized faba bean genotypes.

Conclusion

The combined analysis of variance for thousand seed weight indicated that there were highly significant ($p < 0.01$) differences among genotypes over locations. Genotype EH 06007-2 ranked highest in thousand seed weight followed by EH 06088-6 and EH 06007-4 as 1111, 971.5 and 938.5 g, respectively. The standard check (Hachalu) recorded low thousand seed weight (696 g) as compared with most faba bean genotypes under study. In addition, genotypes that out yielded the standard checks (Walki and Hachalu) in grain yield also had better seed size and lower disease reaction. In 2015, the faba bean crop was not attacked by diseases in Hashenge location and severity scores at Aiba location for Chocolate Spot and Ascochyta Blight were generally low to moderate.

Based on the inter-cluster Gower distance and relationships, the highest distance (0.692) was between cluster V and IV, which suggested that the members of these clusters diverge on most of the studied traits and could be used in breeding programs. The lowest distance (0.148) was between cluster III and cluster I, suggesting that their maternal origin may be very closely related. PCA showed that the first 4 PC accounted for 83.7% of the variability.

Conflict of Interests

The authors have not declared any conflict of interest.

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Allelopathic activity of extracts of *Citharexylum spinosum* L. from Tunisia

A. El Ayeb-Zakhama* and F. Harzallah-Skhiri

Laboratory of Genetic Biodiversity and Valorisation of Bioressources (LR11ES41), High Institute of Biotechnology, Tahar Haddad street, Monastir 5000, University of Monastir, Tunisia.

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Allelopathic potential of *Citharexylum spinosum* L. (Verbenaceae) an exotic tree introduced in Tunisia many years ago was evaluated. Organic extracts using hexane, ethyl acetate and methanol solvents together with aqueous extracts at different concentrations were prepared from different parts of the plant (roots, stems, leaves and flowers). Yields in the 12 organic extracts together with their phenol contents were reported. Leaves methanol extract showed the highest yield and amount in total phenols (6.43%; 617.93±1.12 mg gallic acid equivalent/100 g MS, respectively). All extracts were tested on germination and early growth of two crops: (*Lactuca sativa* L.) and (*Triticum aestivum* L.) and two weeds: (*Peganum harmala* L.) and (*Silybum marianum* L.). Twelve parameters were established and used to the principal components analysis (PCA) and the hierarchical clusters (HCA) analysis. Three groups of extracts were separated according to their allelopathic potentiality. Almost of organic extracts were totally opposed to seed germination of peganum and thistle.

Key words: Allelopathy, bioherbicides, *Citharexylum spinosum* L., crops, extracts, polyphenols, weed management.

INTRODUCTION

The overuse of herbicides has provoked increasing incidences of herbicide resistance in weeds (Valverde et al., 2000) and so disappearance of some susceptible species, which affect biodiversity (Itoh, 2004). Moreover, herbicides cause environmental pollution, unsafe agricultural products and human health concerns (Kohli et al., 1998; Xuan et al., 2004, 2005; Khanh et al., 2005). In response to this problem; the adverse effect of herbicides on people and environment, and the interest in environmentally friendly alternatives for weed control have rapidly increased in recent years (Amossé et al., 2013; Dommanget et al., 2014; Kruidhof et al., 2014). This research mainly focused on strategies of Integrated Weed Management System. Among the possible new strategies, agronomic solutions based on the use of plant natural compounds have been suggested (Dudai et al., 1999; Tworkoski, 2002; Campiglia et al., 2007). This approach would mainly rely on the exploitation of allelopathic effects. Allelopathy is defined as “any process that involves secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of biological systems” (IAS, 1996). Chemicals that impose allelopathic influences are called allelochemicals or allelochemics (Einhelling, 1996).

*Corresponding author. E-mail: ayeb.asma@gmail.com. Tel: +21653787605.

Table 1. Aqueous and organic extracts used tested for allelopathic tests and their abbreviations.

Aqueous and organic extracts for allelopathic test	Abbreviation
Control for aqueous extracts	AC
Aqueous Root extracts at 1, 2, 3 and 4 g/l	AR1, AR2, AR3, AR4
Aqueous Shoot extracts at 1, 2, 3 and 4 g/l	AS1, AS2, AS3, AS4
Aqueous Leaves extracts at 1, 2, 3 and 4 g/l	AL1, AL2, AL3, AL4
Aqueous Flowers extracts 1, 2, 3 and 4 g/l	AFl1, AFl2, AFl3, AFl4
Control for organic extracts	OC
Root, stem, leaf, flower hexane extracts	Rhex, Shex, Lhex, Flhex
Root, stem, leaf, flower ethyl acetate extracts	Reac, Seac, Leac, Fleac
Root, stem, leaf, flower methanol extract	Rmet, Smet, Lmet, Flmet

These chemicals are largely classified as secondary plant metabolites (Rice, 1984). Allelochemicals are present practically in all plant tissues. They may be released from plants into their immediate environment (El-Khawas and Shehata, 2005; Bulut et al., 2006). These chemicals may exert their phytotoxic effect directly or indirectly as they selectively inhibit the growth of other plants, soil microorganisms or both (Lorenzo et al., 2013; Saraf et al., 2014). Originally, classified as waste products, allelochemicals more recently have been investigated extensively by ecologists and pharmacologists, and many complexes biological functions have been discovered (Hadacek, 2002). Now it has been established that allelopathic properties of plants can be exploited successfully as a tool for weed control (Mahajan and Chauhan, 2013). The advantage of utilizing natural compounds in sustainable agriculture patterns such as organic farming depends on their rapid decomposition in the environment (Tworkoski, 2002; Campiglia et al., 2007). *Citharexylum spinosum* L is one tree among many that produces sufficient biomass with allelopathic extracts that can be exploited for weed control purposes.

Citharexylum spinosum (syn. *Citharexylum quadrangular* Jacq. and *Citharexylum fruticosum* L.) (Verbenaceae Family) (Wagner et al., 1999) is native to the Caribbean (Turner and Wasson, 1997) introduced in Tunisia for many years and cultivated along the roadsides and in gardens. This tree possesses medicinal properties and was useful in the treatment of various ailments. A decoction of young twigs was used for children thrush and bark decoction for treating colds (Cordero, 1978; Lachman-White et al., 1992). The leaves were used as a source of an antiallergic and as an alternative in hepatic disorders (Balázs et al., 2006). *C. spinosum* was used with other plants as anthelmintic (Lans, 2007).

Research information on the allelopathic potential of *C. spinosum* is relatively paltry. So, the aim of this present study was to determine the allelopathic potential of *C. spinosum* aqueous and organic extracts on the seed germination and early growth of four target seeds; wheat (*Triticum aestivum* L.), lettuce (*Lactuca sativa* L.), harmal (*Peganum harmala* L.) and milk thistle (*Silybum marianum* L.).

MATERIALS AND METHODS

Plant material

C. spinosum different organs (roots, stems, leaves and flowers) were collected in the garden of the High Institute of Biotechnology of Monastir (latitude 35° 46' 0''N, longitude 10° 59' 0'' E, coastal region, East of Tunisia, with a sub humid climate). A voucher specimen (CQV 12) was deposited at the Herbarium of the Laboratory of Botanic in the Institute. Roots were cleaned with tap water, and all the plant parts were air-dried in a shaded area at ambient temperature. Dried material was ground into a powder to pass through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and stored at 4°C until use.

Preparation of aqueous and organic extracts

One hundred grams of powder from each dried plant part (roots, stems, leaves and flowers) were separately extracted by soaking in 1 L distilled water at ambient temperature for 24 h (Khanh et al., 2005). The aqueous extracts were filtered through a double layered muslin cloth followed by Whatman no. 1 filter paper and then passed through 0.22 µm micro-filter pore size to remove bacteria. Filtrates were preserved at 4ºC. Each crude aqueous extract at 10% (m/v) was diluted with sterile distilled water to give final concentrations of 1, 2, 3 and 4% (m/v) (Table 1). The 16 extracts were used freshly within a week (Omezzine et al., 2011; El Ayeb et al., 2013).

Sequential extraction was carried out in organic solvents with rising polarity: hexane, ethyl acetate and methanol. One hundred grams of powder were immersed in the appropriate solvent for 7 days at room temperature. The 12 organic extracts (Table 1) were evaporated to dryness under reduced pressure in a rotary evaporator at 45°C, to remove the solvent. After determination of the yield the extracts were stored at 4°C until use.

Determination of the total polyphenol content

The content in total polyphenol in each organic extract was measured by spectrophotometric method based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was of the Folin-Ciocalteu's phenol reagent (Merck) (Singleton and Rossi, 1965; AOAC, 1984). 50 µL of the diluted extract (1 mg/1 mL of

methanol) was added to 750 µL of distilled water/Folin-Ciocalteu solution (28:2 v:v). After 3 min, 200 µL of sodium carbonate solution (20% in distilled water) was added and the test tubes were properly shaken before incubating in a boiling water bath for 1 min. The tubes were then allowed to cool in the dark at ambient conditions for 30 min to complete the reaction. For the control sample, 50 µL of methanol was used. The absorbance was measured at 765 nm. Tests were carried out in triplicate. Quantification was obtained by reporting the absorbance in the calibration curve prepared with gallic acid solutions ranging 0.01 to 0.1 mg/ml, results are expressed as mg of gallic acid equivalent (GAE) per gram of extract.

Allelopathic bioassays

Bioassays with aqueous extracts

Four target species; two crops, lettuce and wheat and two weeds; milk thistle and harmal were used to test germination and early growth responses. Lettuce has been used as a test plant because it was too sensitive to chemicals at low concentration (Olofsdotter, 2001). Wheat has been used because it was one of the most important agricultural foods and feed crops worldwide (Högy and Fangmeier, 2008) and milk thistle is a well known competitive weed for crops.

Five millilitres of each diluted aqueous extract was added onto three layers of Whatman no.1 sterilized filter paper, lined on the bottom of a sterile Petri dish (90 mm) and allowed to dry under reduced pressure. All target seeds were surface sterilized by immersing in 0.525 g L^{-1} of sodium hypochlorite for 5 min, rinsed in sterile deionised water four times and soaked in the last water bath at 22°C for 4 h. Preliminary essays prove that the bleach did not inhibit germination. Thirty swollen seeds of each species were sown in each Petri dish where the filter paper was moistened with 5 mL of sterile distilled water and kept in a growth chamber to germinate in the dark with an average temperature of 23 ± 2°C for 7 days. Distilled water was the control (AC) (Table 1). The experimental design was a randomized complete block replicated three times. A seed was considered germinated when the radical protruded ≥ 2 mm.

Bioassays with organic extracts

The 12 dried organic extracts were dissolved in methanol to compare their phytotoxic effects. Five millilitres of each extract dissolved at 6000 ppm (6 mg mL^{-1}), were added to 3 sheets of filter paper displayed in a Petri dish (90 mm) and evaporated to dryness for 24 h at 24°C. The filter paper was moistened with 5 mL of sterile distilled water and then thirty imbibed seeds from each target species were arranged in each Petri dish and allowed to grow in a growth chamber in the dark at 23 ± 2°C for 7 days. Treatments were arranged in a completely randomized design with three replications. Test conditions were identical to the previous bioassay. Control Petri dishes contained only methanol and distilled water (OC) (Table 1).

Statistical analysis

Percentage of germinated seeds was recorded and the root and shoot lengths were measured for all seedlings in each Petri dish on day 7 after placing the seeds on the medium. The data were transformed to percent of control for analysis. Data from the experiments were transformed using arcsin-square root (arcsine √x) to conform with assumptions of normality for analysis of variance (ANOVA) using SPSS 12.0, for Windows program. The significance of the differences between means was determined at $P < 0.05$ using *Duncans's* multiple range tests. We evaluated whether the type of extract (or group of extracts) was useful in reflecting its phytoxic effect on the germination and the early growth of each target seed species. The data obtained for all parameters in accordance with all extracts tested were subjected to Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) using SPSS 12.0 software (SPSS Inc. Chacago, IL, USA).

RESULTS

Yields and total polyphenol contents in the organic extracts

Yields in the 12 organic extracts together with their polyphenol contents were reported in Table 2. Leaves have the highest yield with the three organic solvents; hexane, ethyl acetate, and methanol (0.91, 1.31 and 6.43%, respectively) followed by flowers then stems and finally roots. On the other hand, methanol gave the highest yield in all the plant parts analyzed. According to Folin Ciocalteau test, the different extracts from the different organs contained phenols. Leaf, flower, root and stem methanol extracts showed the highest amount with 617.93±1.12, 346.85±2.38, 134.95±0.69 and 94.77±0.78 mg GAE/g extract. For all the other extracts contents in total phenol were low and varied between 3.72±0.02 and 32.51±1.21 mg GAE/g.

Allelopathic activity of aqueous and organic extracts

To evaluate the allelopathic effects of organic and aqueous *C. spinosum* extracts, data recorded were subjected to Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). The results indicated that, extracts have varying degree of inhibitory or stimulatory effect on germination and seedling growth. Effects of organic and aqueous extracts on germination and early growth of the 4 target seeds tested were reported in Table 3.

The HCA (data not given) based on the *Euclidean* distance between groups indicated three groups of extracts; Groups 1, 2 and 3, identified by the parameters with which they correlate. Data were reported in Table 3 according to the HCA analysis. The 3 groups were separated according to their allelopathic activity. Those groups clearly stand out forming separate groups and responses for tested parameters were different from one group to another. The group 3 was divided into 2 subgroups 3A and 3B, 3A was divided into subgroups 3Aa, 3Ab, 3B was subdivided into subgroups 3Ba and 3Bb. The later (3Bb) still divided into subgroups 3Bb1 and 3Bb2.

Group 1 and subgroups 1A and 1B

The group 1 represented by controls (AC, OC) and root

Table 2. Yields and total polyphenol contents in organic extracts from the different organs of *C. spinosum* L.

Organ and solvent extract	Total phenols (mg gallic acid equivalent/100 g MS)	Yield (%)
*Rhex	3.72±0.02	0.27
Reac	5.33±0.96	0.32
Rmet	134.95 ±0.69	1.60
Shex	4.92±0.04	0.27
Seac	18.27±0.00	0.88
Smet	94.77±0.78	2.05
Lhex	22.33±1.73	0.91
Leac	29.28±6.03	1.31
Lmet	617.93±1.12	6.43
Flhex	14.37±0.21	0.53
Fleac	32.51±1.21	1.23
Flmet	346.85±2.38	5.94

*For abbreviation see Table 1.

aqueous extracts at 1 and 2% (AR1 and AR2) correlated with all parameters related to germination and seedlings growth. We note however that the germination percentage of harmal seeds was between 88.3 to 100%, for milk thistle it was equal to 90 and 85%, in AC and OC, respectively. In contact with AR1 and AR2 extracts, the germination percentages of target seeds were slightly reduced compared to control (93.3-95, 100, 83.3-93 and 83.3, respectively for lettuce, wheat, harmal and milk thistle seeds). Root elongation was also close to control for lettuce (92.6 to 100.7% of control), wheat (93.6 to 95.6% of control), and milk thistle (82 to 90% of control). Root elongation of harmal was more reduced (67.8 to 69.3% of control). Those extracts highly correlate with the elongation of lettuce, wheat and harmal shoot seedlings and so stimulate their development in percentages exceeding the control (126.8-138.3, 109.3-115.4 and 148.7-156.7, respectively). Nevertheless development of milk thistle was slightly less than the control (74.4-95.5% of control).

Group 2 (Rmet, Smet, Flmet)

The group 2 consisting of the three methanol extracts from roots (Rmet), stems (Smet) and from flowers (Flmet), was totally opposed to the germination of harmal and milk thistle seeds and so there is no seedling development. On the other side, those extracts correlated with the germination of wheat seeds (90-100%) and least for the germination of lettuce seeds (88.3-96.7%) and with shoot and root lettuce seedlings elongation. The development of these seedlings (16.5-41.8% of control for root, 23.8-31.9% of control for shoot), was more important than that of wheat (7.2-15.4 and 11.4-22.9% of control).

Group 3Aa (Rhex, Reac, Shex)

Ethyl acetate and hexane root extracts and hexane stem extract were totally opposed to seed germination of milk thistle and harmal (0%) but correlated with wheat and lettuce seeds germination (95-96.7 and 100%, respectively). The development of lettuce and wheat root seedlings was moderately reduced (93.9-74.0% of control, and 53.7-71.6% of control, respectively). Unlike the shoot of wheat seedlings was more elongated than this of lettuce ones (99.2-99.5% of control and 60-79% of control, respectively).

Groupe 3Ab (Lmet, Fleac, Seac)

Germination of lettuce and wheat seeds were weakly inhibited (75-88.3%) in contact with those extracts, which were almost totally opposed to germination of milk thistle seeds (0-6.7%). Germination of harmal seeds was correlated with Fleac and Seac extracts and reached 90 and 83.3%, respectively, but was weakly correlated with Lmet extract (12.2%), solely all harmal seedlings grow slightly. The development of lettuce seedlings was highly reduced in Lmet and Fleac extracts (14.4-27.8% of control for roots; 16.7-49.1% of control for shoots) compared to those of wheat (39.3-49.4% of control for roots 89.6-97.5% of control for shoots). Seedlings of the two target plants (lettuce and wheat) were less sensitive to Seac extract.

Group 3B

All extracts (17) from this group correlate with germination percentages of lettuce, wheat and harmal

Table 3. Percentages germination (%G) of *L. sativa* (Lac), *T. aestivum* (Tri), *P. harmala (*Peg), and *S. marianum* (Sil) seeds tested in presence of *C. spinosum* organic (6 mg/ml) and aqueous extracts (1, 2, 3 and 4%; m/v) and root (Re) and shoot (Se) elongation of their seedlings in percent of control. The table was established according the Hierarchical Clusters Analysis (HCA).

Groups/ subgroups			Aq/Org. Ext.		*Lactuca sativa* (Lettuce)			*Triticum aestivum* (Wheat)			*Peganum harmala* (Harmal)			*Silybum marianum* (Milk thistle)		
					%GLac	LacRe	LacSe	%GTri	TriRe	TriSe	%GPeg	PegRe	PegSe	%GSil	SilRe	SilSe
Group 1	1A		*AC		100±0.0^{h}	100±0.0^{k}	100±0.0^{g-i}	100±0.0^{d}	100±0.0^{n}	100±0.0^{cd}	88.3±1.3^{cde}	100±0.0^{m}	100±0.0^{f}	90±0.0^{fg}	100±0.0^{i}	100±0.0^{h}
			OC		100±0.0^{h}	100±0.0^{k}	100±0.0^{g-i}	100±0.0^{d}	100±0.0^{n}	100±0.0^{cd}	100±0.0^{f}	100±0.0^{m}	100±0.0^{f}	85±0.0^{fg}	100±0.0^{i}	100±0.0^{h}
	1B		AR1		95.0±0.0^{e-h}	100.7±6.4	126.8±3.7^{j-m}	100±0.0^{d}	95.6±0.6^{k-n}	109.3±5.3^{c-e}	83.3±0.5^{cd}	67.8±7.7^{hi}	148.7±5.2^{ij}	83.3±0.1^{i}	90±0.7^{h}	95.5±4.6^{h}
			AR2		93.3±2.8^{d-g}	92.6±8.3^{jk}	138.3±9.9^{mn}	100±0.0^{d}	93.6±6.0^{j-n}	115.4±7.7^{f}	93.3±1.2^{de}	69.3±7.6^{hi}	156.7±3.5^{ik}	83.3±5.8^{i}	82±1.2^{g}	74.4±3.7^{g}
Group 2			Rmet		96.7±0.1^{f-h}	41.8±6.6^{de}	31.9±1.6^{a}	100±0.0^{d}	9.3±1.5^{a}	17.0±3.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	05±1.0^{bc}	0.3±0.5^{a}	0±0.0^{a}
			Smet		88.3±0.$^{5b-d}$	32.6±1.8^{cd}	23.8±3.3^{a}	100±0.0^{d}	7.2±1.9^{a}	22.9±3.4^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0.0±0.0^{a}	0±0.0^{a}
			Flmet		90±0.0^{b-e}	16.5±2.1^{ab}	29.2±5.7^{a}	90±0.0^{a}	15.4±4.5^{a}	11.4±2.6^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0.0±0.0^{a}	0±0.0^{a}
			Rhex		95.0±0.1^{e-h}	93.9±7.2^{jk}	75.4±6.3^{d-f}	100±0.0^{d}	58.9±1.5^{de}	99.5±0.3^{cd}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
	3Aa		Shex		96.7±1.6^{f-h}	88.9±0.1^{h-k}	79.0±2.5^{ef}	100±0.0^{d}	71.6±5.5^{fg}	99.3±0.1^{cd}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
			Reac		95.0±2.1^{e-h}	74±4.1^{fg}	60±9.7^{b-d}	100±0.0^{d}	53.7±7.5^{cd}	99.2±0.3^{cd}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
3A			Lmet		88.3±3.3^{b-d}	27.8±0.5^{bc}	49.1±1.6^{b}	100±0.0^{d}	49.4±2.1^{cd}	97.5±1.0^{cd}	12.2±0.2^{b}	0.6±0.4^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
	3Ab		Fleac		86.7±2.8^{bc}	14.4±2.3^{a}	16.7±6.1^{a}	100±0.0^{d}	39.3±0.5^{b}	89.6±2.1^{bc}	90±0.0^{de}	2.5±0.8^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
			Seac		75.0±5.1^{a}	90.0±2.3^{i-k}	67.4±3.2^{c-e}	100±0.0^{d}	70.1±2.0^{fg}	97.4±0.2^{cd}	83.3±2.3^{cd}	5.7±2.1^{ab}	4.9±4.5^{a}	6.7±0.6^{ab}	0.4±0.3^{a}	2.5±4.2^{a}
			AR3		95±0.0^{e-h}	86.6±4.4^{g-k}	150.2±7.0^{n-p}	100±0.0^{d}	84.1±4.3^{h-j}	117.7±6.2^{f}	95.0±0.3^{ef}	82.3±8.1^{jk}	190.9±1.2^{o}	53.3±2.9^{f}	37.2±9.9^{e}	42.6±1.1^{e}
			AFl1		95±0.6^{e-h}	91.9±3.3^{jk}	136.1±9.8^{mn}	98.3±2.8^{cd}	87.9±6.4^{h-l}	111.3±1.2^{c-e}	100±0.0^{f}	73.4±3.7^{ij}	113.6±9.5^{g}	36.7±5.7^{de}	12.4±6^{b}	24.6±6.9^{c}
	3Ba	3Ba1	AS1		95±5.0^{e-h}	94.9±0.5^{jk}	131±2.1^{k-m}	96.7±5.7^{b-d}	99.6±9.6^{mn}	98.7±0.6^{cd}	95.0±5.2^{ef}	89.6±8.2^{kl}	183.1±2.3^{no}	70.0±9.9^{h}	45±5.5^{f}	53.5±4.2^{f}
			AS2		93.3±5.7^{d-g}	84.2±2.2^{g-j}	144.2±2.2^{m-o}	93.3±3.3^{a-d}	96.6±2.6^{i-n}	98.6±5.2^{cd}	93.3±0.8^{ef}	93.3±5.7^{lm}	167.3±3.2^{k-m}	63.3±5.8^{gh}	24.1±2.7^{d}	42.8±3.4^{e}
Group 3			AS3		91.7±0.3^{c-f}	77.9±0.5^{f-i}	163.9±1.4^{p}	92.2±5.0^{a-c}	88.7±3.6^{h-m}	88.2±2.9^{bc}	83.3±0.3^{cd}	66.7±8.5^{hi}	130.5±2.9^{h}	40±0.0^{e}	18.4±2.0^{c}	39.6±0.6^{de}
		3Ba2	AR4		88.3±1.2^{b-d}	75.9±4.2^{f-h}	158.1±7.7^{op}	100±0.0^{d}	78.6±4.6^{gh}	117±1.2^{f}	95±0.0^{de}	86.1±3.4^{kl}	208.2±9.3^{p}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
				AL2	95.0±0.5^{e-h}	54.1±5.1^{e}	117.2±6.2^{i-l}	92.2±6.9^{a-c}	71.3±1.6^{fg}	110±0.5^{c-e}	95.0±0.0^{ef}	74.2±1.7^{ij}	172.7±4.1^{l-n}	6.7±5.8^{ab}	3.2±1.6^{a}	5.4±5.3^{a}
			3Bb11	AL3	90.0±1.6	49.9±2.7^{e}	113±3.5^{h-j}	93.3±0.0^{a-d}	64.7±5.9^{ef}	100.8±5.2^{c-e}	95.0±0.0^{ef}	55.8±9.5^{fg}	162.9±5.2^{kl}	6.7±5.8^{ab}	1.2±0.5^{a}	2.3±4.0^{a}
		3Bb1		AL1	95.0±0.8^{e-h}	51.2±3.6^{e}	106.9±5.7^{g-i}	91.1±9..6^{ab}	91.4±2.8^{i-n}	113.8±1.5^{ef}	100±0.6^{f}	93.8±4.3^{lm}	178.7±5.5^{m-o}	30±0.0^{d}	5.3±0.6^{a}	14.7±3.2^{b}
				AL4	86.7±1.6^{bc}	33.8±3.8^{cd}	92.3±4.2^{fg}	88.9±5.7^{a}	44.7±2.3^{bc}	93.1±3.6^{bc}	90.0±0.6^{de}	52.6±6.7	140.6±7.3^{hi}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
			3Bb12	AS4	90±2.8^{b-e}	33.8±3.8^{cd}	92.3±4.2^{fg}	91.1±1.9^{ab}	82.4±2.6^{hi}	87.4±3.3^{bc}	80.0±0.0^{c}	61.8±6.6^{gh}	83.7±2.5^{e}	16.7±5.8^{c}	17.2±0.1^{bc}	36.7±7.2^{d}
3B	3Bb			Leac	93.3±2.5^{d-g}	74.4±1.6^{fg}	80.4±2.8^{ef}	100±0.0^{d}	85.4±2.9^{h-k}	93.6±3.1^{bc}	83.3±0.6^{cd}	18.5±5.6^{c}	24.5±8.6^{b}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
			3Bb21	Flhex	91.7±2.8^{c-f}	50.7±9.5^{e}	53.4±7.7^{bc}	100±0.0^{d}	65.5±1.0^{ef}	99.7±1.0^{cd}	90±1.1^{de}	11.8±2.3^{cd}	35.2±3.3^{bc}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
				Lhex	98.3±2.6^{gh}	99.3±2.3^{k}	98.4±1.2^{gh}	100±0.0^{d}	97.2±2.1^{l-n}	89.3±0.1^{bc}	93.3±0.5^{ef}	35.3±1.1^{d}	59.3±7.8^{d}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
		3Bb2		AFl2	90.0±1.1^{b-e}	68.5±4.1^{f}	132.8±6.1^{l-n}	95±8.6^{a-d}	79.1±7.2^{gh}	109.5±0.9^{c-e}	95.0±0.0^{ef}	50.5±4.7^{ef}	79.5±0.5^{e}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
			3Bb22	AFl3	88.3±0.3^{b-d}	47±7.7^{e}	114.4±7.1^{h-k}	98.3±2.8^{cd}	69.2±6.2^{e-g}	107.8±0.1^{c-e}	93.32±2.8^{ef}	44.6±3.6^{e}	86.4±9.6^{e}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}
				AFl4	85.0±2.1^{b}	26.5±1.4^{a-c}	91.2±7.2^{fg}	96.7±2.8^{b-d}	50.7±6.0^{cd}	81.8±0.6^{b}	86.7±5.7^{cde}	19.3±3.6^{c}	47.0±5.8^{cd}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}

Means ±SE followed by different letters differ significantly at P < 5%, as established by Duncan's test. *For abbreviation see Table1.

seeds and with the development of their seedlings. We note also that several extracts stimulate the development of shoots; percentages of shoot elongation exceed that of the control. On the other side, the percentages germination of thistle seeds and the development of seedlings were low or equal to zero, with the exception for extracts for the subgroup 3Ba1.

Subgroups 3Ba1 and 3Ba2 (AR3,4, AFl1, AS1-3)

When grown in contact with those extracts, germination percentages for lettuce, wheat, and harmal seeds reached 88.3-95.0, 92.2-100 and 83.3-100, respectively. The development of root seedlings was important (75.9-94.9, 78.6-99.6 and 66.7-93.3% of control, respectively). The development of shoot seedlings was higher than that of control and harmal shoot was more stimulated (113.6-208.2% of control), than this for lettuce (131-163.9% of control). The shoot of wheat seedlings was stimulated by AR3 and AR4 and by AFl1 (from 111.3 to 117.7% of control). Although, AR4 extract was highly correlated with shoot elongation of harmal seedlings which reached 208.2% of control.

Extracts from subgroups 3Ba1 and 3Ba2 were less correlated with thistle seed germination and with their seedling development. In presence of AR3, AFl1, AS1-3 (3Ba1 subgroup), milk thistle seed percentage germination was between 36.7 and 70% and root and shoot elongation varied from 12.4 to 45.0% of control and from 24.6 to 53.5% of control, respectively. Germination of this target seeds was completely inhibited by AR4, the only representative of 3Ba2 subgroup.

Subgroup 3Bb1 and 3Bb2

Group 3Bb, was shared in 3Bb1 and 3Bb2, and all extracts reduced the development of lettuce, wheat and harmal more than those from 3Ba group. The subgroup 3Bb1 consisted of extracts AL1-4, AS4 which correlated with lettuce, wheat and harmal seeds germination (86.7-95%, 88.9-93.3 and 80-100%) but less with milk thistle seeds germination (6.7-30%). More, AL4 inhibited milk thistle seed germination. Extracts from 3Bb1 subgroup were moderately correlated with the development of wheat root and harmal seedlings (44.7-91.4% of control; 52.6-93.8% of control, respectively) and less with that of lettuce (33.8-54.1% of control). Contrary to the results obtained for wheat and harmal, a low correlation was reported between those extracts and the root elongation of milk thistle seedlings (1.2-17.2% of control).

In presence of AL1-3 extracts from 3Bb11 subgroup, the development of lettuce, wheat and mainly harmal shoots was enhanced (106.9-117.2, 100.8-113.8 and 162.9-178.7% of control). Milk thistle seedling shoot elongation was reduced in the presence of those extracts (2.3-14.7% of control). In AL4 the shoot elongation of harmal was also highly stimulated (140.6% of control). AS4, the only representative of subgroup 3Bb12, stand out of the group 3Bb1 by its moderate effect on milk thistle seedling development (17.2-36.7%).

All extracts from the subgroup 3Bb2 (Leac, Flhex, Lhex, AFl2, AFl3 and AFl4) inhibited the germination of milk thistle seeds. Nevertheless, percentages of germination of lettuce and wheat seeds were close to the effect reported in presence of extracts from subgroup 3Bb1. When seeds where in contact with Leac, Flhex, Lhex (3Bb21 group extracts), the development of roots (50.7-99.3 and 65.5-97.2% of control, respectively) and of shoots (53.4-98.4 and 89.3–99.7% of control) of lettuce and wheat seedlings was important but less than the control. The development of harmal seedlings was less important (for the root 11.8-35.3% of control; for the shoot 24.5–59.3% of control). When grow in contact with AFl2-4% (subgroup 3Bb22), elongation of lettuce and wheat root seedlings varied from 26.5 to 68.8% and from 50.7 to 79.1% of control, respectively. Nevertheless, the development of their respective shoot was important (91.2-132.8, 81.8-109.5% of control) and was higher than the control. On contrary, the development of the harmal seedlings was reduced (for the root 19.3-50.5% of control, for the shoot 47-86.4% of control).

DISCUSSION

Our findings were supported by previous reports that demonstrated the allelopathic effects of many other trees such as *Melia azedarach* (Hong et al., 2003, 2004), *Azadirachta indica* (Neem) (Al-Charchafchi et al., 2007; Ashrafi et al., 2008 ; Abdus Salam and Kato-Noguchi, 2010), *Sesbania sesban* (Mubarak et al., 2009), *Acacia cyanophylla* (El Ayeb et al., 2013), and more recently for six tree from South Africa (Sunmonu and Staden, 2014). For *C. spinosum*, to the best of our knowledge, findings that indicate about its allelopathic effects are not available. Hence, it was for the first time that we have systematically evaluated and demonstrated the allelopathic inhibitory effects of *C. spinosum* on seed germination and early growth of milk thistle and harmal.

Phenol compounds are well known as potential phytotoxins (Seal et al., 2004). In our study we demonstrate that the inhibition of seed germination and the reduction of seedling elongation were not only related to the content in polyphenols of the extract (Table 2) but probably with the presence in all plant parts of one or more of phenol compound responsible for the activity (Inderjit, 1996; Khan and Siddique, 2012). In fact, we demonstrate that extracts containing low quantities in total phenol show a great inhibitor power and that the toxic metabolites are distributed in all plant parts in various concentrations (Harborne, 1977).

The effect of *C. spinosum* extracts varied with the kind of organ, concentration and target species. Roots of the different target species appeared to be more sensitive to *C. spinosum* extracts than shoots, presumably because of their more ultimate contact with the treated filter paper (Ahn and Chung, 2000). Similar results were reported with other crops by Maharjan et al., 2007, Ashrafi et al. (2008) and Wakjira et al., (2009). Abdus Salam and Kato-Noguchi (2010) reported that the extracts of allelopathic plants had more inhibitory effect on root growth than on

shoot growth because the root is the first organ to absorb allelochemicals from the environment. Germination inhibition would be attributed to those allelochemicals (Bulut et al., 2006). Furthermore, the permeability of allelochemicals to root tissues was reported to be greater than that to shoot tissues (Nishida et al., 2005) due to the direct contact between the root and phytotoxic compounds present in extract. Those compounds might inhibit or reduced rate of cell division (Wang et al., 2002; Qin et al., 2006) which is highly active at meristematic tissue of the growing root tip.

In all organic extracts germination of lettuce and wheat seeds was not or weakly reduced and germination percentages were proximate to control in the majority of cases (75.0-96.7 and 88.9-100%, respectively). Harmal seeds germination was strongly reduced (12.2%) or totally inhibited by all methanol extracts from stems, roots, leaves and flowers, hexane extract from roots and stems and ethyl acetate extract from roots. However, the two weeds: harmal and milk thistle were more sensitive than lettuce and wheat and the flowers extracts were the most toxic and milk thistle was more susceptible to extracts, than harmal. Organic extracts were more toxic than aqueous ones. Those later extracts, at high concentrations and all organic extracts reduced strongly or inhibited totally germination of milk thistle seeds. Contrariwise, the aqueous extracts at 1-3% (from leaves, stems and roots) stimulated elongation of lettuce, wheat, and harmal shoot seedlings in percentages exceeding control.

Conclusion

The present study demonstrated that aqueous and organic extracts of *C. spinosum* possess allelopathic potential and contain inhibitory substances. Allelopathic substances present in *C. spinosum* under favourable conditions should release into the environment and likely act synergistically to affect the growth of weed plants. These results suggest that *C. spinosum* could be one of the useful natural resources for developing bioherbicides for weed management and crude extracts of this tree could be a cost effective way for crops protection against weeds. Further research in order to know the growth inhibitory substances from *C. spinosum* organs are underway.

Conflict of Interest

The authors have not declared any conflict of interest.

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Pathogenic and genetic diversity in *Puccinia hordei* Otth in Australasia

K. S. Sandhu*, H. Karaoglu and R. F. Park

Plant Breeding Institute, Faculty of Agriculture and Environment, The University of Sydney, 107 Cobbitty Rd., Cobbitty, NSW 2570, Australia.

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Two PCR-fingerprinting primers, $(GACA)_4$ and M13, were tested across 22 pathotypes of *Puccinia hordei* Otth collected from Australasia over a 30 year period, to assess their usefulness in revealing genetic variability in this pathogen. Both primers revealed polymorphisms among the pathotypes, with $(GACA)_4$ generating a higher level of polymorphism. Molecular analyses revealed evidence of clonality among the *P. hordei* pathotypes, supporting the hypothesis that some arose from mutational changes in the pathogenicity of a founding pathogen genotype. Evidence was also obtained of sexual recombination within *P. hordei* in Australia on the alternate host *Ornithogalum umbellatum*. This is the first study of genetic variation among Australasian pathotypes of *P. hordei* using a PCR-fingerprinting technique.

Key words: *Puccinia hordei*, genetic diversity, fingerprinting, $(GACA)_4$, M13.

INTRODUCTION

The fungus *Puccinia hordei* (*Ph*) belongs to the genus *Puccinia*, the largest genus of the order Pucciniales with 3,000 to 4,000 species (Littlefield, 1981). *Ph* is the casual agent of barley leaf rust, an economically important disease which affects barley production in many parts of the world (Clifford, 1985). The pathogen is present in all barley growing regions of Australia (Park et al., 2003), reaching epidemic levels in Queensland during 1978, 1983, 1984 and 1988 (Cotterill et al., 1995). A severe epidemic of leaf rust can reduce the yield of a susceptible cultivar by up to 62% (Cotterill et al., 1992), and significant yield losses have been experienced in Australia (Cotterill et al. 1995; Cotterill et al., 1992; Waterhouse, 1927), New Zealand (Arnst et al., 1979), Europe and the USA (Griffey et al., 1994; Melville et al., 1976). *Ph* is a macrocyclic and heteroecious rust pathogen that forms its aecial stage on various species of *Ornithogalum*, *Leopoldia* and *Dipcadi* in the family Liliaceae (Clifford, 1985).

Different barley genotypes with resistance genes, known collectively as a differential set, were used by Levine and Cherewick (1952) and Clifford (1977) to characterise pathotypes (pts) among different isolates of *Ph*. The differential set used to characterise pts of *Ph* at the University of Sydney, Plant Breeding Institute (PBI) comprises 30 different barley genotypes with one or more resistance (*Rph*) genes (Park, 2003). The first assessment of pathogenic variability in *Ph* in Australia

*Corresponding author. E-mail: karanjeet.sandhu@sydney.edu.au. Tel: +61 2 93518821. Fax: +61 2 93518875.

was made in 1920 by Waterhouse (1927), who detected two pts, one similar to a European pt and another that differed in virulence on some genotypes compared to a pt found in North America (Waterhouse, 1952; Watson and Butler, 1947). In a later Australian study, Cotterill et al. (1995) found substantial pathogenic variation among *Ph* isolates collected between 1966 and 1990. This study identified 11 different pts among 154 isolates, of which pt 210P$^+$ was the most common. Up to 1995, virulence was detected for the leaf rust resistance genes *Rph1*, *Rph2, Rph4, Rph5*, *Rph6*, *Rph8*, *Rph9* and *Rph12*, and the genes *Rph3* and *Rph7* remained effective (Cotterill et al., 1995). Pathotype 4610P$^+$ virulent on *Rph12* was first detected in 1991 from Tasmania, after which (1996 to 2002), more pathogenic variation was detected in *Ph* including the identification of two new *Rph12* virulent pathotypes (pts) with added virulence for the resistance gene *Rph10* (viz. pts 5610P$^+$ and 5453P$^-$) (Park, 2003). While no virulence was detected in these studies for genes *Rph3*, *Rph7*, *Rph11*, *Rph14*, *Rph15* and *Rph18* (Park, 2003), virulence for *Rph3* was detected in 2009 (pt 5457P$^+$) in northern New South Wales (NSW) (Park, 2010). This pathotype is believed to have arisen from pt 5453P$^-$, first detected in Western Australia in 2001 (Park, 2006), via sequential single step mutations for virulence to *Rph19* (pt 5453P$^+$) and then *Rph3* (pt 5457P$^+$) (Park et al., 2015; Park, 2010).

While annual surveys of pathogenic variability in rust pathogens that infect cereal crops in Australia have provided evidence that variation arises via either the introduction of exotic genotypes, simple mutation, and asexual hybridisation (Wellings and McIntosh, 1990), sexual recombination is also thought to contribute to variability in the case of *P. hordei* (Park, 2008; Park et al., 1995). The alternate host *Ornithogalum umbellatum* occurs in Australia, where it is present on the Yorke Peninsula of South Australia (SA) (Wallwork et al., 1992) and in the Murrumbidgee catchment areas including Henty and Junee in NSW. While six pts of *Ph* were identified among uredinial isolates derived from aeciospores collected from infected plants of *O. umbellatum* from the Yorke Peninsula (Wallwork et al., 1992), the contribution of sexual recombination to overall genetic variability in *Ph* in Australia is largely unknown.

Although information on variability obtained from pathogenicity on differential genotypes is important in the genetic control of rusts, it is of limited use in assessing genetic variation in these pathogens. Both biochemical and molecular markers have been applied to evaluate genetic diversity among various plant pathogens (McDermott and McDonald, 1993). Amplified fragment length polymorphism (AFLP) analyses were used to study genetic diversity among isolates of *Ph* in relation to their virulence (Sun et al., 2007). This study revealed an association between molecular diversity and virulence patterns in *Ph* isolates collected from different geographical regions of the world. Keiper et al. (2003) studied the genetic structure of several cereal rust pathogens using various polymerase chain reaction (PCR) based tools like AFLP, selectively amplified microsatellites (SAM) and sequence-specific amplification polymorphisms (S-SAP). This study was able to discriminate fungal pathogens from five rust taxa [*P. triticina* (*Pt*), *P. graminis* f. sp*. tritici* (*Pgt*), *P. striiformis* f. sp*. tritici* (*Pst*), barley grass stripe rust caused by *P. striiformis* f. sp*. pseudohordei* (*Psph*) and *P. graminis* f. sp*. avenae* (*Pga*)], although the level of polymorphism observed within individual taxa was low. In a separate study that used AFLPs and random amplified polymorphic DNA (RAPDs), Steele et al. (2001) found no polymorphism among Australian and New Zealand isolates of *Pst*. However, the same AFLP primers showed five to 15% polymorphic fragments among isolates of *Pst* from the UK, Denmark and Colombia. These results were consistent with clonality in Australian populations of *Pst*. Microsatellites, or simple sequence repeats (SSRs) have also been developed and applied to study polymorphisim among different rust pathogens (Dambroski and Carson, 2008; Kolmer et al., 2011; Ordoñez et al., 2010; Mantovani et al., 2010; Keiper et al. 2006; Visser et al., 2011; Karaoglu and Park, 2014).

Another useful tool for assessing genetic diversity is “PCR-fingerprinting”. This technique uses microsatellites $(GACA)_4$ and $(GTG)_5$ and the minisatellite M13 derived from the core sequence of the wild type phase M13 bacterium, as single primers in PCR to amplify hypervariable DNA sequences (Meyer et al., 2001). The PCR-fingerprinting technique has been used successfully to reveal polymorphism among various fungal and bacterial pathogens. For example, Vuyst et al. (2008) used $(GTG)_5$ to identify acetic acid bacteria in cocoa beans and the primers GTG, GACA and M13 were used to study population dynamics in several human pathogens (Cogliati et al., 2007; Delhaes et al., 2008; Meyer et al., 2001; Roque et al., 2006; Trilles et al., 2008). Selective amplification of the microsatellite polymorphic loci (SAMPL) markers $(GACA)_4$ + H-G and R1 + H-G were used to study polymorphism among 44 (25 Australasian and 19 European) isolates of *Phragmidium violaceum* (causal agent of blackberry rust), revealing more diversity in European isolates than in Australasian isolates, with 37 and 22% polymorphic loci, respectively (Gomez et al., 2006). In all of these studies, the primers GACA and M13 generated the most discriminating and informative DNA profiles. Efforts have been made for the first time to study genetic variation in Australasian populations of *Ph* using PCR-fingerprinting profiles with primers $(GACA)_4$ and M13.

MATERIALS AND METHODS

Isolates of pathogens and DNA extraction

A total of 22 pts of *Ph*, comprising 20 from Australia and two from

Table 1. Details of *Puccinia hordei* pathotypes and control pathotypes of *P. triticina*, *P. graminis* f. sp*. tritici*, *P. striiformis* f. sp*. tritici*, *P. graminis* f. sp*. avenae* and *P. striiformis* f. sp*. pseudohordei* analysed using PCR-fingerprinting markers $(GACA)_4$ and M13.

Isolate ID	Pathogen	Pathotype	Culture No.	Origin	Host/Cultivar	Year
1-*Ph*	*P. hordei*	$211P^+$	484	Coonamble, NSW	Barley/O'Connor	1992
2-*Ph*	*P. hordei*	$220P^+$	485	Yanco, NSW	Barley/Nigrinudum	1992
3-*Ph*	*P. hordei*	$253P^-$	490	Grafton, NSW	Barley/?	1992
4-*Ph*	*P. hordei*	$243P^+$	537	Grafton, NSW	Barley/?	1999
5-*Ph*	*P. hordei*	$200P^+$	570	Yanco, NSW	Barley/Gus	2002
6-*Ph*	*P. hordei*	$232P^+$	506	Balaclava, SA	Barley/Galleon	1994
7-*Ph*	*P. hordei*	$201P^-$	480	St Leonards, VIC	Barley/?	1992
8-*Ph*	*P. hordei*	$201P^+$	481	Rochester, VIC	Barley/?	1992
9-*Ph*	*P. hordei*	$242P^+$	531	Borung, VIC	Barley/?	1998
10-*Ph*	*P. hordei*	$5653P^-$	569	Byaduk, VIC	Barley/Franklin	2002
11-*Ph*	*P. hordei*	$243P^+$	489	Monto, QLD	Barley/?	1992
12-*Ph*	*P. hordei*	$243P^-$	507	Toowoomba, QLD	Barley/Dampier	1994
13-*Ph*	*P. hordei*	$5453P^-$	560	Esperance, WA	Barley/Schooner	2002
14-*Ph*	*P. hordei*	$5653P^+$	584	Wongan Hills, WA	Barley/?	2004
15-*Ph*	*P. hordei*	$4610P^+$	491	Cressy, TAS	Barley/Franklin	1992
16-*Ph*	*P. hordei*	$5653P^+$	542	Glen Esk, TAS	Barley/Gairdner	2000
17-*Ph*	*P. hordei*	$211P^-$	483	Aorangi, NZ	Barley/?	1992
18-*Ph*	*P. hordei*	$231P^+$	486	Aorangi, NZ	Barley/?	1992
19-*Ph*	*P. hordei*	$5610P^+$	520	Ravensthorpe, WA	Barley/?	1997
20-*Ph*	*P. hordei*	$220P^+$	577	SA	*O. umbellatum*	2003
21-*Ph*	*P. hordei*	$200P^-$	518	SA	Barley/?	1995
22-*Ph*	*P. hordei*	$5457P^+$	612	Legume, QLD	Barley/?	2009
23-*Pt*	*P. triticina*	104-2,3,(6),(7),11	423	Mt Derimut, VIC	Wheat/Nebraska	1984
24-*Pgt*	*P. graminis* f. sp*. tritici*	194-2,3,7,8,9	344	Hermitage, QLD	Wheat/?	1980
25-*Pst*	*P. striiformis* f. sp*. tritici*	110 E143 A^+	444	Richmond, TAS	Wheat/Hartog	1987
26-*Psph*	*P. striiformis* f. sp*. pseudohordei*	981549	589	Turretfield, SA	Barley/?	1998
27-*Pga*	*P. graminis* f. sp*. avenae*	41+*Pg9*	496	Rutherglen, VIC	Oat/?	1993

Source: Cereal Rust Collection, University of Sydney, PBI, Cobbitty.

New Zealand, along with isolates of five control pathogens (*Pt*, *Pgt*, *Pst*, *Psph* and *Pga*) were included in this study (Table 1). All the pts used in the study were sourced from the rust collection maintained in liquid nitrogen at PBI, University of Sydney. The *Ph* pts used were selected to represent those identified in different regions within Australia and New Zealand in annual pathogenicity surveys conducted from 1980 to 2009.

Freshly collected urediniospores were desiccated over silica for 12 h. A sample of 25 to 30 mg of urediniospores of each rust isolate was put in labelled Lysing Matrix C tubes (Impact resistant tubes with 1.0 mm silica spheres, Mp Biomedical, Ohio, USA). One milliliter of 2x Cetyl-trimethylammonium bromide (CTAB) extraction buffer [(CTAB 2% (w v^{-1}), 20 mM EDTA (pH 8.0), 1.4 M NaCl, Polyvinylpyrrolidone (PVP; 40000 MW) 1% (w v^{-1}), 100 mM Tris-HCl (pH 8.0) and ddH_2O (double distilled autoclaved water)] was added to each sample, mixed well by inversion and tubes were submerged in ice for 2 min. Tubes were then shaken for 15 s on a FastPrep® Cell Distrupter (M.P. Biomedicals, Irvine, CA, USA) at speed 6, returned to ice for 3 min and shaken again for 20 s at the same speed. Tubes were kept in a pre-warmed water bath at 65˚C for 30 min and inverted every 10 min, after which they were removed, mixed well by inversion and the solution in each tube/sample was divided (~500 µl in each tube) into two new 1.5 ml Eppendorf tubes to generate duplicate extractions. DNA extraction was carried in a fume hood by adding ~ 250 µl of cold phenol, followed by ~ 250 µl of cold chloroform: isoamyl alcohol (24:1 v v^{-1}), to each tube. Samples were mixed gently by inverting (~ 100 times) the tubes until a thick emulsion formed. Tubes were centrifuged at 13,000 rpm for 15 min and the supernatant was transferred into sterile 1.5 ml Eppendorf tubes. The process of phenol and chloroform: isoamyl alcohol extraction was repeated. About 50 µl of 3 M NaOAc and ~ 500 µl of cold isopropanol were added to each tube and tubes were then stored at -20˚C. The following day, the tubes were centrifuged at 13,000 rpm for 30 min and the DNA pellet thus formed was drained carefully. The pellets were washed with 500 µl of ethanol (70% v v^{-1}), centrifuged at 13,000 rpm for 15 min, drained carefully and allowed to air dry. The dried pellet was re-suspended in 100 µl ddH_2O and stored overnight at 4˚C. The following day, 5 µl of Rnase-A (10 µg $µl^{-1}$) was added to each tube and incubated at 37˚C for 2 h. All DNA samples were quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop® Technologies) and diluted to working dilution of 10 ng $µl^{-1}$ using ddH_2O.

PCR-fingerprinting

Two oligonucleotide primers were used in fingerprinting the isolates

of *Ph* and control pts: The microsatellite-specific [$(GACA)_4$ (5'GACAGACAGACAGACA3')] (Ali et al., 1986; Meyer et al., 2001) and [M13 (5'GAGGGTGGCGGTTCT3') minisatellite specific core sequence derived from the wild-type phage M13 vector] (Vassart et al., 1987; Meyer et al., 2001).

PCR reactions were performed in a final volume of 50 µl which contained 3.0 µl of genomic DNA (10 ng μl^{-1}), 5.0 µl of dNTPs (0.2 mM), 5.0 µl of 10x PCR buffer (NH4 Reaction buffer, Bioline), 3.0 µl of 50 mM $MgCl_2$ (Bioline), 5.0 µl of primer (2 mM), 0.5 µl (5 u μl^{-1}) of *Taq* DNA (Immolase DNA polymerase from Bioline) and 28.5 µl of ddH_2O. PCR amplification profile comprised of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s denaturation at 94°C, 60 s annealing at 47°C if M13 or at 40°C if $(GACA)_4$ primer was used, 30 s extension at 72°C and a final extension of 7 min at 72°C. Reactions were performed in a 96-well DNA theromocycler (Eppendorf Mastercycler, Germany). PCR products were concentrated to 30 µl by placing in a fan forced oven for 45 min at 65°C and resolved on 2% high resolution agarose (MetaPhor® Agarose, Lonza, Rockland Inc.USA) gels at 80 V electrophoresis for 6 h. Five kilobite DNA marker HyperLadder™ III (Bioline) was used as reference. The separated fragments were visualised under an ultra violet light unit fitted with a GelDoc-IT UVP Camera (Bio-rad, Australia Pty. Ltd. Gladesville NSW).

Data analyses

Gel images were scored and analysed using the software GelCompar II (6^{th} edition, Applied Maths, Belgium). Fragment position optimisation and tolerance was set to 1 and 1.5%, respectively. Fragments were selected automatically by the GelCompar and unclear fragments were deselected manually. Based on the standard DNA ladder used, molecular weights of selected fragments were assigned automatically. Fragment scoring for the both primers ranged from 500 to 2500 bp. Genetic diversity among the *Ph* pts examined was evaluated using Unweighted pair group method for arithmetic averages (UPGMA) cluster analyses based on a distance matrix calculated using the Dice coefficient of similarity. The quality of similarity clusters was tested using the cluster validity index Cophenetic correlation coefficient (CPCC) using software GelCompar II. The CPCC was used to test the efficiency of the similarity clusters that resulted from the individual analyses of markers M13 and $(GACA)_4$. The CPCC is a simple correlation coefficient between the original dissimilarity matrix and the final dissimilarity matrix (Cophenetic matrix) produced after the clustering algorithm recalculates the dissimilarities (Lessig, 1972). Dendrograms were constructed and based on similarity clusters of both primers $(GACA)_4$ and M13, the *Ph* pts were clustered accordingly.

RESULTS

Both oligonucleotides $(GACA)_4$ and M13 amplified all pts, producing fragments in the range of 500 to 2500 bp. After deselecting unclear fragments manually, a total 27 and 28 fragments were scored automatically for markers $(GACA)_4$ and M13, respectively (Table 3). The UPGMA similarity dendrograms produced from the cluster analyses based on markers $(GACA)_4$ and M13 data grouped all 22 *Ph* pts and control pathogens (Figures 1 and 2). Both primers $(GACA)_4$ (Figure 1) and M13 (Figure 2) out-grouped representative control isolates of *Pt*, *Pgt*, *Pst*, *Psph* and *Pga* from the *Ph* pts examined. Both fingerprinting primers produced distinct clades for *Pst* and *Psph*, *Pgt* and *Pga*, while *Pt* was in a standalone group (Figures 1 and 2).

Cluster analysis based on marker M13 produced seven groups among the *Ph* pts with 75.9% to 100% similarities (Figure 2), while marker $(GACA)_4$ revealed higher variability among the *Ph* pts and produced 10 different groups with 70.5 to 100% similarities (Figure 1). Markers clustered pts $211P^-$ and $231P^+$ together (Figures 1 and 2), both of which originated from New Zealand.

Marker $(GACA)_4$ resolved the greatest genetic variation among the *Ph* pts and different "GACA" and "M13" groups were defined (Table 2). GACA group one (^{GGP1}) contained pts $211P^+$, $220P^+$, $253P^-$, $243P^+$, $200P^+$, $232P^+$, $201P^-$, $201P^+$, $242P^+$ and $243P^-$. All 10 pts have virulence for *Rph8* in common (Park 2003). Marker M13 also grouped these pts in one group (^{MGP2}), except pts $201P^+$ and $243P^-$, which were grouped in ^{MGP1} (Table 2). Both markers out-grouped pts $200P^-$ (^{GGP8} and ^{MGP3}), $5653P^+$ (^{GGP3} and ^{MGP4}) and $5653P^-$ (^{GGP6} and ^{MGP5}) from all others (Table 2). Pathotype $200P^-$ is virulent to *Rph8* only whereas pt $5653P^-$ carries aditional virulence for genes *Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph9*, *Rph10* and *Rph12*. Pathotype $5653P^+$ posses additional virulence for *Rph19* compared to pt $5653P^-$ (Park, 2003). In both cases, pts $211P^-$ and $231P^+$ were grouped together in distinct groups of GACA (^{GGP7}) and M13 (^{MGP7}) as detailed in Table 2. Marker $(GACA)_4$ produced distinct clusters for pts $5453P^-$ (Isolate 13-*Ph*) and $5457P^+$ (Isolate 22-*Ph*) but with 90.9% similarity (Figure 1), whereas these two pts were shown to be 100% similar (Figure 2) when genotyped using the fingerprinting marker M13. Pathotype $5457P^+$ carries additional virulence for *Rph3* and *Rph19* as compared to the pt $5453P^-$ though both pts share virulence for genes *Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12* (Park et al., 2015). Marker $(GACA)_4$ grouped pts $243P^+$, $4610P^+$ and $5653P^+$ together in a single group (^{GGP4}) and discriminated pt $220P^+$ in a distinct group ^{GGP5} (Table 2), but genotyping based on M13 marker grouped these four pts in a group (^{MGP2}) with other pts (Table 2).

DISCUSSION

The evolution of new virulent pts of *Ph* is a significant constraint in the economical production of barley in Australia and worldwide. Understanding genetic diversity in *Ph* is fundamental in the efforts to develop cultivars of barley with resistance to this pathogen. For example, genetically diverse fungal pathogens may have a greater potential to evolve new pts with the ability to overcome resistance. In earlier work, six pts of *Ph* were identified from aeciospores collected from infected plants of *O. umbellatum* in SA (Wallwork et al., 1992). Furthermore, high diversities of *Ph* pts have been reported in SA in pathogenicity surveys, suggesting that sexual recombination is contributing to pathogen diversity (Park, 2010).

Table 2. Groups of *P. hordei* pathotypes based on the cluster analyses using PCR-fingerprinting markers $(GACA)_4$ and M13.

Isolate ^{MGP}	Pathotype	Isolate ^{GGP}	Pathotype	Virulence to *Rph* genes*
8 ^{MGP1}	201P$^+$	1^{GGP1}	211P$^+$	*Rph1*, *Rph4*, *Rph8*, *Rph19*
12 ^{MGP1}	243P$^-$	2 ^{GGP1}	220P$^+$	*Rph5*, *Rph8*, *Rph19*
16 ^{MGP1}	5653P$^+$	3 ^{GGP1}	253P$^-$	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*
1 ^{MGP2}	211P$^+$	4 ^{GGP1}	243P$^+$	*Rph1*, *Rph2*, *Rph6*, *Rph8*, *Rph19*
2 ^{MGP2}	220P$^+$	5 ^{GGP1}	200P$^+$	*Rph8, Rph19*
3 ^{MGP2}	253P$^-$	6 ^{GGP1}	232P$^+$	*Rph2*, *Rph4*, *Rph5*, *Rph8*, *Rph19*
4 ^{MGP2}	243P$^+$	7 ^{GGP1}	201P$^-$	*Rph1*, *Rph8*
5 ^{MGP2}	200P$^+$	8 ^{GGP1}	201P$^+$	*Rph1*, *Rph8*, *Rph19*
6 ^{MGP2}	232P$^+$	9 ^{GGP1}	242P$^+$	*Rph2*, *Rph6*, *Rph8*, *Rph19*
7 ^{MGP2}	201P$^-$	12 ^{GGP1}	243P$^-$	*Rph1*, *Rph2*, *Rph6*, *Rph8*
9 ^{MGP2}	242P$^+$	19 ^{GGP2}	5610P$^+$	*Rph4*, *Rph8*, *Rph9*, *Rph10*, *Rph12*, *Rph19*
11 ^{MGP2}	243P$^+$	14 ^{GGP3}	5653P$^+$	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph10*, *Rph12*, *Rph19*
15 ^{MGP2}	4610P$^+$	11 ^{GGP4}	243P$^+$	*Rph1*, *Rph2*, *Rph6*, *Rph8*, *Rph19*
19 ^{MGP2}	5610P$^+$	16 ^{GGP4}	5653P$^+$	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph10*, *Rph12, Rph19*
20 ^{MGP2}	220P$^+$	15 ^{GGP4}	4610P$^+$	*Rph4*, *Rph8*, *Rph9*, *Rph12*, *Rph19*
21 ^{MGP3}	200P$^-$	20 ^{GGP5}	220P$^+$	*Rph5*, *Rph8*, *Rph13*, *Rph19*
14 ^{MGP4}	5653P$^+$	10 ^{GGP6}	5653P$^-$	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph10 Rph12*
10 ^{MGP5}	5653P$^-$	17 ^{GGP7}	211P$^-$	*Rph1*, *Rph4*, *Rph8*
13 ^{MGP6}	5453P$^-$	18 ^{GGP7}	231P$^+$	*Rph1*, *Rph2*, *Rph4*, *Rph5*, *Rph8*, *Rph19*
22 ^{MGP6}	5457P$^+$	21 ^{GGP8}	200P$^-$	*Rph8*
17 ^{MGP7}	211P$^-$	13 ^{GGP9}	5453P$^-$	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12*
18 ^{MGP7}	231P$^+$	22 ^{GGP10}	5457P$^+$	*Rph1*, *Rph2*, *Rph3*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12*, *Rph19*

Isolate: Isolate ID as given in Table 1; ^{MGP}Groups of *P. hordei* pathotypes based on M13 analysis; ^{GGP}Groups of *P. hordei* pathotypes based on GACA analysis; *with respect to the resistance genes listed in Park (2003), virulence to *Rph* genes shown in last column is corresponding to the pathotypes shown in the previous column.

Prior to the current study, no attempt had been made to study the genetic diversity of *Ph* in Australia, using PCR-fingerprinting. The usefulness of the PCR-fingerprinting primers M13 and GACA in discriminating fugal pathogens has been shown in several studies (Cogliati et al., 2007; Delhaes et al., 2008; Meyer et al., 2001; Roque et al., 2006; Trilles et al., 2008). In view of this, PCR-fingerprinting primers M13 and $(GACA)_4$, were assessed for their utility in *Ph*.

Cluster analyses of marker data revealed seven to 10 clusters among the 22 *Ph* pts and both markers out-grouped the control pathogens. As expected, a high percentage of similarity was observed among the *Ph* clusters, whereas the control pathogens were more diverse. Both PCR-fingerprinting primers $(GACA)_4$ and M13 clearly differentiated *Pt*, *Pgt*, *Pst*, *Psph*, *Pga* from each other and from the pts of *Ph*. Markers M13 and $(GACA)_4$ revealed only 26.4 and 33.3% genetic similarities between *Ph* and the control rust pts. These findings are in accordance with earlier studies in which isolates of *Pgt* were clearly differentiated from isolates of *Ph* using AFLP markers (Sun et al., 2007).

Both markers distinguished *Pst* and *Psph* with 57.1 to 83.3% genetic similarities, which is in accordance with an earlier study of these rust pathogens by Keiper et al. (2003) in which *Pst* and *Psph* were distinct but more similar compared to other rust pathogen species. Both markers M13 and $(GACA)_4$ formed distinct clades of *Pga* and *Pgt* and differentiated these two from the wheat rust pathogens *Pst* and *Pt*, also consistent with earlier results of an AFLP study on these rust pathogens (Keiper et al., 2003). The current results support the informative value and usefulness of the PCR-fingerprinting markers in differentiating species of rust pathogens.

The PCR-fingerprinting primer M13 clustered the 22 *Ph* pts into seven groups, while the marker $(GACA)_4$ resolved 10 groups among the *Ph* pts (Table 2) and detected more polymorphism. Interestingly, both markers grouped *Ph* pts 211P- and 231P$^+$ with 100% similarity (^{GGP7} and ^{MGP7}, Table 2) and differentiated them from all other *Ph* pts. Both pts originated from New Zealand and differ only in virulence on *Rph2*, *Rph5* and *Rph19*. It is therefore possible that these two pts are simply related and their distinctiveness from the Australian pts indicates that *Ph* populations in the two countries are distinct. This contrasts with results from long-term surveys of pathogenic variability in wheat rust pathogens across Australia and New Zealand, which have provided

Table 3. GelCompar selected fragments accross the amplifications produced by PCR-fingerprinting markers $(GACA)_4$ and M13 where unclear fragments were deselected manually.

S/N	$(GACA)_4$ fragments (bp)	M13 fragments (bp)
1	510	543
2	550	557
3	572	571
4	584	668
5	675	768
6	707	811
7	741	853
8	765	895
9	790	914
10	839	950
11	956	999
12	961	1023
13	1061	1084
14	1089	1122
15	1126	1195
16	1173	1255
17	1278	1327
18	1276	1410
19	1380	1542
20	1502	1574
21	1568	1629
22	1639	1699
23	1852	1787
24	2000	1875
25	2078	2014
26	2250	2140
27	2480	2268
28		2485

substantial evidence of rust migration between the two land masses (Luig, 1985). These studies have also provided evidence that wheat rust movement is predominantly from west to east (Luig, 1985; Wellings et al., 2003). In view of this, the distinctiveness of the two pts of *Ph* from New Zealand from those in Australia suggests that they may have originated from a region outside Australasia and that they have remained localized to New Zealand.

Based on pathogenicity, Cotterill et al. (1995) suggested that the appearance of a group of pts distinct from pt $243P^-$ and typified by pt $200P^-$ and its subsequent single-step mutations in the form of pts $201P^-$, $210P^-$ and $220P^-$ in the 1980s, may have resulted from an exotic incursion. The present results support this hypothesis.

Studies of pathogenic variability in all three wheat rust pathogens in Australia have provided strong evidence of clonality, with presumed clonal lineages comprising closely related pts derived by sequential single-step mutations from a common ancestor (Keiper et al., 2006). In contrast, pts of *Ph* detected in Australia between 1992 and 2001 did not appear to be so simply related based on pathogenicity (Park, 2003). Of the pts examined in the present study, pt $5457P^+$ is believed to have originated from pt $5453P^-$ via step-wise mutation for virulence for *Rph19* and then for *Rph3* (Park, *unpublished*). Surprisingly, while markers $(GACA)_4$ and M13 grouped these two pts and separated them from all other pts, they were not identical (Figures 1 and 2, respectively). These results show that the relationship between these two pts is not as simple as thought.

The molecular analyses in the present study did, however, provide some evidence of clonal lineages in *Ph* in Australasia. Marker $(GACA)_4$ revealed pts $201P^+$ and $201P^-$ to be 100% genetically similar (Figure 1) and given that pt $201P^+$ differs from $201P^-$ only in being virulent for *Rph19*, together these results are consistent with pt $201P^+$ arising via a single step mutation in pt $201P^-$ with

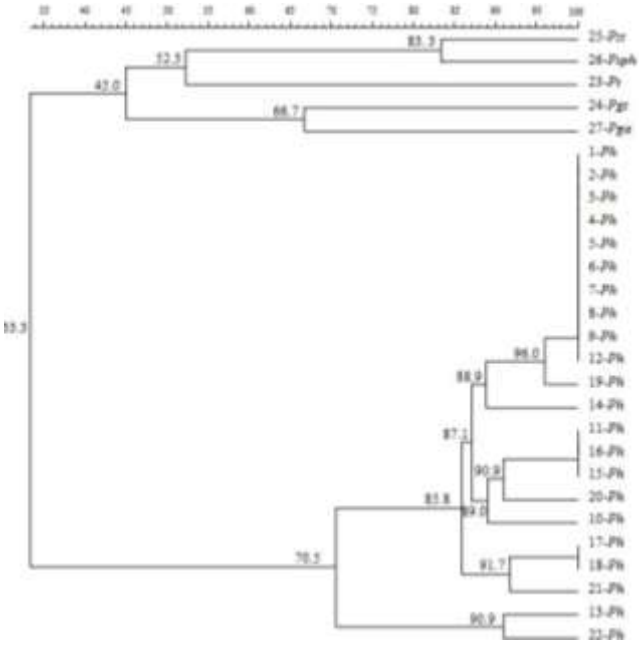

Figure 1. Genetic similarity dendrogram of 22 *P. hordei* pathotypes and five control pathotypes (*Pt*, *Pgt*, *Pst*, *Psph* and *Pga*) based on PCR-fingerprinting marker $(GACA)_4$ data. UPGMA cluster analyses conducted using Dice coefficient of similarity. Similarity percentage values are shown on the left hand side of the group nodes. Pathotypes detail is provided in Table 1.

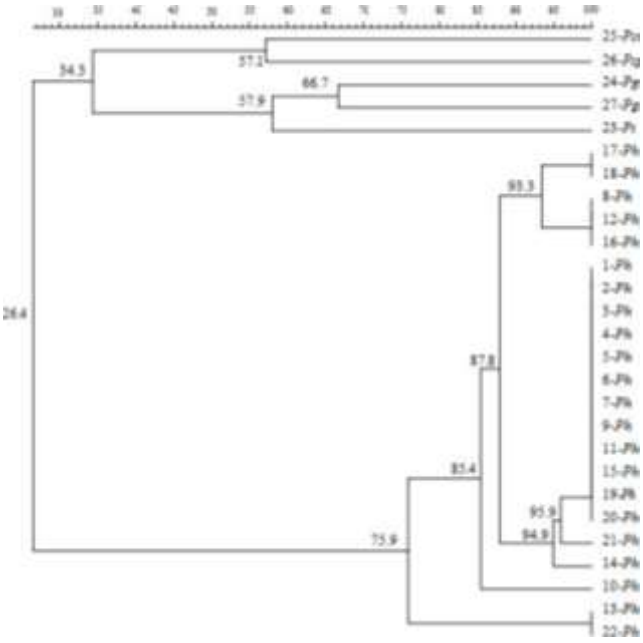

Figure 2. Genetic similarity dendrogram of 22 *P. hordei* pathotypes and five control pathotypes (*Pt*, *Pgt*, *Pst*, *Psph* and *Pga*) based on PCR-fingerprinting marker M13 data. UPGMA cluster analyses conducted using Dice coefficient of similarity. Percent similarity values are shown on the left hand side of the group nodes. Pathotypes detail is provided in Table 1.

added virulence for *Rph19*. The lack of molecular variation among some of the pts studied support the hypothesis of single-step mutation being an important source of pathogenic variation in *Ph*, which is consistent with the results published by Steele et al. (2001) who found a similar situation among Australian isolates of *Pst*. Marker $(GACA)_4$ revealed more informative fragments compared to the M13. So PCR-fingerprinting technique using marker $(GACA)_4$ can be a very efficient and an effective tool to find genetic variations in *Ph* and other rust pts.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Tomato (*Lycopersicon esculentum* Mill.) varieties evaluation in Borana zone, Yabello district, southern Ethiopia

Desalegn Regassa*, Wakene Tigre and Addis Shiferaw

Oromia Agricultural Research Institute, Yabello pastoral and Dry-land Agriculture Research Center, P. O. Box: 85, Yabello, Ethiopia.

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Tomato is one of the most important vegetable in Ethiopia as well as in the world in irrigated regions. It can be produced in a wide range of climatic conditions and many types of soils. Borana zone has potential to produce the crop, but not well done due to technical (production technology) and socio-economic problems. Among technical problem unavailability of seeds of adapted and improved tomato varieties is the most limiting factor. Therefore, the objective of this research is to test a range of improved varieties under Borana condition to identify genotypes with relatively better performance. To this effect, four improved and recommended varieties of tomato, namely: Fetan, Melkashola, Miya and Cochoro introduced from Melkassa Agricultural Research Center and one variety called CAL-J introduced from Kenya were tried for their adaptation at Yabello under irrigation using tap water in the year 2014. The experiment was single factor (varieties) single season experiment in RCBD with three replications. Parameters like Plant height, number of primary branch, days to 50% flowering, days to first harvest, Total fruit yield (ton/ha), marketable fruit yield (ton/ha), unmarketable fruit yield (ton/ha), fruits number per plant and single fruit weight (gram) were collected and analyzed. The result showed that there was significant (P ≤ 0.05) variation among the varieties for parameters including Plant height, number of primary branch, Total fruit yield (ton/ha), marketable fruit yield (ton/ha), unmarketable fruit yield (ton/ha) and single fruit weight (gram), but there was no significant variation for parameters days to 50% flowering, days to first harvest and fruit number per plant. Variety Miya gave significantly higher marketable fruit yield (22.95 ton/ha) and higher average of single fruit weight (85.84 gram) than other varieties. The least fruit marketable yield was obtained from the variety Fetan (11.61 ton/ha). Based on the result of the experiment, Variety Miya was recommended for further popularization in Yabello district under irrigation.

Key words: Tomato, variety introduction, variety evaluation, yield components, fruit yield.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetable crops in the world, second to potato. It originally came from tropical area from Mexico to Peru (Maerere et al., 2006; FAO, 2005).

*Corresponding author. E-mail: amensisa.regassa@gmail.com.

Table 1. Soil physical and chemical properties at Yabello YPDARC research site, southern Ethiopia in 2014.

% OC	EC mmhos/cm at 25ºC	pH	Av.P in ppm	CEC in meq/100g soil	texture			
					% sand	% clay	% silt	classes
2.262	0.112	7.03	15.36	20.400	46	36	18	sandy

Its use as a food originated in Mexico, and spread throughout the world following the Spanish colonization of the Americas (Wikipedia, 2016). In Ethiopia, there is no exact information as to when tomato was first introduced; however, the crop is cultivated in different major growing areas of the country. In 2015 cropping calendar, tomato production in Ethiopia was about 22,788 tons from harvested area of 3,677 ha (CSA 2015). It is used as canned vegetable having multiple uses and supplies essential nutrients in human diets (Choudhury, 1979). It is popularly used for both commercial and home use purposes. The fresh produce is sliced and used as salad. It is also cooked for making local saucer (‘watt’). The processed products like tomato paste, tomato juice, tomato catch-up and whole peel-tomato are produced in the country for local market and export. It was recognized as quality product for both local and export markets and providing a route out of poverty for small scale producers who live in developing countries in general and in Ethiopia in particular (Tewodros and Asfaw, 2013).Despite the importance of this crop, the production and productivity is constrained by different biophysical and socio-economic reasons, such as lack of adapted and improved tomato technologies, land shortage, inadequate knowledge on production and management (processing) systems, poor extension services, poor marketing system and proper utilization of the crop are a few to mention (Mersha, 2008).

Agriculture forms the basis for livelihood and creates job opportunity for more than 85% of Ethiopian population. It accounts for 50% of the Gross Domestic Product (GDP) and 90% of the national export earnings (MoARD, 2007). Vegetables took up about 1.18% of the area under all crops at national level. Productions of vegetables contribute 2.0% of the total crops production. Holders living near to urban centers largely practice vegetable farming. In Ethiopia, tomato ranks fourth in total production (5.45%) after Ethiopian cabbage, red pepper and green pepper are third in area coverage (4.49%) next to red pepper and Ethiopian cabbage from vegetable crops cultivated. Its national mean yield is 6.2ton/ha (CSA 2015). This is by far below the world average 34.84 ton/ha (FAO 2009).This is due to shortage of varieties and recommended information packages, poor quality seed, poor irrigation systems, lack of information on soil fertility, disease and insect pests, high post harvest loss, and poor marketing system (Lemma, 2002). Tomato can be produced in a wide range of climatic conditions and many types of soils. It is mainly produced under irrigation during off season because under rainy condition, it is susceptible to a disease complex. Successful cultivation of tomato is based essentially upon the choice of suitable varieties for a particular location (Chaerani, 2006). Tomato shops are mushrooming in towns of Borana zone, and investors and farmer associations have started to produce those crops (Personal observation). There is high potential to produce tomato in the zone, but not well done due to technical (production technology) and socio-economic problems. Among technical problems, unavailability of seeds of adapted and improved tomato varieties is the most limiting factor. Therefore, the objective of this research is to test a range of improved varieties under Borana condition to identify genotypes with relatively better performance and to know the pattern and extent association among yield and yield related traits collected.

MATERIALS AND METHODS

Description of study area

The experiment was conducted at Yabello YPDARC research site, in southern Ethiopia under irrigation, using tap water. The site is located at N04.88006 and E038.14761 with altitude of 1615 m.a.s.l. The soil at the site is characterized as sandy with PH of 7.03 (Table 1). It is located at 570 km along Addis Ababa-Moyale main road. It has annual mean precipitation of 500 mm. The area is characterized by bimodal rainfall where 60% rainfall occurs during main rainy season (March to May) and the remaining 40% goes to the short rainy season (September to November). The mean annual temperature varies from 19 to 24°C. Livelihood of the people is basically Agro pastoralist. OC = organic carbon; EC = electro conductivity; Av.P = available Phosphorus; CEC = Cation Exchangeable Capacity NB: PH: in water suspension with soil to water ratio 1:2.5 by PH meter; EC: in water suspension with soil to water ratio 1:2.5 by electro Conductivity meter; % OC: Walkey and Black method; Av.P by Olsen etal; Texture by Hydometer; CEC = CationExchangable Capacity by Ammonium Acatate (1 M NH4OAC).

Planting materials and experiment methodology

Four improved and recommended varieties of tomato, namely: Fetan, Melkashola, Miya and Cochoro, introduced from Melkassa Agricultural Research Center and one variety called CAL-J introduced from Kenya, were tried for their adaptation using tap water. The experiment design was a RCBD with three replications. Six rows and six plants per row with 70 cm between rows and 30 cm between plants were used for this experiment. Four middle rows were used for data per plot leaving the two rows as border. A fertilizer rate of 200 kg/ha of Di Ammonium Phosphate (DAP) (23-46-0) and 50 kg/ha of Urea (46-0-0) were applied at transplanting

Table 2. Mean squares due to varieties and error for yield and yield components of 5 tomato varieties grown in 2014 in Borana zone at YPDARC.

Traits	Df = 4	Df = 22
	Varieties	Error
Days to 50% flowering	5.17n.s.	4.22
Days to first harvest	1.00 n.s.	5.80
Plant height (cm)	159.53***	2.07
Number of primary branch	1.70**	0.15
Marketable fruit yield (ton/ha)	56.64*	10.98
Unmarketable fruit yield (ton/ha)	0.87*	0.21
Total fruit yield (ton/ha)	71.46*	14.04
Fruit number per plant	11.59n.s.	5.28
Single fruit weight (g)	987.67**	122.72

*, **, *** = Significant at 5, 1 and 0.1% probability level respectively, Df = degree of freedom, n.s. = non- significant.

time and 50 kg/ha urea was applied at early flowering stage. The seedlings were raised on nursery bed at 10 cm distance between rows and about 2 cm between plants at thinning. The nursery bed was kept moist but not wet. Transplanting was done at 36 days after sowing when the seedlings were about 13 to 15 cm length and at 2 to 3 leaf stage. Frequency of irrigation on field was at 7 days interval using tap water and harvested rain water until the soil in the root zone (30 cm depth) is moist but not wet. All the agronomic practices were as per the recommendation from Melkasa Agriculture research center. No serious diseases were observed during this experiment, but bird attack was a potential problem so that the guard was assigned at the research site.

Data collected and statistical analysis

Data were collected on parameters like Plant height, number of primary branch, days to 50% flowering, days to first harvest, Total fruit yield (ton/ha), marketable fruit yield (ton/ha), unmarketable fruit yield (ton/ha), fruits number per plant and single fruit weight (gram). Damaged, sunburn and fruits with a weight of less than 25 gram were recorded as unmarketable (Lemma, 2002).

1. Plant height (cm): Plant height was recorded by measuring the height of 5 randomly selected plants in each plot from the ground to the main apex.
2. Number of Primary branch: Counted at maturity from 5 randomly selected plants in each plot.
3. Days to 50% flowering: transplanting date to the day on which 50% of the plant in each plot is flowered.
4. Days to first harvest: the number of days from transplanting to the first picking day.
5. Fruit yield (ton/ha): Sum of fruit weight per plot from successive harvest (kg) was taken and converted to ton per hectare.
6. Fruit number per plant: The number of fruit in successive harvest per plant.
7. Single fruit weight (gram): Calculated by dividing fruit yield per plot to total number of fruits harvested per plot.

Analysis of variance for the collected parameters was performed as per the methods described by Gomez and Gomez (1984) using SAS computer software (SAS, 2009) for randomized complete block design and treatment mean comparison is done by Fisher's list significance difference (LSD) at 5%. Pearson's correlations among all the collected parameters were also evaluated.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) was done for the collected parameters. The result of ANOVA showed that there was significant ($P \leq 0.05$) variation among the varieties for parameters including Plant height, number of primary branch, Total fruit yield (ton/ha), marketable fruit yield (ton/ha), unmarketable fruit yield (ton/ha) and single fruit weight (gram), but there was no significant variation for parameters like days to 50% flowering, days to first harvest and fruit number per plant (Table 2). Baliyan and Rao (2013) also found significance variability in yield produced by six tomato varieties evaluated for pest and disease and productivity in Botswana. Variety Miya gave significantly higher marketable fruit yield (22.95 tons ha^{-1}) and higher average of single marketable fruit weight (85.84 gram) than other varieties. The least mean marketable fruit yield was obtained from the variety Fetan (11.61 tons ha^{-1}) (Figure 1 and Table 3). The mean marketable fruit yield obtained (11.61 to 22.95 ton/ha) is comparable to the result of other literatures. Researchers on tomato (Palada and Allison 2001; Znidarcic etal., 2003; Lemma, 2002) got a mean marketable fruit yield between 7.21 to 48.80 ton/ha. The variety CAL-J was the tallest (52 cm) whereas the variety Cochoro is the shortest (32.24 cm) (Table 2). Eshteshabul et al. (2010) and Kaushik et al. (2011) also obtained tomato plant with plant height in the range of 36.80 to 126.50 cm.

Marketable fruit yield was significantly and positively correlated with fruit number per plant (r = 0.75) and single fruit weight (r = 0.51) (Table 4). This indicates that varieties with higher fruit number per plant and single fruit weight gives high marketable fruit yield. Marketable and unmarketable fruit yields are also highly and positively correlated (r = 0.99). Number of primary branch was negatively significantly correlated (r = 0.54) with days to first harvest indicating that late maturing varieties have more number of primary branches than early maturing

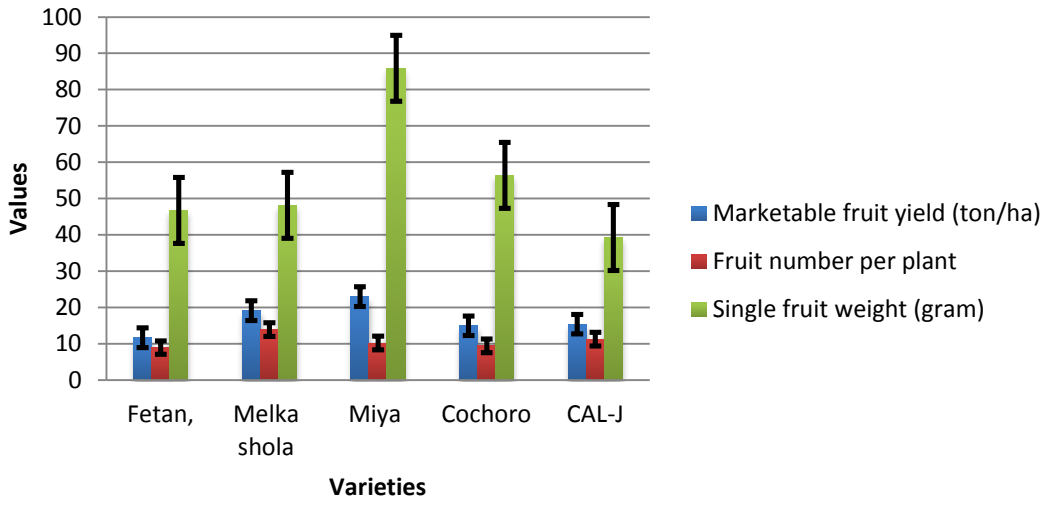

Figure 1. Value of Marketable fruit yield, Fruit number per plant and single fruit weight of 5 tomato varieties grown at Yabelloin 2014.

Table 3. Mean value of yield and yield components of 5 tomato varieties grown in 2014 in Borana zone at YPDARC.

S/N	Varieties	Dfl	Dfh	Ph	Npb	Mfy	Ufy	Tfy	Fnpp	Sfw
1	Fetan,	71.67	86.67	36.60^{d}	3.15^{b}	11.61^{c}	1.92^{c}	13.53^{c}	8.91	46.70^{b}
2	Melka shola	71.33	86.33	39.73^{c}	4.15^{a}	19.11^{ab}	2.79a^{b}	21.90^{ab}	13.85	48.14^{b}
3	Miya	71.33	86.33	44.27^{b}	3.88^{a}	22.95^{a}	3.30^{a}	26.25^{a}	10.17	85.84^{a}
4	Cochoro	74.33	87.67	33.24^{e}	2.47^{b}	14.94b^{c}	2.20b^{c}	17.14b^{c}	9.38	56.35^{b}
5	CAL-J	71.33	86.33	52.00^{a}	4.23^{a}	15.38b^{c}	2.41b^{c}	17.79b^{c}	11.27	39.24^{b}
	Varieties Mean	72.00	86.67	41.17	3.58	16.80	2.52	19.32	10.71	55.25
	CV (%)	2.85	2.78	3.50	10.67	19.73	18.27	19.39	21.44	20.05
	SED	1.68	1.97	1.18	0.31	2.71	0.38	3.06	1.88	9.05
	LSD(0.05)	n.s.	n.s.	2.71	0.72	6.24	0.87	7.05	n.s.	20.86

SED = standard error of difference; CV= coefficient variation; LSD= least significant difference; n.s.= non significant; Dfl=Days to 50% flowering, Dfh=Days to first harvest, Ph=Plant height (cm), Npb= Number of primary branch, Mfy=Marketable fruit yield (ton/ha), Ufy= Unmarketable fruit yield (ton/ha),, Tfy= Total fruit yield (ton/ha), Fnpp=Fruit number per plant, and Sfw= Single fruit weight, Varieties mean in the same column with similar letter are not significantly different from each other.

Table 4. Pearson's correlation (r) of yield and other collected parameters of 5 tomato varieties grown in 2014 in Borana zone at YPDARC.

Parameters	MFY	UFY	TFY	Fnpp	Sfw	Dfl	Dfh	Ph	Npb
MFY	-	-	-	-	-	-	-	-	-
UFY	0.98***	-	-	-	-	-	-	-	-
TFY	0.99***	0.99***	-	-	-	-	-	-	-
Fnpp	0.75***	0.74**	0.75***	-	-	-	-	-	-
Sfw	0.51*	0.48n.s.	0.51*	-0.13n.s.	-	-	-	-	-
Dfl	0.24n.s.	0.19n.s.	0.24n.s.	0.28n.s.	0.01n.s.	-	-	-	-
Dfh	0.28n.s.	0.21n.s.	0.27n.s.	0.43n.s.	-0.18n.s.	0.80***	-	-	-
Fh	0.16n.s.	0.18n.s.	0.16n.s.	0.10n.s.	-0.02n.s.	-0.43n.s.	-0.26n.s.	-	-
Npb	0.18n.s.	0.16n.s.	0.18n.s.	0.25n.s.	-0.08n.s.	-0.54*	-0.31n.s.	0.76**	-

*;**;*** = Significant at 5%, 1% and 0.1% probability level respectively n.s.= non significant; Dfl = Days to 50% flowering, Dfh=Days to first harvest, Ph=Plant height (cm), Npb= Number of primary branch, Mfy=Marketable fruit yield (ton/ha), Ufy= Unmarketable fruit yield (ton/ha),, Tfy= Total fruit yield (ton/ha), Fnpp=Fruit number per plant, and Sfw= Single fruit weight.

ones.

CONCLUSION AND RECOMMENDATIONS

Tomato need to be cultivated in Borana zone for both commercial and home use purposes. To increases production and productivity of the crop appropriate varieties has to be looked for beside agronomic and plant protection activities. Evaluation of varieties for local adaptation continued to part of strategic approach of Oromia Agricultural Research Institute in developing and promoting appropriate crop technologies for Oromia region of Ethiopia. In the present experiment, variety Miya was found superior in economic yield (marketable yield) and other parameters that it was recommended for further popularization in Yabello district under irrigation. Other agronomic and plant protection trials should be done for the success of production and productivity of tomato in the area. Post harvest management activities also have to be researched to increase its shelf life. Since the experiment is one site one season experiment, further studies using combination of locations and seasons is required to generate more reliable information on performance of varieties across location and year.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agro-morphological characterization of Fonio millet accessions (*Digitaria exilis* Stapf.) collected from Boukoumbé, Northwest of Benin

Emmanuel Sekloka[1]*, Cyrille Kanlindogbe[1], Samadori Sorotori Honoré Biaou[2], Hubert Adoukonou-Sagbadja[3], Albert Kora[1], Fidèle Tchossi Motouama[2], Moudjaidou Seidou[1], Valérien Amégnikin Zinsou[1], Léonard Afouda[1] and Lamine Baba-Moussa[4]

[1]Laboratory of Phytotechny, Plant Breeding and Plant Protection (LaPAPP), Department of Sciences and Techniques of Vegetal Production (STPV), Faculty of Agronomy, University of Parakou, Benin.
[2]Laboratory of Ecology, Botany and Plant Biology (LEB), Department of Natural Ressources Management, Faculty of Agronomy, University of Parakou, Benin.
[3]Laboratory of the Genetic Resources and Breeding of Species, Department of Genetics and Biotechnology, Faculty of Sciences and Technologies, University of Abomey-Calavi, Benin.
[4]Laboratory of Biology and Molecular Typing in Microbiology, Department of Biochemistry and Cell Biology, Faculty of Sciences and Technologies, University of Abomey-Calavi, Benin.

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Fonio is a cereal food of great socio-economic and cultural importance in south Sahara African Countries. Unfortunately, it is practically absent from National Agricultural Research Programs. To characterize the agro-morphological diversity of fonio ecotypes grown in Benin, twenty accessions collected from Boukoumbé were evaluated in a randomized complete block design (RCBD) with three replicates in Parakou. Significant variability was detected for several characters. The early accessions matured at approximately 90 days after sowing with yields below 800 kg/ha. The late accessions matured in 100 days and the most productive recorded more than 1.5 t/ha. Factor analysis of mixed data helped to classify the accessions into four morphological groups. Chi-square independence test showed that collar color, green color of foliar limb, anthocyanin coloration and its distribution in different aerial organs, type of panicle and panicle exsertion were the most discriminating qualitative parameters. The λ-wilk test revealed that date of flowering, plant height, length of panicle leaf, length of racemes and grain yield were the most discriminating quantitative traits. This study enabled a better knowledge of cultivated ecotypes and distinguishing criteria. The variability observed offered interesting perspectives for genetic progress through breeding programs of these ecotypes. However, it is important to improve our understanding on the floral biology and reproductive system for this species to create new and efficient varieties.

Key words: Genetic variability, neglected plant, morphotype, crop phenology, growth parameters.

INTRODUCTION

Fonio (*Digitaria* spp.) is one of the neglected and underutilized crops of West Africa. It is grown in an area stretching from Senegal to Lake Chad (Cruz et al., 2011). Well adapted to local pedo-climatic conditions, fonio

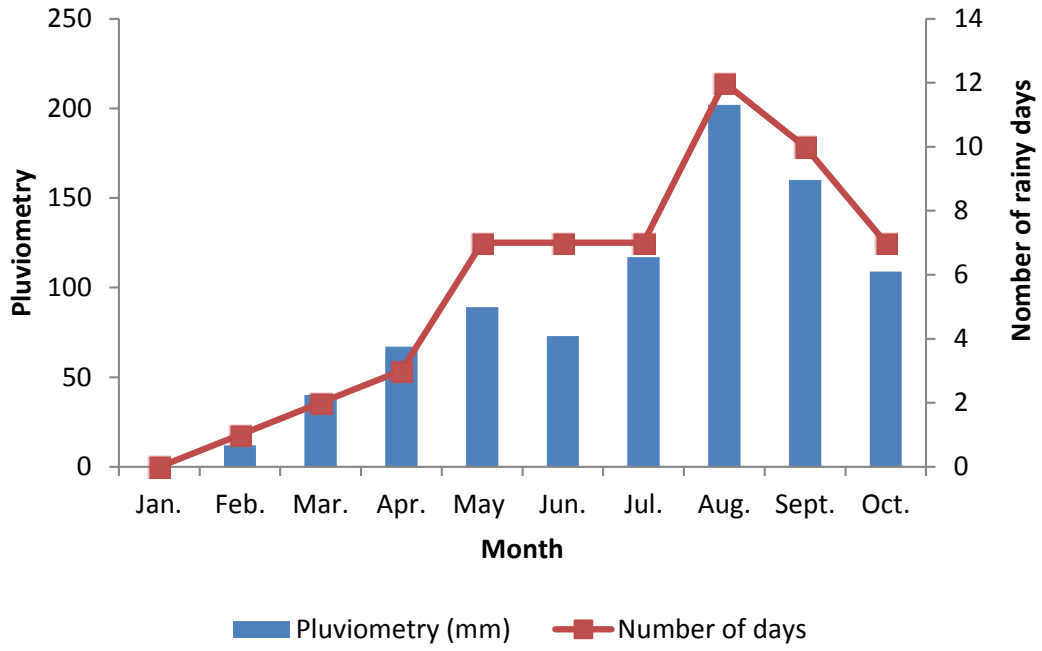

Figure 1. Distribution of rainfall during the trial period.

resists drought due to its C4 metabolism and contributes to the maintenance of the environment by ensuring vegetal cover on ecologically sensitive and undervalued soils (Vall et al., 2008; Cruz et al., 2011). Poor in gluten, fonio is indicated for diabetics and those suffering from overweight and breastfeeding women (Vodouhe and Achigan Dako, 2006). It is rich in methionine and cysteine, two essential amino acids for humans and deficient in wheat, rice, maize and sorghum (Vietmeyer et al., 1996; Ballogoun, 2013).

In Benin, unlike other cereals such as maize, sorghum, millet and rice, that are cultivated everywhere throughout the country, fonio appears as an essentially local or endemic crop to the Atacora department in the northwest of Benin (Vodouhe et al., 2003). This crop has a socio-cultural importance for the Otamari ethnic group in Boukoumbé, the main producer community providing 74% of the national production (Dramé and Cruz, 2002; Ballogoun, 2013; Paraïso et al., 2013). It also plays an important role in food security in the population especially during the lean season when early varieties are used to curb famine.

Despite its potentials, fonio remains a marginal plant and long neglected in national research programs. Most of the research has put emphasis on the management of harvest and post-harvest. Little knowledge is available on varietal breeding of this crop. The crop is thus poorly known as far as the morphological, agricultural and biological characteristics are concerned. Today, it is hard to determine with accuracy, the varieties of fonio that are grown in Benin. The objective of this study was to assess and structure the morphological and agronomic variability of fonio ecotypes cultivated in Benin in order to improve their performance.

MATERIALS AND METHODS

The trial was conducted in 2015 at the experimental farm of the Faculty of Agronomy, the University of Parakou (9° 18' 57" North latitude, 2° 42' 5" East longitude, 362 m of altitude). The soil is poor in organic matter; it is of tropical ferruginous type and consists of about 22.40% of clay and silt, 1.43% of total carbon and 0.167% total nitrogen, or a C/N ratio of 8.56 (Azontondé et al., 2009).

Rains were regular and well distributed during the period of the trial (Figure 1). The wettest months were July, August, September and October. The total annual rainfall obtained in 56 days was 869 mm. The average daily temperature varied between 20 and 25 °C with a daily average of 22°C over the period of the study.

Twenty (20) accessions of fonio collected in the commune of Boukoumbé were evaluated in a randomized complete blocks design (RCBD) with three replicates. The experimental units consisted of 3 lines of 4 m each. Sowing was done at a spacing of 0.20 x 0.20 m (20 holes/line) with a pinch of seeds. The plants were reduced to one per hole 25th day after sowing. The alley between two consecutive experimental units was 0.4 m. The plots were kept clean by regular manual weeding until harvest. The trial block was

*Corresponding author. E-mail: emmanuelsekloka@hotmail.com. Tel: (229) 97163598 or 95853417.

Table 1. Qualitative variables used for the evaluation of accessions studied.

Cycle phases	Qualitative variables	Meaning	Modalities
vegetative phase	Coul_Bas_Pl	Color of base of the plants	Green, purple green, light purple
	ColAnt_GF	Coloration of anthocyanin at foliar sheath	Absent, present
	ColAnt.LF	Coloration of anthocyanin at foliar limb	Absent, present
	DistrAnt-LF	Distribution of anthocyanin color at foliar limb	Absent, extremity only, spotted
	Pubs_LF	Pubescence of foliar limb	Glabrous, hairy
	Coul_LF	Intensity of green color of foliar limb	Medium green, light green, dark green
	Coul_Aur	Color of auricle	Absent, present
	Coul_Collet	Color of collar	Green, purple green, purple
	Att_LF	Attitude of foliar limb	Erected, horizontal, descending
Flowering phase	Form_Lig	Form of ligula	Absent, truncated, pointed, bifid
	Coul_Lig	Color of ligula	Absent, whitish, purple, light purple
	Att_FP	Attitude of panicle leaf	Erected, horizontal, descending
	Port_Tige	Port of the stem (angle)	Semi erected, open, very open
	ColAnt_Nd	Anthocyanin colouration at the nodes of the stems	Absent, light purple, purple
	ColAnt_SA_Nd	Color of underlying portion of node of the stems	Absent, light gold, green
Maturation phase	ColAnt_EnNd	Anthocyanin coloration of internodes of the stems	Absent, light purple
	ColAnt_SA_Nd	Color of the underlying portion of internodes of the stems	Absent, light gold, green
	Exs_Pan	Panicle exsertion	Good exsertion, very good exsertion
	Type_Pan	Type of panicle	Open, horizontal, falling
	Form_Ap	Apex form of grains	Pointed, curved
	Coul_raceme	Color of racemes	Brown, red

fenced with wood and protected with mosquito nets so as to exclude rodents and domestic animals. No fertilizer and pesticide treatments were applied. Harvesting was performed by mowing stems using sharp scissors for cutting the panicles. The plants were characterized using a descriptor adapted from the one utilized for the description of rice (Tia and Iliath, 2014). In total, 18 qualitative variables and 21 quantitative variables were analyzed so as to describe and partition the varieties in morphological groups. Qualitative variables were observed at the plot level. They consisted of visual observation of the coloration of different organs (stem, node, internode, leaf and panicle) during the vegetation, the flowering and the maturation phases (Table 1). Quantitative variables were measured, some at the plot level and the others at the plant level. These were collected from ten plants randomly chosen on the central lines of the elementary plots. The measures were phenology, plant growth and plant production parameters (Table 2).

R software version 3.1.3 was used for data analysis. Qualitative variables were studied by calculating, for each of them, the proportions of the accessions belonging to each category. The variability of quantitative traits was investigated through an analysis of variance. After an analysis of the correlation matrix, quantitative variables not correlated significantly ($p>0.05$) and differentiated the accessions as well as qualitative variables differentiated the accessions were submitted to a factor analysis of mixed data (FAMD) to identify morphotypes. The identified morphotypes were eventually described and the main differentiating traits for these morphotypes were determined by using Chi-squared test for categorical variables and λ-Wilk test for quantitative traits. For the later, the Tukey test was used to compare means when differences between morphotypes were significant.

RESULTS

Qualitative description of accessions

Among the 18 evaluated variables, a dozen enabled distinctions between accessions. Some of the collars were purple green while others were purple. Also, some of the foliar limbs, internodes and stem nodes showed anthocyanin coloration while others were devoid of it. The panicles, whether horizontal or open, were either brown or red at maturity. The stems had an open port, semi-erected, or very open. There were also differences in the color of the plant base, the foliar limb, the ligula and the panicle exsertion between the accessions (Table 3).

Variability of morphology measurable characters

Differences in the accessions were only observed with

Table 2. Quantitative variables used for the evaluation of accessions studied.

Phases	Quantitative variables	Meaning	Descriptions
Vegetative Phase	50% levée	Date of emergence (days)	Number of days after sowing when 50% of plants emerge
	50% tallage	Date of Start tillering (days)	Number of days from sowing to tillering of 50% of plants
	Nb talles	Number of tillers	Mean number of tillers on 10 plants of center lines
	Nb_feuilles	Number of leaves	Mean number of leaves on 10 plants of center lines
Flowering Phase	50%CSE	cycle sowing to flowering (days)	Number of days after sowing when 50% of plants let discover their flowers
	LongF	length of the leaf under panicle leaf (cm)	Measured from the level of insertion of the ligula to the top of the foliar limb
	largF	width of the leaf under panicle leaf (cm)	Measured in the middle of foliar limb
	LongFP	length of panicle leaf (cm)	Measured from the insertion level of the ligula to limb top
	LargFP	width of panicle leaf (cm)	Measured in the middle of the foliar limb
Maturation Phase	50%CSM	cycle sowing to maturation (days)	Number of days after sowing when 50% of the plants reach maturity without the grains are dry
	HP	Plant height(cm)	Measured from the soil level to the top of longest panicle
	HIP	Insertion height of panicle (cm)	Measured from the soil to insertion panicle level
	NN_plt	Number of nodes	Counting of the number of node on the main stem (longest)
	NIP	node of panicle insertion	Node of panicle Insertion on the main stem (longest)
	pan_plt	Number of panicles	Measurement taken on 10 plants of the central lines
	rac_pan	Number of raceme per panicle	Mean of number of racemes per panicle taken on 10 plants
	Lg_pan	Panicle length(cm)	Measured from the insertion panicle level to the top of longest raceme
	Lg_rac	Raceme length (cm)	Length from beginning of racemes to the top of longest raceme
Post-harvest	Rdt_grain	Grain yield (kg/ha)	Ratio of grain weight of each accession by plot after threshing, winnowing per plot area
	Rdt_biom	Yield of aerial dry biomass (kg/ha)	Ratio of Weight of aerial dry biomass (stem, leaf and panicle) per plot area
	IR	Harvest index (%)	Ratio of grain yield per biomass yield

plant height and panicle length ($P < 0.01$). Plant height, either in centimeters or number of nodes varied from simple to more than double. The shortest accession was AS1 and the longest was AS5. The same trend was observed for the height and node of panicle insertion. The shortest panicles were noted on AS12 and the longest on AS5. For the remaining variables, differences among accessions were not significant (Table 4). The analysis of the correlation matrix showed significant associations between some of these morphological variables (Table 5).

Table 3. Proportions of the categories of each qualitative variable observed.

Qualitative variables	Categories	Percentages (%)	Accessions numbers
Coul.collet	Purple green	63.16	1, 3, 5, 6, 7, 8, 9, 10, 11, 13, 17, 19
	Purple	36.84	12, 14, 18, 2, 20, 15, 16
ColAnt.LF	Absent	52.63	1, 5, 6, 7, 8, 9, 11, 12, 17, 18
	Present	47.37	2, 3,10, 14, 13, 15, 16, 19, 20
ColAntEnNd	Absent	63.16	1, 5, 6, 8, 9, 10, 11, 12, 13, 14, 17, 18
	Light purple	36.84	2, 3, 7, 15, 16, 19, 20
ColAntNd	Purple	31.58	3, 7, 15, 16, 20, 13
	Light purple	68.42	1, 2, 5, 6, 8, 9, 10, 11, 12, 14, 17, 18, 19
Coul.bas.pl	Green,	21.05	3, 5, 8, 17
	Purple green	63.16	1, 2, 6, 7, 9, 10, 11, 12, 13, 16, 19, 20
	Light purple	15.79	14, 15, 18
Coul.LF	Light green	47.37	2, 7, 11, 12, 14, 16, 18, 19, 20
	Medium green	52.63	1, 3, 5, 6, 8, 9, 10, 13, 15, 17
Coul.lig	Whitish	68.42	1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 17, 18, 20
	Light purple	31.58	3, 13, 14, 15, 16, 19
coul.raceme	Brown	73.68	1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, 20
	Red	26.32	2, 19, 13, 14, 15
DistrAnt.LF	Extremity only	31.58	2, 3, 13, 16, 19, 20
	No distribution	52.63	1, 5, 6, 7, 8, 9, 11, 12, 17, 18
	Spotted	15.79	10, 14, 15
Exs.Pan	Good exsertion	68.42	1, 6, 7, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20
	Verygoud exsertion	31.58	2, 3, 5, 9, 10, 11
Port.Tige	Open	57.89	5, 7, 8, 9, 10, 11, 12, 15, 16, 18, 19
	Semi erected	26.32	1, 2, 6, 14, 20
	Very open	15.79	3, 13, 17
Type.Pan	Horizontal	89.47	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20
	Open	10.53	12, 14

Coul.collet: Color of collar; ColAnt.LF: coloration of anthocyanin at foliar limb; ColAnt_EnNd: anthocyanin coloration of internodes of the stems; ColAnt_Nd: anthocyanin colouration at the nodes of the stems; Coul.bas.pl: color of the base of the plant; Coul_LF: intensity of green color of foliar limb; Coul_Lig: color of ligula; Coul_raceme: color of racemes

Plant height (HP) was significantly ($p<0.05$) correlated with the width of the leaf under the panicle leaf (LargF), the height of panicle insertion (HIP), the node of panicle insertion (NIP), the number of nodes on the main stem (NN_Plt) and the number of racemes per panicle (rac_plt).

Width of panicle leaf (LargFP) was significantly ($p<0.05$) correlated with the width of the leaf under the panicle leaf (LargF), the length of the leaf under the panicle leaf (LongF) and the length of panicle leaf (LongFP). Panicle length (Lg_pan), raceme length (Lg_rac) and the number of panicles per plant (Pan_plt)

Table 4. Variability of measurable characters of plant morphology.

Variables	Minimum		Maximum		Mean	Standard deviation	CV (%)	Prob.	
LongF (cm)	8.6	(AS14)	11.6	(AS8)	10.0	0.9	8.6	0.729	
LongFP(cm)	6.1	(AS14)	9.6	(AS17)	7.5	0.9	11.4	0.139	
LargF (cm)	0.5		0.5		0.5	0.0	4.2	0.409	
LargFP (cm)	0.3		0.4		0.3	0.0	4.2	0.344	
HP (cm)	66.1	(AS1)	112.7	(AS5)	97.6	12.6	12.9	<0.001	***
NN_plt	5.0	(AS1)	12.5	(AS19)	10.2	2.3	22.5	<0.001	***
HIP (cm)	24.1	(AS1)	83.6	(AS5)	68.1	19.0	27.9	<0.001	***
NIP	5.0	(AS1)	12.5	(AS19)	10.2	2.3	22.5	<0.001	***
Pan_plt	32.0	(AS11)	54.6	(AS20)	42.3	6.0	14.2	0.385	
rac_pan	3.5	(AS1)	4.4	(AS9)	4.1	0.2	5.1	0.084	
Lg_rac (cm)	11.7	(AS1)	16.3	(AS15)	13.3	1.0	7.7	0.127	
Lg_pan (cm)	23.9	(AS12)	30.3	(AS5)	26.4	1.5	5.5	0.008	*

LongF: length of leaf under panicle leaf; LongFP: length of panicle leaf; largF: width of leaf under panicle leaf; largFP: width of panicle leaf; HP: plant height; NN_plt: number of nodes on the main stem; HIP: height of panicle insertion; NIP: node of panicle insertion; Pan_plt: number of panicle per plant; rac.pan: Number of raceme per panicle; Lg_rac: raceme length; Lg_pan: Panicle length. *** Very highly significant (<0.001), ** highly significant (<0.01), * Significant (<0.05).

Table 5. Matrix of Pearson correlations between quantitative variables.

	HIP	HP	LargF	LargFP	Lg.pan	Lg.rac	LongF	LongFP	NIP	NN.plt	Pan.plt	Rac.pan
HIP	1.0											
HP	**1.0**	1.0										
LargF	**0.5**	**0.5**	1.0									
LargFP	0.0	0.1	**0.5**	1.0								
Lg,pan	0.4	0.4	0.2	0.0	1.0							
Lg,rac	0.0	0.1	0.2	0.1	0.2	1.0						
LongF	0.2	0.4	**0.7**	**0.6**	0.1	0.1	1.0					
LongFP	-0.1	0.1	0.4	**0.7**	0.0	0.1	**0.8**	1.0				
NIP	**0.8**	**0.7**	0.3	-0.1	0.4	0.0	-0.1	-0.3	1.0			
NN.plt	**0.8**	**0.7**	0.3	-0.1	0.4	0.0	-0.1	-0.3	**1.0**	1.0		
Pan.plt	-0.1	-0.1	-0.2	0.0	-0.2	0.1	-0.1	0.0	0.0	0.0	1.0	
Rac.pan	**0.7**	**0.6**	0.3	-0.2	0.2	0.0	0.2	-0.1	**0.5**	**0.5**	0.1	1.0

LongF: Length of leaf under panicle leaf; LongFP: length of panicle leaf; largF: width of the leaf under panicle leaf; largFP: width of panicle leaf; HP: plant height; NN_plt: number of nodes; HIP: height of panicle insertion; NIP: node of panicle insertion; Pan_plt: number of panicle per plant; rac.pan: number of raceme per panicle; Lg_rac: raceme length; Lg_pan: panicle length. The correlation coefficients in bold are significantly different from zero at the 5% threshold.

were not correlated with any other variables. For descriptions of morphotypes, five variables were retained: length of panicle leaf (longFP), plant height (HP), panicles length (Lg.pan), raceme length (Lg.rac) and number of panicles per plant (Pan.plt). These variables were considered sufficient to account for the accessions of morphometric variability since each of the other variables was correlated with either of these five.

Variability of agronomic characterization parameters

Despite the large variations observed in the date of emergence (4 to 10 days after sowing), number of tillers per plant (15 to 37), number of leaves per plant (51 to 134), and the harvest index (9 to 36%), there was no significant difference among accessions ($p>0.05$) for these parameters (Table 6). Nevertheless, there were significant variations among accessions for other parameters such as flowering and maturation dates. The early matured varieties were AS1 and AS8, whereas AS15 and AS10 matured late.

Grain and biomass yields also showed significant variations between accessions. AS2 was the most productive accession, with more than 2 t/ha of grain yield, which was almost nine times the yield of the least

Table 6. Variability of agronomic characters.

Variables	Minimum		Maximum		Mean	Standard deviation	CV (%)	Prob.	
50% level (das)	4.3	(AS16)	10.0	(AS1)	5.8	1.8	32	0.080	
50% tallage (das)	24.0	(AS15)	25.7	(AS19)	24.4	1.0	4.3	0.646	
50% CSE (das)	64.0	(AS1)	92.0	(AS15)	83.3	10.1	12.1	<0.001	***
50% CSM (das)	79.7	(AS8)	104.7	(AS10)	99.4	7.3	7.4	<0.001	***
Nb_talles	15.3	(AS1)	36.6	(AS2)	27.1	14.3	52.6	0.979	
Nb_feuilles	50.8	(AS1)	133.9	(AS2)	104.4	52.0	49.8	0.957	
Rdt_grain (kg/ha)	286.3	(AS1)	2452.8	(AS2)	1220.2	700.0	57.4	0.012	*
Rdt_biom. (kg/ha)	1279.3	(AS1)	11905.6	(AS5)	7381.5	3437.4	46.6	0.000	***
IR (%)	8.9		36.4		19.2	6.5	33.7	0.377	

50% level: Date of emergence 50% tallage: Date of Start tillering; Nb_talle: Number of tillers; Nb_feuilles: Number of leaves; 50%CSE: cycle sowing to flowering; 50%CSM: cycle sowing to maturation; Rdt_grain: Grain yield; Rdt_biomasse: yield of aerial dry biomass; IR: Harvest index; das: days after sowing. *** Very highly significant (<0.001), ** highly significant (<0.01),* Significant (<0.05).

Table 7. Pearson correlation matrix between agronomic parameters.

	50% CSE	50% levée	50% CSM	IR	50% tallage	Rdt_biom	Rdt_grain	Nb_feuilles	Nb_talles
50% CSE	1.0								
50% level	-0.3	1.0							
50% CSM	0.9	-0.3	1.0						
IR	-0.3	-0.1	-0.4	1.0					
50% tallage	0.1	-0.2	0.0	0.4	1.0				
Rdt_biom	0.5	0.0	0.5	-0.5	-0.5	1.0			
Rdt_grain	0.2	-0.2	0.2	0.3	-0.2	0.5	1.0		
Nb_feuilles	-0.1	0.2	0.0	-0.3	-0.5	0.4	0.1	1.0	
Nb_talles	-0.1	0.2	0.0	-0.3	-0.4	0.4	0.1	0.9	1.0

50% level: date of emergence 50%tallage: date of start tillering; Nb_talle: number of tillers; Nb_feuilles: number of leaves; 50%CSE: date of flowering; 50%CSM: date of maturity; Rdt_grain: grain yield; Rdt_biom: yield in aerial dry biomass; IR: harvest index. The correlation coefficients in bold are significantly different from zero at the 5% threshold.

productive, AS1 ($p < 0.05$). Significant differences ($p < 0.01$) were observed among accessions for the biomass yield as well. AS1 recorded the lowest biomass yield, while AS5 recorded the highest (Table 6).

Pearson correlation matrix showed that the grain yield (Rdt_grain) was little correlated with the other variables; it was only correlated with the biomass yield (Rdt_biomasse). The biomass was correlated with most of the other variables except for the date of emergence (50% level), the number of leaves (Nb_feuilles) and the number of tillers (Nb_talle). There was also a strong correlation ($p<0.01$) between the date of flowering (50% CSE) and date of maturity (50% CSM), and between the number of leaves and number of tillers (Table 7).

For the identification of morphotypes, the date of emergence (50% level), date of flowering (50% CSE), harvest index (IR), grain yield (Rdt_grain) and number of tillers (Nb_talles) proved to be sufficient to describe agronomic variability of the studied accessions, given that each of the other variables was correlated with one or more of these selected parameters.

Regrouping of accessions in morphotypes

The categorical variables that distinguished accessions and quantitative parameters selected from the analysis of the correlation matrix were submitted to a factor analysis of mixed data (FAMD).

The first five axes explained 73.2% of the total information (cumulated variance) with all Eigen values greater than 1. The first two axes explained 43.37% of the total variability (Table 8). The first axis was correlated with plant height (HP), earliness (50% CSE and 50% levée) and grain yield (Rdt_grain). It is mostly an axis characterizing vigor and earliness. It also describes the coloration of different parts of the plant, in particular, anthocyanin coloration of the foliar limb (ColAnt.LF) and, to a lesser extent, coloration of the internodes of the stem (ColAntEnNd), racemes (coul.racème) and ligula

Table 8. Variance explained by the main axes from the factor analysis of mixed data (FAMD).

Variables	Axis 1	Axis2	Axis3	Axis 4	Axis 5
Eigen values	6.4	4.01	2.96	2.25	1.94
Explained variance (%)	26.66	16.71	12.34	9.39	8.07
Cumulative variances (%)	26.66	43.37	55.7	65.09	73.16

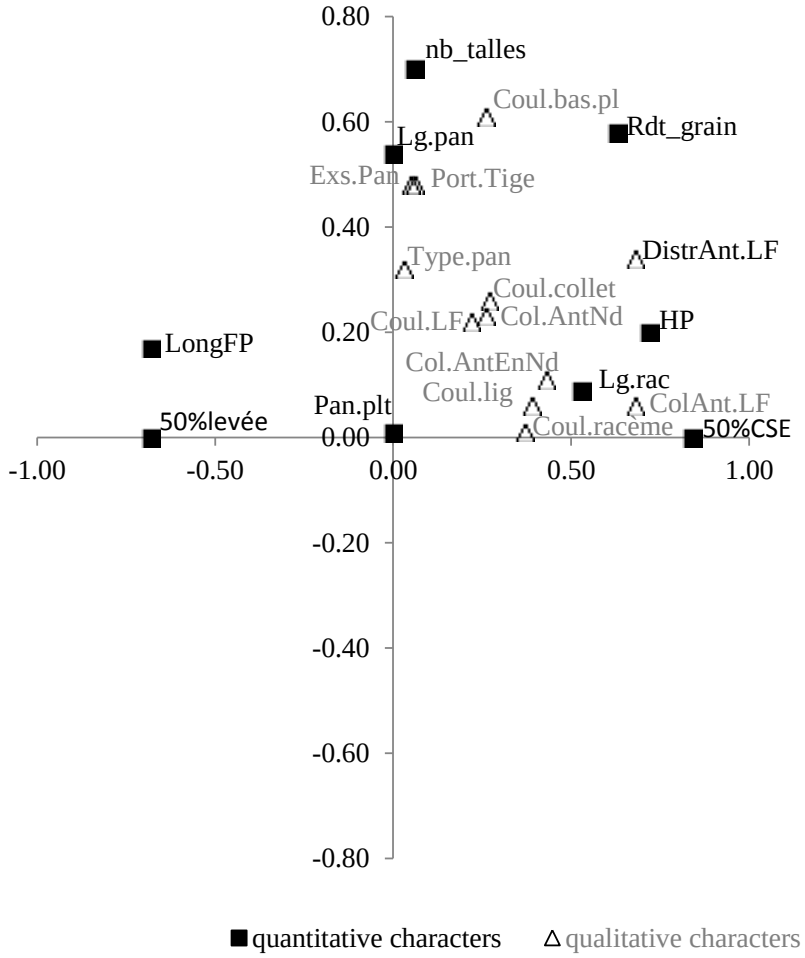

Figure 2. Projection of the variables in the factorial plane formed by the first two axes. Coul_LF: intensity of green color of foliar limb; Coul.bas.pl: color of the base of the plant; ColAnt.LF: coloration of anthocyanin at foliar limb; DistrAnt-LF: distribution of anthocyanin at the foliar limb; Coul.collet: color of collar; Coul_Lig: color of ligula; ColAnt_Nd: Anthocyanin colouration at the nodes of the stems; ColAnt_EnNd: Anthocyanin coloration of internodes of the stems; Port_Tige: port of the stem; Type_Pan: type of panicle; Exs_Pan: exsertion panicle; Coul_racème: color of racemes; HP: plant height; Lg.pan: panicle length; Lg.rac: raceme length; 50% CSE: date of flowering; Rdt_grain: grain yield; 50% levée: date of emergence; Nb_talles: number of tillers; pan.plt: number of panicles per plant; Long FP: length of panicle leaf.

(Coul.lg) (Figure 2). The second axis was correlated with the number of tillers (nb_talles), the panicles length (Lg.pan) and grain yield (Rdt_grain). Regarding qualitative characters, it describes the color of the base of the plant (Coul.bas.pl), the port of the stem (Port.Tige) and panicles form (Exs.Pan and Type.Pan) (Figure 2).

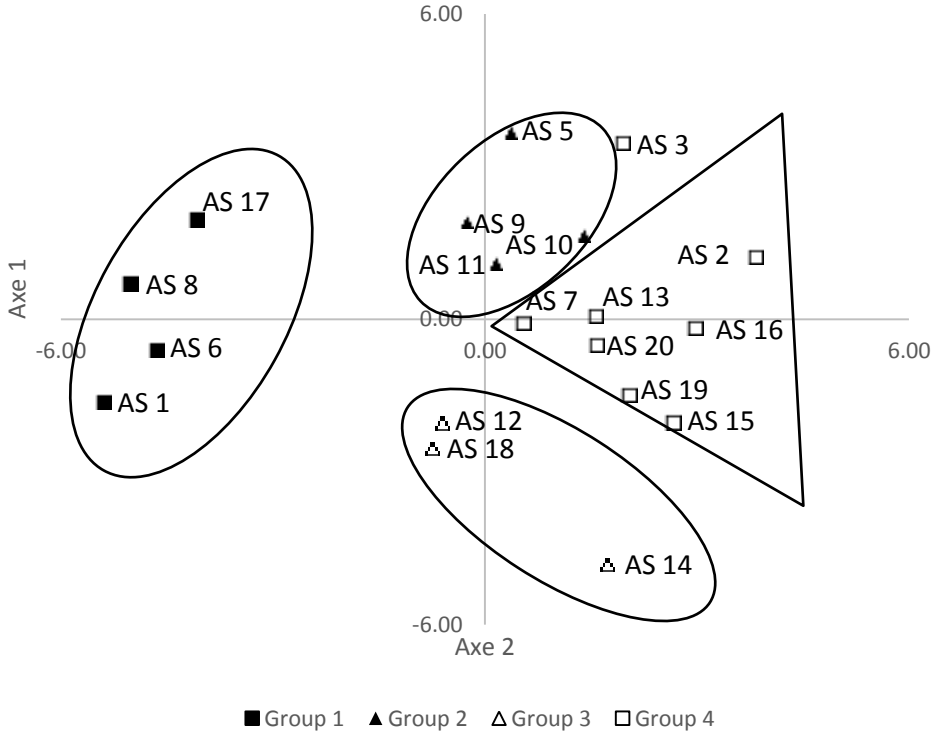

Figure 3. Projection of accessions in the factorial plane formed by the first two axes.

The projection of accessions in the factorial plane formed by the first two axes allowed separation of the accessions into four groups (Figure 3). Axis 1 opposed the accessions of group 1, earlier, of short height and longest panicle leaves to those of group 4, later and more slender. Regarding qualitative traits, this axis also opposed accessions devoid of anthocyanin coloration at foliar limb and internodes (Group 1) to others which displayed anthocyanin coloration (Group 4).

The second axis opposed the accessions of group 2 and group 3, respectively located on the upper and lower side of this axis (Figure 3). Accessions of group 2 have a higher tillering aptitude, longer panicles and higher yields than accessions of group 3. Regarding qualitative traits, this axis opposed accessions equipped with horizontal panicles and very good exsertion (Group 2) to those equipped with open panicles and good exsertion (Group 3).

Identification of discriminating traits

Chi-square tests (χ^2) showed that eight qualitative traits are significantly related to the classification of the accessions in morphological groups (Table 9). λ-Wilk test revealed that five (5) quantitative characters allowed discrimination of the identified morphotypes. These were the date of flowering (50%CSE), plant height (HP), length of panicle leaf (LongFP), raceme length (Lg.rac) and grain yield (Rdt_grain) (Table 10).

DISCUSSION

The morphological and agronomic characterization of a crop is an important step in the management of genetic diversity (Manzano et al., 2001; Yobi et al., 2002; Radhouane, 2004). It is also a prerequisite towards the selection of improved varieties (Smith et al., 1991; Fraleigh, 1987). This work had helped to characterize for the first time in station in Benin, the diversity of ecotypes of fonio grown in Boukoumbé, one of the regions described as one of the areas of origin of this crop (Adoukonou-Sagbadja et al., 2006, 2007). The results showed significant differences between several morpho-phenological and agronomic characters. This demonstrates a high variability between accessions, which could be explained by the farmers' practices of seed management coupled with the proximity to the study area with Togo, another region of origin for fonio where genetic diversity was described as very high (Adoukonou-Sagbadja et al., 2007). Indeed, several authors have shown that farmers' practices of seed management, including the exchange of varieties among farmers, are the source of an important diversity among populations of cultivated plants (Mckeye et al., 2001; Delaunay et al., 2008; Missihoun et al., 2012). The exchange of varieties among farmers, which has been described as the main mode of access to fonio seeds in this region (Sekloka et al., 2015), has been able to contribute to a significant increase in the diversity of ecotypes of fonio grown in Boukoumbé. This substantial phenotypic variability could

Table 9. Qualitative variables significantly related to the classification of the accessions in morphological groups (Chi-square test, χ^2).

Qualitative variables	χ^2	Probability	
ColAntEnNd	15.240	0.002	**
ColAntNd	12.058	0.007	*
Exs.Pan	12.058	0.007	
Type.Pan	11.922	0.008	*
Coul.collet	10.405	0.015	
ColAnt.LF	9.808	0.020	*
DistrAnt.LF	14.382	0.026	*
Coul.LF	8.471	0.037	*
coul.bas plant	9.566	0.144	
coul.lig	7.237	0.065	
Coul.racème	5.248	0.155	
Port.Tige	5.815	0.444	

Coul_LF: Intensity of green color of foliar limb; Coul.bas.pl: color of the base of the plant; ColAnt.LF: coloration of anthocyanin at foliar limb; DistrAnt-LF: distribution of anthocyanin at the foliar limb; Coul.collet: color of collar; Coul_Lig: color of ligula; ColAnt_Nd: anthocyanin colouration at the nodes of the stems; ColAnt_EnNd: anthocyanin coloration of internodes of the stems; Port_Tige: port of the stem; Type_Pan: type of panicle; Exs_Pan: exsertion panicle; Coul_racème: color of racemes. **Highly significant (<0.01), *Significant (<0.05).

Table 10. Quantitative variables significantly related to the classification of the accessions in morphological groups (λ-Wilk test).

Variables	λ-Wilk	Statistique F	probability	
LongFP	0.285	12.525	<0.001	***
Pan.plt	0.940	0.319	0.812	
Lg.rac	0.506	4.885	0.015	*
HP	0.118	37.303	<0.001	***
Lg,pan	0.633	2.895	0.070	
50% level	0.607	3.239	0.052	
Nb_talles	0.894	0.593	0.629	
50%CSE	0.024	200.678	<0.001	***
Rdt_grain	0.544	4.185	0.024	*

HP: Plant height; Lg.pan: panicle length; Lg.rac: raceme length; 50% CSE: date of flowering; Rdt_grain: grain yield; 50% levée: date of emergence; Nb_talles: number of tillers; pan.plt: number of panicle per plant; Long FP: length of panicle leaf. *** Very highly significant (<0.001), ** highly significant (<0.01), *Significant (<0.05).

be an expression of a strong genotypic heterogeneity. Such morphological and phenological dissimilarities between accessions are often generated and maintained by diverse evolutionary processes. Agroecosystems are likely to exert widely varying selective pressures on genotypes (Sadiki and Jarvis, 2005). This is also the case with anthropic pressures (Robert et al., 2004). Indeed, the way seed is managed by farmers such as selective sorting, post-harvest technologies and agricultural practices lead to a selection involving the maintenance, and even the creation of a remarkable phenotypic diversity (Robert et al., 2005).

Our results revealed that the studied collection encompasses extra-early accessions of less than 90 days and late accessions of more than 100 days. These results consolidate and complement those found by Vodouhe and Achigan Dako (2003) which identified in Benin short cycle varieties (90 days on average) and long cycle varieties (about 120 days). Similar results regarding earliness of Fonio were also found in Niger by Saidou et al. (2014). Identifying early accessions is of great agronomic importance for varietal breeding of fonio in the current context where climatic variations are becoming recurrent. However, the floral biology of fonio remains poorly known and the mode of reproduction, preferentially autogamic for some varieties (Cissé, 1975), allogamic for others (Vodouhe and Achigan Dako, 2006) and essentially apomictic for Adoukonou-Sagbadja et al. (2010), has not make unanimity within the scientific community yet (Cruz et al., 2011). A good understanding of its floral biology and possibilities of achieving chromosomal mixings will enable to exploit this variability better and create more efficient varieties.

In this study, four fonio morphotypes were identified, whereas Dansi et al. (2010) found five morphotypes in Boukoumbé and surrounding areas. Indeed, Dansi et al. (2010) had done their ranking based on the results of field surveys and not experimentation in station, which already made a fundamental difference in the methodological approach used for the evaluation of accessions. Also, their work had covered the entire region of Atacora where fonio is grown in Benin and not only the main production commune of Boukoumbé which was the focus of this study. These reasons could be the differences in the number of morphotypes identified. Similar to these findings, Saidou et al. (2014) identified four morphological groups of fonio in Niger for the species *Digitaria exilis* through an experimentation of fonio accessions in station.

The results of this study suggest that the accessions grown in Boukoumbé were rather of *D. exilis* and not of *Digitalia iburua*. Indeed, the descriptions obtained in this study are similar to those found by Cissé (1975) for the species *D. exilis*. Moreover, referring to the work of Portères (1955), the leaves of *D. iburua* have long lashes near the basis, leaving behind the ligula which is membranous, round, wide, long of 1 to 2 mm, with a terminal panicle consisting of 4-10 sub-racemes. These results differ from this description and suggest that our accessions were rather of *D. exilis*. The factor analyses made it possible to identify a set of quantitative and qualitative characters that discriminate the accessions. Among these traits, descriptors of earliness, vegetative development and grain productivity ranked high.

Examples of these descriptors were the date of flowering, plant height, length of foliar limb and racemes length. The importance of these types of characters in structuring the diversity of vegetal populations has been demonstrated on maize (Moreno et al., 2006; N'da et al. 2014), cotton (Sekloka et al., in review), sorghum (Koffi et al., 2011) and many other crops. Although subject to environmental variations, these parameters should not be neglected by plant genetic resources managers since they have always been important in farmers' environment and constitute important criteria for mass selection.

CONCLUSIONS AND SUGGESTIONS

This study on agro-morphological characterization showed a significant diversity of ecotypes of fonio cultivated in Benin. They are diverse in their port, the color of their raceme, the presence or absence of anthocyanin at different levels of the stems and leaves. The earliest accessions had a sowing-maturity cycle of about three months, but grain yields were mostly less than or equal to 800 kg/ha. The most productive accessions recorded more than 1.5 t/ha of grain yield, with more than 2 t/ha for AS2. These accessions can be separated into four distinct morphological groups based on discriminating traits. The most discriminating qualitative traits were the color of collar, the intensity of green coloration of foliar limb; anthocyanin coloration and its distribution across different aerial organs of the plant, and the type and exsertion of panicles. As for quantitative parameters, the most discriminating were the date of flowering, plant height, length of foliar limb and racemes length. The morphological, phenological and agronomic variability demonstrated is sufficient to implement a program of varietal breeding based on varietal homogenization followed by multiple sites evaluation of the improved ecotypes. To sum up, these results contribute to a better knowledge of fonio ecotypes and therefore, allow a better management of the species variability for the benefit of the producers. However, it is important to improve understanding onthe floral biology and reproductive system of fonio in order to be able to achieve genetic mixings leading to new genotypes.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of seed source and seed age on yield and yield related traits of malt barley (*Hordium vulgare* L.) varieties at Central Arsi Highlands Ethiopia

Tefera Regasa[1]*, Firew Mekbib[2] and Firdissa Eticha[3]

[1]Madawalabu University, P. O. Box 247, Bale Robe, Ethiopia.
[2]Haramaya University, P. O. Box: 138, Dire Dawa, Ethiopia.
[3]NSERC Post-doctoral Visiting Fellow, Agriculture and Agri-Food, Canada.

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The major constraints in fulfilling the growing demand of malt barley in Ethiopia are limited as a result of the selection of a favourable production environment and long-time seed storage for market advantages among others are few to mention. Besides, few research findings are available on evaluation of the potential differences across sites and crop management practices to meet malting and brewery industry quality standards. The present study was conducted to evaluate the effect of seed sources and seed age on yield and yield related traits of malt barley. The experiment was conducted at Bokoji sub site of Kulumsa Research using 12 treatments consisting of 3 malt barley collections obtained from Debrebrhan Agricultural Research Centre, Ethiopia Seed Enterprise and Oromia Seed Enterprise, two seed age (year 1 and year 2) and two barley varieties (Beka and Holker). Field experiment was laid out using RCBD factorial with three replications. Highly significant (P≤0.01) differences were achieved for days to physiological maturity and number of seeds per spike indicating the presence of variability among varieties due to genetic and environmental influences. Likewise, significant (P≤0.05) variation were obtained for number of seeds per spike and yield (kg/ha). The interaction effect of seed source and variety were found significant (P≤0.05) difference for days to physiological maturity. Positive and highly significant (P≤0.01) correlations were found between days to heading, day to physiological maturity and number of seeds per spike. Our study suggests that seed sources and varietals difference were salient factors in creating field performance variability among malt barley cultivars. Therefore, evaluating the effect of genotypes, environment, varieties and their interaction effect plays an immense role to improve yield and yield related traits in malt barley. Furthermore, the finding of this study shows that further studies should be conducted across sites and years to assess varieties performances which will help as cornerstone to fulfil the growing demand of quality malt barley seed and grain for processing industries.

Key words: Malt barley, seed age, seed source.

INTRODUCTION

Barley (*Hordeum vulgare* L.) has the ability to adapt and survive in a wide range of environmental conditions mainly produced in temperate areas and high altitudes of the tropics and subtropics (Birhane et al., 1996; Ullrich, 2002). In Ethiopia, barley is the fourth most abundant crop grown among major cereal crops in terms of area coverage and yield next to wheat, maize and tef (Harlan, 1969). Barley grain is used for the preparation of different

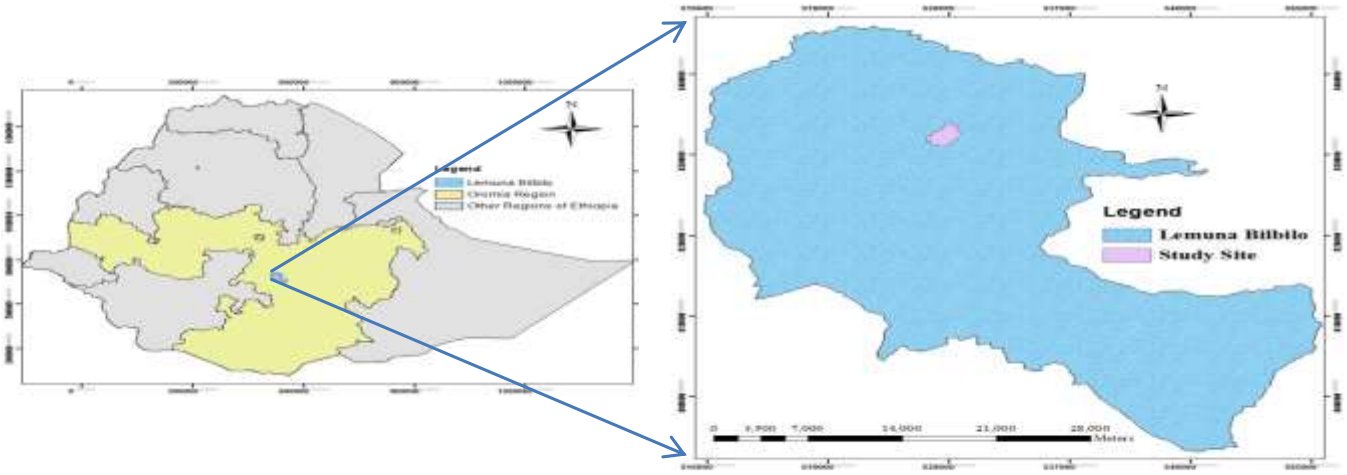

Figure 1. Map of the study site.

foodstuffs (bread, porridge) and malting (beer, gruel) (Zemedie and Bothmer, 1990; Getachew et al., 2007). The malting quality of barley is one of the economically important trait which is controlled by many genes and strongly influenced by environmental factors (Fox et al., 2003). Even though there is favorable environment for malt barley production in Ethiopia, the country on an average imports about 67,453 tons annually valued at 22.7 million Birr (Mohammed and Getachew, 2003). There is heavy financial penalty to malting industry and breweries due to short supply to meet the demand. Therefore, import substitution with local malt barley production is salient for sustainable supply for processing industries which will minimize importation cost and delivery time of produce and also generate income for farmers. Accordingly, improves quality malt barley production and minimize dependence on other countries (Mohammed and Getachew, 2003). Few studies have been conducted on malt barley variety performances across locations and on seed aging. Few years back, Altenbach et al. (2003) reported the significance of variety testing across sites or years and found higher explained percentage of environmental effect on the actual performance of varieties. These studies suggested that for sustainable improvement of malt barley production and to meet brewery companies seed quality standards, evaluating varieties across sites play an immense role to systematically identify which variety win. Therefore, in view of the important investigation of the effect of production site, seed age and varieties on yield and yield components of malt barley is useful for future malt barley improvement programs.

MATERIALS AND METHODS

The field experiment was conducted at Bekoji sub site Kulumsa Agricultural Research Centre. It is located in Arsi zone of Oromia National Regional state, Ethiopia during 2011 main cropping season. The study was conducted to investigate the effect of seed sources and seed age on yield and yield related traits and seed quality of malt barley varieties. The experimental site is situated at 231 km from Addis Ababa at 07° 32' 37'' N latitude and 39° 15' 21'' E longitudes. Different varieties of malt barley seeds were obtained from Ethiopia Seed Enterprise (ESE), Oromia seed enterprise (OSE) and Debrebirhan Agricultural Research Centre (DARC). Factorial experiment was used based on randomized complete block design with three replications. The station soil type is classified as Eutric Nitosol, exhibiting a clay content of approximately 48.5% (Tanner et al., 1993) and is relatively deficient in phosphorus with pH of 5.3. The station is situated at an altitude of 2780 m.a.s.l. The site receives an annual average rainfall of 1020 mm. It has a long term mean minimum and maximum annual average temperatures of 8 and 20°C, respectively (Figure 1).

Description of experimental materials

Seed samples of two improved malt barley varieties, namely Beka and Holker, harvested in 2009 designated as year two and 2010 designated as year one and three malt barley collection sites. Representative samples were obtained from DARC, ESE, and OSE. The class of seeds employed for this experiment was certified cycle one.

Experimental design and procedure

Field experiment was laid out in Randomized Complete Block Design (RCBD) using factorial arrangement in three replications. Treatments were assigned to each plot in random manner. The plot size was 3 m2 which accommodates six rows of 2.5 m length and

*Corresponding author. E-mail: tefera.regasa@mwu.edu.et.

1.2 m width. Spacing between rows was 0.2 m, while plots and blocks were separated by 0.5 and 1 m, respectively to prevent inter-plot interference. In both sides of the plots one row was left to control border effect while the remaining four rows were used to measure grain yield.

Field management

Land preparation was done after the onset of rains. Seeds were sown in rows by hand using recommended seeding rate of 100 kg/ha in 2011. Diammonium phosphate (DAP) fertilizer was applied at a rate of 100 kg/ha during sowing time. All the agronomic practices recommended for seed production, such as hand weeding, cultivation and rouging were undertaken from land preparation through seed processing and drying.

Agronomic parameters

Days to heading and maturity, number of fertile tillers and plant height

Days to heading and maturity, number of fertile tillers and plant height were recorded when 50 and 95% of the plants attained their respective phenological stages, respectively. Two middle rows were randomly selected from each plot at late tillering and physiological maturity stages to determine number of fertile tillers and plant height from 1 m row length.

Number of seeds per spike

Number of seeds per spike was determined from 10 randomly selected plants of each plot at maturity. Plants from the central four rows of each plot were subjected to yield evaluation and seed quality analysis.

Seed moisture content

Seed moisture content was determined using the digital moisture meter in the field so as to determine harvesting. After harvest, threshing, and drying, seeds of the same treatments from the three replications were separately weighed and bulked together whereby sampled for laboratory analysis.

Seed yield and 1000 kernel weight

Seed yield and 1000 kernel weight were determined in kg ha-1 and g, respectively at adjusted moisture content (MC%) of 12.5% using the following relationship and converted to ha-1 (Gassim, 1988). Data on days to heading and maturity were recorded on a plot basis and plant height was averaged over five plants randomly selected from each plot.

RESULTS AND DISCUSSION

Agronomic parameters, yield components and yield

Analysis of variance indicated that days to maturity and number of seeds per spike were highly (P≤0.01) affected by main effect of production site. This is in agreement with the result of Delouche (1980) who reported significant effect of environmental factors on seed quality and growth of some plants. Mati et al. (1989) also reported that the centrality of some special regions for the production of some products is a convincing reason for the effect of the environment on growth and quality of seeds. Environmental interactions during grain filling alter the time course for grain development and influence final grain weight, protein and starch contents (Altenbach et al., 2003). Meanwhile, no detectable variations were demonstrated on days to heading, productive tiller number and plant height due to main effect of seed age. Hence, the longest days to maturity was recorded in seed samples collected from ESE (143 days) followed by samples collected from DARC, while the shortest duration to maturity were recorded in samples collected from OSE (Table 1). This indicates harvesting of seeds at physiological maturity is better to have maximum dry weight, higher viability and vigor, besides higher seed yield and yield attributing parameters (Vasudevan et al., 2008). This indicates that barley varieties tend to maintain their relative days to maturity regardless of seed age, which intern indicated that environmental effect played a significant role in determining barley maturity irrespective of seed age. According to Waddington et al. (1986), modern genotypes had the highest rates of total grain sink filing period, reflecting their larger grain sink. Intact barley spikes sampled randomly from the field at different dates during the maturation period can be used for determining the number of days to maturity where it was affected by genotype. Higher numbers of seeds per spikes were recorded in Holker (112 seeds) as compared to Beka variety with (103 seeds).

Since there was no interaction effect between seed age, site and variety, it tends to maintain their relative days to maturity from year to year and from location to location as well as from variety to variety. Days to physiological maturity can change depending on weather conditions prevailing in a given year. In consistent to this result, Bayeh and Grando (2011) stated that the interaction effect of variety by seed age was not significant, indicating that malting barley can be produced without yield fluctuation risk in the study area. The main effect of variety had resulted in significant (P≤0.05), while site had posed highly significant (P≤0.01) on seeds per spike effect. Similarly, other reports have shown the variation of number of kernels per spike as a function of genotypes and environment (Martin, 1987; Schulthess, 1992; Zewdu et al., 1992; Tilahun et al., 1996).

Analysis of variance depicted that the interaction effect of site by variety significantly (P≤0.05) affected days to physiological maturity while the rest measured traits did not show any significant variation. Nevertheless, the longest duration of maturity (142 days) were recorded in seed samples collected from DARC as compared to the other seed sources (Table 2). According to Tekrony and Egli (1997) physiological maturity is a good sign of achieving maximum seed quality on the mother plant.

Table 1. Main effect of seed age, site and varieties on some agronomic traits of barley.

Treatment	Parameter					
Age	**DH**	**PTN**	**PH**	**DPM**	**SPS**	**GY**
Year 1	82.56[a]	5.01[a]	95.89[a]	141.94[a]	109.59[a]	2712.4[a]
Year 2	80.00[a]	4.90[a]	95.08[a]	142.28[a]	105.27[a]	2604.6[a]
P≤0.05	ns	ns	ns	ns	ns	ns
Site						
DARC	84.08[a]	5.03[a]	96.47[a]	142.25[ab]	116[a]	2683.50[a]
ESE	80.33[a]	4.83[a]	95.60[a]	142.50[a]	100[b]	2605.10[a]
OSE	79.42[a]	5.03[a]	94.37[a]	141.58[c]	107[ab]	2686.70[a]
P≤0.05	ns	ns	ns	0.77	10.2	ns
Variety						
Beka	80.61[a]	5.03[a]	94.62[a]	141.67[b]	103[a]	2575.1[b]
Holker	81.94[a]	4.89[a]	96.34[a]	142.56[a]	112[a]	2741.8[a]
P≤0.05	ns	ns	ns	0.63	ns	13.55

DARC: Debrebrhan Agricultural Research Centre; ESE: Ethiopia Seed Enterprise; OSE: Oromia Seed Enterprise; DH: days to heading; PTN: productive tiller number; PH: plant height (cm); DPM: Days to physiological maturity; SPS: seeds per spike; GY: grain yield (kg/ha); LSD (0.05%): least significant difference at 0.05% probability level.

Table 2. Interaction effect of variety x site on days to physiological maturity.

Variety	Sites		
	DARC	**ESE**	**OSE**
Beka	142.0	141	140
Holker	142.67	142.33	142.50
P≤0.05	-	1.1	
CV (%)	-	0.66	

DARC: Debrebrhan Agricultural Research Centre; ESE: Ethiopia Seed Enterprise; OSE: Oromia Seed Enterprise; DPM: Days to Physiological maturity; LSD (0.05%): Least significant difference at 0.05% probability level, CV (%): coefficient of variation.

Likewise, Bekele (1990), Tesfahun (2000), and Hailemikaiel (2000) observed the existence of high variability in barley genotypes for agronomic traits studied such as days to physiological maturity. Similarly, it is in coherent with (Djekic et al., 2011) who reported that yield is largely dependent on the genetic potential, which could be defined as yield of variety which was grown in conditions on which it had been adapted, with adequate amount of water, nutrients and efficient control of pests, diseases, weeds and other stresses.

Knowledge on interrelationship among agronomic parameters, their relationship with grain yield and their effect on grain yield plays significant role in the effort to improve malt barley meet quality requirement for seed and processing industries. Our study result indicated that, there exists positive and highly significant (P≤0.01) association between days to heading and day to physiological maturity. Similarly, days to physiological maturity and number of seeds per spike were also depicted positive and strong association at (P≤0.01) probability level (Table 3).

Conclusion

The existence of significant variation in days to physiological maturity and number of seeds per spike due to independent effect of seed sources and varieties in malt barley implies there is high performance variability in the studied quantitative traits. Analogously, considerable variation observed in number of seeds per spike and grain yield due to main effect of variety might indicate those traits are a function of genotypes. The positive and strong association found between days to physiological maturity and number of seeds per spike with days to physiological maturity might confirm that days to maturity

Table 3. Correlation coefficient between agronomic parameters.

Parameter	DH	DPH	GY	PH	PTN
DPM	0.53**	1			
GY	-0.09^{ns}	-0.08^{ns}	1		
PH	-0.04^{ns}	-0.16^{ns}	0.0107^{ns}	1	
PTN	0.13^{ns}	0.20^{ns}	-0.14^{ns}	-0.17^{ns}	1
SPS	0.59**	0.54**	-0.11^{ns}	0.14^{ns}	0.23^{ns}

DH: Days to heading; DPM: Days to physiological maturity; GY: grain yield (kg/ha); PH: plant height (cm); PTN: productive tiller number; SPS: seeds per spike.

depend on duration of heading. The number of seeds per spike also depends on the duration of maturity. In general, the main and communal effect of varieties and seed sources as well as association of agronomic parameters are major cause of variability in malt barley yield attributes and yield. Therefore, consideration of the existing phonological variability and association is substantially useful in designing efficient selection strategies in malt barley improvement. Hence, evaluating the effect of genotypes, environment, varieties and their interaction effect plays an immense role in the effort to improve yield and yield related traits in malt barley. With this, our research result ascertain that further studies should be conducted across sites and years to assess varieties performances which helps as cornerstone to fulfill the growing demand of quality malt barley seed and grain for processing industries in Ethiopia.

Conflict of Interests

The authors have not declared any conflict of interests.

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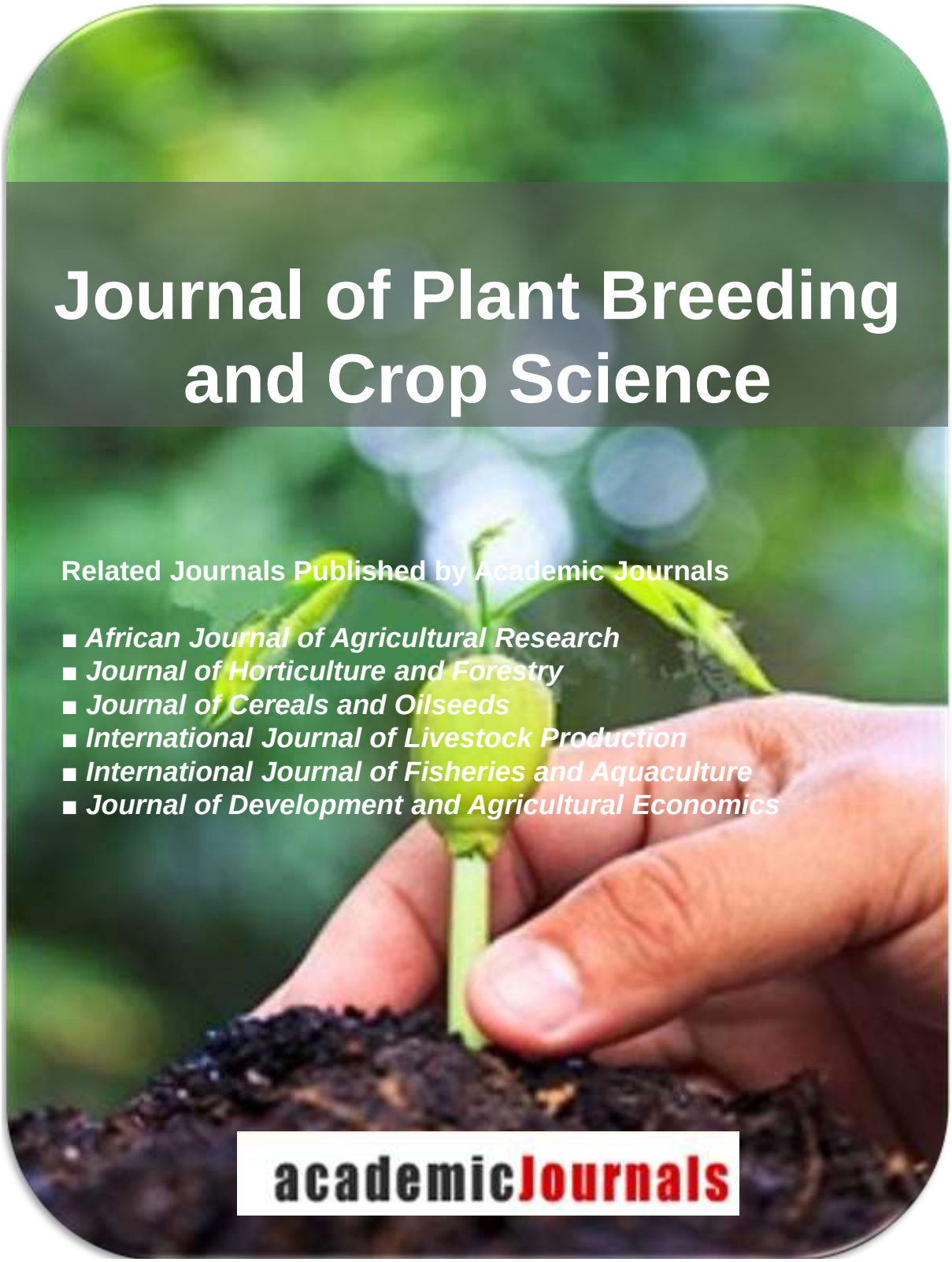

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