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Full Length Research Paper

Evaluation of Growth, Yield and Quality of Potato (*Solanum tuberosum* L.) Varieties at Bule, Southern Ethiopia

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This experiment was aimed to evaluate the performance of potato varieties in respect to growth, yield and quality attributes. A field experiment was carried out in Bule, Southern Ethiopia during the summer of 2017 under rain fed condition. The experiment was a single factor and arranged with randomized complete block design with 3 replicates. Treatments included different potato varieties (Bule, Gudenie, Jalenie, Belete, Degemegni and Local variety "Key Dench"). The results showed statistically significant variations in almost all of the parameters. The highest total yield (36.533 t ha^{-1}) and marketable tuber yield (33.985 t ha^{-1}) were obtained from Belete variety, unmarketable tuber yield from Degemegni (5.0370 t ha^{-1}) and Key Dench (8.5036 t ha^{-1}), average tuber weight from Gudenie (58.67g) and Belete (63.38g), large-sized tuber yield from Bule (54.716%), Gudenie (53.81%) and Belete (59.446 %), small-sized tuber from Key Dench (59.576%), days to flowering and maturity from Gudenie (65.7 and 113.3 days, respectively) and Belete (66.3 and 115.67 days, respectively), plant height on Bule (100 cm), stem number in Bule (4.917) and Jalenie (4.583), total and marketable tuber number from Bule (841473 and 477032 ha^{-1}, respectively), unmarketable tuber number on Key Dench (571846 ha^{-1}), tuber dry matter from Bule (22.331%), Gudenie (22.495%), Jalenie (22.653%), Belete (24.088%) and Key Dench (21.961%) varieties. Yield of tuber per hectare was significantly and positively correlated with plant height, days to physiological maturity, large-sized tuber, marketable tuber number and yield. In conclusion, results of the experiment revealed that Belete variety resulted as best total (36.533 t ha^{-1}) and marketable (33.985 t ha^{-1}) tuber yields in Bule, Southern Ethiopia during 2017 rainy season.

Key words: Bule, potato, rainfed, yield, variety.

INTRODUCTION

Potato is one of the main tuber crops grown in Ethiopia. World annual production of potato is about 381, 682, 144 tonnes with area coverage of 19, 098,328 ha. In Africa, total production of potato is about 25, 354, 279 tonnes with total area coverage of 1, 735, 533 ha. In Ethiopia total production is around 921, 832 tonnes with area coverage of 67, 362 ha. Ethiopia has suitable climatic and endemic conditions for potato production. However, the national average yield is about 13.68 t ha^{-1}, which is low compared to the Africa's and world's average production of 14.61 and 20.09 t ha^{-1}, respectively (FAOSTAT, 2014). The major production problems that account for such low yield are unavailability and high cost of seed tubers, lack of well adapted cultivars, poor agronomic practices, diseases, insect pests, inadequate storage, transportation and marketing facilities (Tekalign, 2005). The potential of the potato crop has not been adequately exploited as is clearly illustrated by the low national yield.

None of the variety or cultivar, had many potential which suits in all environments and for all uses (Bradshaw et al., 2007). Different researchers also reported that different potato varieties had different potential on yield and yield components across locations (Berhanu and Tewodros, 2016; Habtamu et al., 2016).

One of the major problems resulting in lower potato productivity in the study area is still many farmers who grow not well adapted (low yielder) varieties according to Bule Worda's Agricultural Office evaluation. Evaluation of selected varieties, are therefore, one of the considerations to ease the existing problems of obtaining the desired varieties for which the output of this study was likely to assist and sensitize potato growers, and which has a great contribution for increment of national average yield. Therefore, to address this problem the study was initiated with the objective of evaluating the performance of potato varieties on growth, yield and quality components at Bule, Southern Ethiopia.

MATERIALS AND METHODS

Study site

The experiment was conducted in 2017 rainy season of the year in which potato is mostly produced at Bule, Southern Ethiopia; which is located 28 km from Dilla town (6 °24'30'' North latitude and 38 °18'30'' East longitude with altitude of 1820 to 3060 m a.s.l). The mean annual temperature of the site is ranges between 12.5-22.5°C and mean annual average rainfall 849.8 mm.

Experimental set up

The treatments consisted of six potato varieties namely Bule, Gudenie, Jalenie, Belete, Degemegni and Key Dench (local cultivar).

The design was a single factor experiment arranged in a randomized complete block, replicated three times. The plot size was 3.75 x 3.6 m. Medium-sized and well-sprouted potato tubers were planted at the spacing of 75 x 30 cm distances. Spacing between plots and replications were separated by 1 and 1.5 m, respectively. Potato tuber was planted in May 5, 2017. Agronomic practices were applied during growing period of the crop (110 Kg nitrogen ha^{-1} and 92 Kg phosphorous ha^{-1} fertilizer and 3 hilling until canopy closure for weed control). Harvesting was done at physiological maturity when the leaves of the potato plants senesced.

Description of cultivars

The selected varieties of potato named 'Bule, Gudenie, Jalenie, Belete and Degemegni collected from Hawasa Agricultural Research Center and Key Dench from local farmers were used for the experiment (Table 1).

Data collection

Time to flowering was recorded when 50% of the population reached the flowering stage. Time to physiological maturity was recorded when 70% of plants leaves turned yellowish. Plant height was determined by measuring stem height from the base of the main shoot to the apex at full blooming. Number of stems per hill was recorded as the average stem count of five hills per plot during the flowering stage. Only stems arising from the mother tuber were considered as main stems. Tuber number per hill was recorded by counting the average number of tubers during harvesting from five sample plants. Average tuber weight per hill was determined on the basis of total tuber weight produced per plant divided by total tuber number counted per plant at harvest. It was taken from 5 plants during harvest. Weight and number of marketable tubers yield were recorded as the weight and the number of marketable tubers that were free from diseases, insect pests, and greater than or equal to 25 g in weight (Lung'aho et al., 2007). These were taken from plants in the net plot area at harvest. Weight and number of unmarketable tubers yield were determined as the weight and the number of unmarketable tubers (culls) of each plot which included rotten, insect attacked and undersized tubers (less than 25 g) (Lung'aho et al., 2007). These were taken from plants in the net plot area at harvest. Total tuber number and yield were recorded as the sum of number and yield of marketable and unmarketable tuber. Size categories of tuber were recorded by taking all tubers from five randomly-selected plants and categorizing them into small (< 39 g), medium (39-75 g), and large (>75 g) according to (Lung'aho et al., 2007). Dry matter content of tuber (%) was taken from five fresh tubers randomly selected in each plot and weighed. Tubers were sliced and dried in an oven at 70°C until constant weight. Dry weight was recorded and dry matter percent was calculated according to Williams and Woodbury (1968) as:

$$\text{Dry matter}\,(\%) = \frac{\text{Weight of sample after drying}}{\text{Initial weight of sample}}\, x100$$

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Table 1. Description of potato varieties.

S/N	Potao Varieties	Released Year	Breeder/Maintainer	Recommended Altitude (m.a.s.l)
1	Bule	2005	Hawassa Agricultural Research Center	1700-2700
2	Gudenie	2006	Holeta Agricultural Research Center	1600-2800
3	Jalenie	2002	Holeta Agricultural Research Center	1600-2800
4	Belete	2010	Holeta Agricultural Research Center	1600-2800
5	Degemegn	2002	Holeta Agricultural Research Center	1600-2700
6	Key Dench	-	Local cultivar	-

Source: (Ministry of Ethiopian Agriculture and Rural Development, 2009)

Table 2. Response of potato varieties in respect to days to 50% of flowering and 70% maturity, plant height, stem and tuber number per hill.

Varieties	Days to 50% flowering	Days to 70% maturity	Plant height (cm)	Stem number	Tuber number per hill
Bule	51.3^{b}	103.3^{b}	100.00^{a}	4.917^{a}	18.933^{a}
Gudenie	65.7^{a}	113.3^{a}	95.92^{ab}	4.333^{ab}	10.333^{c}
Jalenie	53.7^{b}	101.67^{b}	91.667^{bc}	4.583^{a}	12.333^{bc}
Belete	66.3^{a}	115.67^{a}	96.667^{ab}	4.000^{ab}	12.933^{abc}
Degemegni	52.3^{b}	93.3^{c}	84.667^{c}	2.500^{c}	9.800^{c}
Key Dench	46.7^{c}	91.67^{c}	73.00^{d}	3.083^{bc}	18.533^{ab}
Level of Significance	***	***	***	*	*
CV (%)	2.82	2.71	5.22	19.6	26

Means followed by the same letter within a column are not significantly different at 5 % level of significance

Data analysis

All crop data were subjected to analysis of variance, using SAS software version 9.1. Means that differed significantly were separated using the LSD procedure. Simple linear correlations between parameters were computed.

RESULTS

Time to flowering and maturity

Potato genotype differences significantly influenced both days required to attain 50% flowering and 70% of maturity (Table 2). The longest days required to attain 50% flowering and 70% maturity were recorded on Belete (66.3 and 115.6 days) and Gudenie (65.7 and 113.3 days) varieties, while the earliest in local varieties Key Dench (46.7 and91.7 days) and Degemegni (93.3 days to maturity), respectively.

Plant height, stem number and tuber number per hill

Potato genotype differences significantly influenced plant height, stem number and tuber number per hill (Table 2). The highest plant height were recorded for variety Bule (100 cm), Gudenie (95.917 cm) and Belete (96.667 cm) while the shortest in local variety Key Dench (73 cm). The highest numbers of main stem per plant were recorded on Bule (4.917), Gudenie (4.333), Jalenie (4.583) and Belete (4.000) varieties while the lowest on Degemegni (2.500) and local variety Key Dench (3.083) (Table 2). Highertubernumbers per hill were produced by Bule (18.933) and the lowest on Key Dench (9.8) and Gudenie (10.33) (Table 2).

Average tuber weight, total, marketable and unmarketable tuber number

Potato genotypes differed significantly in respect of influenced average tuber weight, total, marketable and unmarketable tuber number (Table 3). Belete (63.38 g) and Gudenie (58.67 g) produced higher average tuber weight while local variety Key Dench (25.41 g) lowest average tuber weight per hill. The higher total and marketable tuber numbers per hectare were obtained on

Table 3. The response of potato varieties in term of average tuber weight per hill, total, marketable and unmarketable tuber number per hectare.

Varieties	ATW (g hill^{-1})	TTN ha^{-1}	MTN ha^{-1}	UMTN ha^{-1}
Bule	37.76^{bc}	841473^{a}	477032^{a}	364441^{b}
Gudenie	58.67^{a}	459255^{c}	314071^{c}	145184^{c}
Jalenie	47.89^{ab}	548143^{bc}	328886^{bc}	219257^{bc}
Belete	63.38^{a}	574809^{abc}	444440^{ab}	130369^{c}
Degemegni	40.62^{bc}	435551^{c}	219257^{c}	216294^{bc}
Key Dench	25.41^{c}	823695^{ab}	251849^{c}	571846^{a}
Level of significance	**	*	**	**
CV (%)	19.87	26	20.86	38.43

Means followed by the same letter within a column are not significantly different at 5 % level of significance. ATW= Average tuber weight; TTN= Total tuber number, MTN= Marketable tuber number, UMTN= Unmarketable tuber number.CV (%) – Coefficient of variation.

Table 4. The response of total, marketable and unmarketable tuber yield per hectare to potato varieties.

Varieties	TTY (t ha^{-1})	MTY (t ha^{-1})	UMTY (t ha^{-1})
Bule	30.696^{ab}	26.992^{ab}	3.7037^{bc}
Gudenie	25.363^{bc}	23.496^{b}	1.8666^{c}
Jalenie	25.481^{bc}	21.778^{b}	3.7037^{bc}
Belete	36.533^{a}	33.985^{a}	2.5481^{c}
Degemegni	17.422^{c}	12.385^{c}	5.0370^{a}
Key Dench	20.355^{c}	11.852^{c}	8.5036^{a}
Level of significance	**	**	***
CV (%)	17.66	20.47	26.19

Means followed by the same letter within a column are not significantly different at 5 % level of significance. TTY= Total tuber yield, MTY= Marketable tuber yield and UMTY= Unmarketable tuber yield. CV (%), Coefficient of variation.

variety Bule (841473 and 477032, respectively) while the lower on variety Gudenie (459255) and Degemegni (435551) for total tuber number and Degemegni (219257) and local variety Key Dench (251849) for marketable tuber number. In another way, the more number of unmarketable tuber numbers was recorded on local variety Key Dench (571846) while the lower on variety Gudenie (145184) and Belete (130369) (Table 3).

Total, marketable and unmarketable tuber yield

Potato genotype differences significantly influenced total, marketable and unmarketable tuber yield (Table 4). The higher total and marketable fresh tuber yield were attained on variety Belete (36.533 t ha^{-1} and 33.985t ha^{-1}) and Bule (30.696 t ha^{-1} and 26.992 t ha^{-1}) and the lower on variety Degemegni (17.422 t ha^{-1} and 12.385 t ha^{-1}) and Key Dench (20.355 t ha^{-1} and 11.852 t ha^{-1}), respectively. The total and marketable tuber yield produced by variety Bule was in statistically parity with Gudenie and Jalenie. On the other hand the higher unmarketable tuber yields were obtained on potato variety Degemegni (5.0370 t ha^{-1}) and Key Dench (8.5036 t ha^{-1}), while the lower on Gudenie (1.8666 t ha^{-1}) and Belete (2.5481 t ha^{-1}) varieties.

In the present study positive and significant correlation was observed between total tuber yield and large-sized tuber percentage (r= 0.52*), positive and highly significant with plant height (r= 0.6**) and days to maturity (r=

Table 5. Simple correlation coefficient among different parameters.

Parameter	DTF	DTM	PH	SN	ATW	TNP	TTN	MTN
DTF	1.00	0.88***	0.56*	0.23NS	0.85***	-0.44NS	-0.44NS	0.29NS
DTM	0.88***	1.00	0.73***	0.57*	0.75***	-0.25NS	-0.25NS	0.50*
PH	0.56*	0.73***	1.00	0.62**	0.53*	-0.11NS	-0.11NS	0.68**
SN	0.22NS	0.57*	0.62**	1.00	0.24NS	0.12NS	0.12NS	0.57*
ATW	0.85***	0.75***	0.53*	0.24NS	1.00	-0.66**	-0.66**	0.16NS
TNP	-0.44NS	-0.25NS	-0.11NS	0.12NS	-0.65**	1.00	1.00***	0.54*
TTN	-0.44NS	-0.25NS	-0.11NS	0.12NS	-0.65**	1.00***	1.00	0.54*
MTN	0.29NS	0.50*	0.68**	0.57*	0.16NS	0.54*	0.54*	1.00
UMTN	-0.70**	-0.62**	-0.55*	-0.21NS	-0.88***	0.85***	0.85***	0.01NS
TTY	0.52*	0.61**	0.60**	0.44NS	0.44NS	0.31NS	0.31NS	0.90***
MTY	0.64**	0.74***	0.73**	0.51*	0.59**	0.14NS	0.14NS	0.87***
UMTY	-0.76***	-0.82***	-0.83***	-0.48*	-0.80***	0.50*	0.50*	-0.37NS
LSTP	0.70**	0.79***	0.74***	0.53*	0.77***	-0.37NS	-0.37NS	0.43NS
MSTP	-0.27NS	-0.26NS	0.10NS	0.02NS	-0.34NS	0.25NS	0.25NS	0.19NS
SSTP	-0.65**	-0.75***	-0.84***	-0.58*	-0.70**	0.30NS	0.30NS	-0.54*
TDMP	0.38NS	0.60**	0.30NS	0.52*	0.22NS	0.16NS	0.16NS	0.45NS

***, ** and *= Correlation is significant at 0.001, 0.01 and 0.05, respectively. NS= non significant; DTF= Days to 50 % of flowering; DTM= Days to 50% of flowering; SN= Stem number; PH= Plant height; ATW= Average tuber weight; TNP= Tuber number per plant; TTN= Total tuber number; MTN= Marketable tuber number; UMTN= Unmarketable tuber number; TTY= Total tuber yield; MTY= Marketable tuber yield; UMTY= Unmarketable tuber yield; LSTP= Large-sized tuber percentage; MSTP= Medium-sized tuber percentage; SSTP= Small sized tuber percentage; TDMP= Tuber dry matter percentage.

0.61**) and positive and very highly significant correlation with marketable tuber number and yield (r= 0.90*** and 0.97***, respectively) (Table 6).

Large, mediumand small-sized tuber yield (%) and tuber dry matter (%)

Potato genotype differed significantly in terms of large, small-sized tuber yield (%) and tuber dry matter (%), while non-significantly in respect of medium-sized tuber (Table 5). The higher, proportion of large-sized tuber yield (%) were recorded on varieties Bule (54.716%), Gudenie (53.818%) and Belete (59.44%) and for small-sized tuber yield (%) in local variety Key denchi (59.576%), while the lowest proportion in Key Dench (8.108 %) for large-sized and Bule (11.532%), Gudenie (15.628%) and Belete (11.519%) for small-sized yield (%). The lowest tuber dry matter (%) was recorded in Degemegni variety, while the higher in others (Table 5).

DISCUSSION

The variation in total and marketable tuber yield of potato varieties might be associated with genotypes difference among varieties. In agreement with the present findings, a significant difference in total and marketable tuber yield among potato varieties was reported by Berhanu and Tewodros (2016) and Habtamu et al. (2016). Also, Elfinesh (2008) stated yield differences among genotypes were attributed both by the inherent yield potential of genotypes and growing environment as well as the interaction of genotype x environment. Yield variation among varieties also indicated that increment of plant height, large-sized tuber yield, marketable tuber number and yield and prolonged time of maturity could be considered as a factor contributing to higher total tuber yield. In line with this study Girma and Niguisse (2015) who reported that total tuber yield was positively and highly significantly correlated with marketable tuber number and large-sized tuber yield. Similarly, Zami et al. (2010) also reported that plant height was positively and significantly correlate with tuber yield.

The variation observed in non-marketable yield of the genotypes in this study may be due to crop adaptability, crop maturity and inherent ability of potato genotypes in producing unmarketable tubers yield. In line with finding, Berhanu and Tewodros (2016), Habtamu et al. (2016) and Elfinesh (2008) reported that unmarketable tuber

Table 6. Simple correlation coefficient among different parameters (continued).

Parameter	UMTN	TTY	MTY	UMTY	LST	MST	SST
DTF	-0.70**	0.52*	0.64**	-0.76***	0.69**	-0.27NS	-0.65**
DTM	-0.61**	0.61**	0.73***	-0.82**	0.79***	-0.26NS	-0.75***
PH	-0.55*	0.6**	0.73***	-0.83***	0.74***	0.10NS	-0.84***
SN	-0.21NS	0.44NS	0.51*	-0.48*	0.53*	0.02NS	-0.58*
ATW	-0.88***	0.44NS	0.59**	-0.80***	0.77***	-0.34NS	-0.70**
TNp	0.85***	0.31NS	0.14NS	0.48*	-0.37NS	0.25NS	0.30NS
TTN	0.85***	0.31NS	0.14NS	0.48*	-0.37NS	0.25NS	0.30NS
MTN	0.01NS	0.90***	0.87***	-0.38NS	0.43NS	0.19NS	-0.54*
UMTN	1.00	-0.19NS	-0.38NS	0.80***	-0.71***	0.17NS	0.69**
TTY	-0.19NS	1.00	0.97***	-0.43NS	0.52*	0.07NS	-0.59*
MTY	-0.38NS	0.97***	1.00	-0.64**	0.67**	0.02NS	-0.73***
UMTY	0.80***	-0.43NS	-0.64**	1.00	-0.84***	0.15NS	0.84***
LST	-0.71***	0.52*	0.67**	-0.84***	1.00	0.02NS	-0.93***
MST	0.18NS	0.07NS	0.02NS	0.15NS	-0.38NS	1.00	0.01NS
SST	0.69**	-0.59*	-0.73***	0.84***	0.02***	-0.37NS	1.00
DMP	-0.09NS	0.52*	0.50*	-0.20NS	0.32NS	-0.05NS	-0.03NS

***; ** and *= Correlation is significant at 0.001; 0.01 and 0.05; respectively. NS= non significant; DTF= Days to 50 % of flowering; DTM= Days to 50% of flowering; SN= Stem number; PH= Plant height; ATW= Average tuber weight; TNP= Tuber number per plant; TTN= Total tuber number; MTN= Marketable tuber number; UMTN= Unmarketable tuber number; TTY= Total tuber yield; MTY= Marketable tuber yield; UMTY= Unmarketable tuber yield; LSTP= Large-sized tuber percentage; MSTP= Medium-sized tuber percentage; SSTP= Small sized tuber percentage; TDMP= Tuber dry matter percentage.

was influenced by varietal difference on potato crop.

The result of this study showed the variations of days required in attaining 50% flowering and 70% maturity among varieties. This result may also be attributed to genetic differences. This result is in agreement with that of Berhanu and Tewodros (2016), Habtamu et al. (2016), Girma and Niguisse (2015) and Tekalign (2005) who have reported that days required in attaining maturity period in potato depends on cultivars and environmental factors. Similarly, Vreugdenhil (2007) reported that days required to attain 50% flowering is highly dependent on gene factors and governed by many environmental factors, mainly temperature and light.

The significant differences in plant height were observed among varieties in this study. This result is in agreement with those of Berhanu and Tewodros (2016), Elfinesh (2008) and Girma and Niguisse (2015) who reported that plant height varied with potato varietal differences. This suggestion is also consistent with that of Sing and Singh (1973) who reported that plant height is a quantitative trait controlled by many genes, and is highly influenced by environmental factors like nutrient status of the soil, available moisture and intercepted radiation.

The observed difference in stem number among varieties in this study might be attributed to genetic differences, which in turn influence the number of sprouts or eyes on the tubers. This result is consistent with those of Berhanu and Tewodros (2016), Habtamu et al. (2016) and Morena et al. (1994) study who reported that the number of stems per plant is influenced by variety. The number of stems in a tuber varies considerably depending on many factors such as variety, storage condition of tuber, size of tuber, inherent variations in the number of buds per tubers or number of viable sprouts at planting, sprout damage at the time of planting, physiological age of the seed tuber and growth conditions (Allen, 1978).

The variation of average tuber weight might be associated to an inherit potential of the genotypes. In line with this study, Habtamu et al. (2016) who reported that average tuber weight varies with potato varieties and the highest recorded on Belete (105.24 g) according to their result. The variation in total, marketable and non-marketable tuber number of potato varieties might be associated with inherent ability of potato genotypes in producing these tubers. In line with this findings, Habtamu et al. (2016) and Khalafalla (2001) reported the difference in number of tubers could have been attributed to the difference in genetic makeup of varieties. Allen (1978) showed that the number of tubers set by plants was determined by stem density, variety, crop management and season.

Large and small-sized tuber yield percentage variations among potato varieties was observed in this findings which might be due to the inherent characteristics of the cultivars used. In line with this study, Berhanu and Tewodros (2016), Habtamu et al. (2016) and Girma and Niguisse (2015) reported that tuber size distribution varied with varieties. In contrast to the current findings, the above authors reported the variation of medium sized tubers on varieties. Also, in confirmation with the findings of Patel et al .(2008) and Kumar et al. (2007) who reported that maximum yield of small size tubers may be due to higher number of tubers as well as varietal character, adaptability or establishment effects of the other growth attributes. Similarly, this study result is in agreement with those of Beukema and Vanderzaag (1990) who observed that the variation larger sized tuber number variation among cultivars could be genetic.

The observed differenece on tuber dry matter production might be attributed to varieties inherent differences. In line with findings, Berhanu and Tewodros (2016), Girma and Niguisse (2015) and Tekalign and Hammes (2005) also reported that cultivars differed significantly with respect to total dry matter production. All varieties except Degemegniproduced tuber dry matter percentage of greater than 20% which is acceptable range for processing. Kabira and Berga (2003) justified that potato tubers containing high dry matter of 20 to 24% produce fried products with high yields, less oil absorption and having better texture than those with lower solids.

In general, this finding indicates the variation of growth, yield and yield components of potato and can be manipulated with proper selection of potato variety in the study area.It could, thus be concluded thatBelete variety leads to optimum production of total (36.5 t ha^{-1}) and marketable (33.99 t ha^{-1}) tuber yields in Bule district Southern Ethiopia, under rain fed condition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of rice (*Oryza sativa* L.) advance uniform lines using multivariate analysis

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Principal component analysis is a multivariate statistical technique used to observe the variance and to assess the relative influence of various traits for aggregate variability. In this study, ten advance uniform rice lines were grown for two consecutive years and morphometric data on eight yield and yield related attributes were collected. The principle component analysis was performed on the means of two years' agronomic data to identify the patterns of variation and to determine the selection criteria. The first five principle components accounted for 97.9% of total variation, with the first three components explaining the cumulative variability of 82.7%. The individual contribution of PC1, PC2 and PC3 were 46.8, 20.7 and 15.2%, respectively. The PC1 and PC2 projected more towards yield contributing traits such as panicle length, number of grains per panicle and 100 grain weight. Days to 50% flowering and days to maturity were grouped under PC3 which regarded as earliness component, as it had the traits which allowed for developing early and late maturing varieties. Panicle length & 100 grain weight showed negative direction to each other, however, both traits grouped under PC2 and genotype under this component were good for further yield improvement. Thus, the prominent traits grouped together in different principal components were causal to describe the variability and breeding consideration. The results of this study will be greatly helpful for the development of early maturing and higher yielding varieties in future breeding programs.

Key words: *Oryza sativa*, Principal Component Analysis (PCA), rotated component matrix, Biplot, Loading plot.

INTRODUCTION

Rice (*Oryza sativa* L.) is an annual crop plant which belongs to the *Poaceae* (previously known as *Gramineae*) family. Rice is a major crop of world and have been grown in diverse ecological zones, with different response to disease, adaption, phenology and yield. It is the staple food of most of the developing countries and ranks second after wheat. Globally, it is cultivated on an area of ~154 million ha with an average yield of 400 million tons per year (Sohgaura et al., 2015). It contributes more than 20% of the daily calorie requirement. In Asia, more than 2 billion people fulfill their 60-70% calorie requirement from rice and its derivatives. Therefore, there is need to enhance the rice yield to meet the food demand of rapidly growing population. To overcome future food shortage, it is direly requisite to develop the high yielding varieties. The success of

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hybridization program depends upon the availability of genetic diversity in the germplasm of crop species (Tenorio et al., 2013). From beginning of crop improvement, evaluation of germplasm on basis of agronomic markers played an important role toward yield enhancement and to broaden the genetic variability for desired traits. For yield improvement in new breeding scheme, it is essential to study the yield and yield contributing traits in existing varieties and advance lines (Seyoum et al., 2012).

Multivariate analysis helps the plant breeders to formulate their selection approaches for improving the desired traits (Ravindra et al., 2012). Principal components analysis (PCA) is a multivariate statistical technique which is used to explore the variation among genotypes and remove redundancy in data sets when the variables are measured in different units (Maji and Shaibu, 2012). Therefore, it becomes appropriate to standardize the variables for relating the variation of a data set. It explores the resemblance among the variable and classify the genotypes on basis of inter-correlated dependent quantity variables (Idris and Khalid, 2013).

The core objective of the PCA is to drive the significant information from the variable data and describe it as new orthogonal variable sets to show the similarity behavior of observations and variables as points in graph. The Eigen value describes and measures the contribution of each component to the total variance while each coefficient of proper vector shows the percentage of participation of each original variable with that each principle component is related (Nachimuthu et al., 2014). The higher the coefficients value, either positive or negative, the more effective it will be in discriminating between entries. The variability among the genotypes of crop species should be uncover to develop new varieties with stability to tolerate severe abiotic and biotic factors (Bhakta and Das, 2008). Therefore, this study was conducted to exploit the genetic differences among the selected agronomic characters of rice advance lines for their possible use in future breeding programs.

MATERIAL AND METHODS

This study was conducted at the experimental fields of Rice Research Institute, Kala Shah Kaku, Pakistan. The experimental material consisted of ten advance uniform lines with Basmati background and showing resistance to Bacterial Leaf Blight (BLB). These advance lines were grown in a randomized complete block design (RCBD) with three replications for two consecutive years (during kharif seasons 2015-2016 and 2016-2017). Thirty days old seedlings were transplanted in four rows of 5m length keeping plant to plant and row to row spacing 21 cm. Data were recorded on 10 randomly selected plants from two middle rows for these traits: Days to 50% flowering, days to maturity, plant height (cm), tillers per plant, panicle length (cm), grains per panicle, 100 grain weight (g) and grain yield (t/ha). The principle component analysis was performed on the means of two years' agronomic data to identify the patterns of variation and determination of selection criteria. Those principle components (PCs) with Eigen values greater than one were selected as proposed by Jeffers (1967). Correlation matrix between observed traits were assessed and factor analysis was done using the MiniTab 15 software (Mohammadi et al., 2012).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) of all the traits under study was found to be highly significant. The days to 50% flowering ranged from 88 (PKBB 1501) to 114 (PKBB 1509) with a mean of 99 days. The days to maturity ranged from 117 (PKBB 1501 and PKBB 1510) to 145 (PKBB 1509) with a mean of 127 days. The lowest plant height (cm) was measured in PKBB 1501 (123 cm) and highest in PKBB 1509 (140 cm) with an average of 131 cm. The average tillers per plant were 15 with highest in PKBB 1508 (16) and lowest in PKBB 1502 (13). The maximum panicle length (29.4 cm) was measured in PKBB 1507 and minimum (22.04 cm) in PKBB 1504 with a mean of 26.8 cm. The number of grains per panicle (NGP) ranged from 109 (PKBB 1501) to 81 (PKBB 1504) with a mean of 96.8. The 100 grain weight (00 GW) ranged from 2.88 g (PKBB 1503) to 1.92 g (PKBB 1504) with a mean of 2.26 g. The maximum yield was measured in PKBB 1501 (4.97 t/ha) and minimum was measured in PKBB 1508 (2.45t/ha) with a mean of 3.62 t/ha (Table 1).

Principal Component Analysis (PCA) was done on eight morphological traits of ten advance uniform rice lines. The first five principle components accounted for 97.9% of total variation with the first three factors explaining the cumulative variability of 82.7%. The individual contribution of PC1, PC2 and PC3 were 46.8, 20.7 and 15.2%, respectively. Only the first three PCs had Eigen values greater than one, 3.74, 1.65 and 1.21 times the variance of original variables and selected for further studies (Table 2).

PC1 contributed 46.85% of the total variation and traits that positively contributed to variation included number of grains per panicle (0.467), yield (0.429), 100 grain weight (0.363), panicle length (0.265) and tillers per plant (0.129). All other characters contributed negatively to the first component. The second principal component (PC2) contributed 20.7% of the total variation. Traits that positively contributed in PC2 includes 100 grain weight (0.426), yield (0.246), plant height (0.185) and maturity days (0.022). The third principal component (PC3) accounted for 15.2% of the total variability and positively contributed traits, included days to 50% flowering (0.577), days to maturity (0.556), yield (0.358), number of grains per panicle (0.262), panicle length (0.188) and 100 grain weight (0.112) (Table 2).

The loading plot between first (PC1) and second principal component (PC2) was constructed using variable observed traits. In PC1, 00 grain weight, yield and number of grains per panicle had more loaded score while panicle length and number of tiller per plant contributed to low variation in PC1 as compare to the

Table 1. Average performance of uniform advance rice lines.

S/ N	Line Name	50% Flowering	Maturity days	Plant Height (cm)	Tillers/ plant	Panicle Length (cm)	Number of Grains/Panicle	100 Grain weight (g)	Yield (t/ha)
1	PKBB 1501	88	117	123.43	16	28.56	109	2.76	4.97
2	PKBB 1502	90	119	138.63	13	23.88	95	2.69	3.99
3	PKBB 1503	105	130	127.87	15	27.06	104	2.88	4.22
4	PKBB 1504	108	135	128.23	15	22.04	81	1.92	2.97
5	PKBB 1505	106	137	131.23	14	24.1	93	1.95	3.34
6	PKBB 1506	103	129	125.37	15	29.12	106	2.06	4.38
7	PKBB 1507	95	120	132.83	15	29.4	102	2.31	3.47
8	PKBB 1508	97	122	131.20	16	27.06	85	1.87	2.45
9	PKBB 1509	114	145	140.53	15	28.76	89	2.05	2.91
10	PKBB 1510	91	117	132.03	16	28.48	104	2.13	3.47
Average		**99.7**	**127.1**	**131.14**	**15**	**26.85**	**96.8**	**2.26**	**3.62**

Table 2. Eigen Values, Factor scores and contribution of the first eight principal component axes to variation in Rice advance lines.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Plant height (cm)	-0.259	0.185	-0.334	0.757	-0.068	-0.392	-0.093	-0.217
Tillers/ plant	0.129	-0.708	-0.043	-0.100	-0.530	-0.364	-0.201	-0.132
Panicle length (cm)	0.265	-0.469	0.188	0.568	0.135	0.335	0.450	0.136
50% days to flowering	-0.392	-0.044	0.577	0.062	-0.073	0.266	-0.116	-0.647
Maturity days	-0.398	0.022	0.556	0.109	-0.122	-0.314	-0.033	0.637
No. of grains/panicle	0.467	-0.011	0.262	0.200	0.378	-0.062	-0.725	0.033
100 grain weight	0.363	0.426	0.112	0.181	-0.728	0.310	-0.084	0.092
Yield (t/ha)	0.429	0.246	0.358	-0.076	0.060	-0.577	0.449	-0.290
Eigen value	3.74	1.65	1.21	0.93	0.28	0.11	0.04	0.01
Proportion	0.468	0.207	0.152	0.117	0.035	0.014	0.006	0.001
Cumulative	0.468	0.675	0.827	0.944	0.979	0.993	0.999	1.000

other traits. In PC2, plant height, maturity days and days to 50% flowering score loaded heavily to the second component (Figure 1).

The rotated component matrix revealed that PC3 contributed to days to 50% flowering, days to maturity, yield and number of grains per panicle (Table 3). This component (PC3) is regarded as earliness factor as it has the traits which allow for development of early and late maturing varieties. Early varieties can escape abiotic and biotic stresses, however, Vishu et al. (2014) was not in agreement with this result. The PC1 and PC2 were also more prominent by yield contributed characters such as panicle length, number of grains per panicle and 100 grain weight (Table 3). Similar findings were reported by Kumar et al. (2012). Panicle length and 100 grain weight are characteristics which show negative direction to each other but both characteristics comes under PC2 simultaneously. This will be very helpful when selection is being carried out for those genotypes which comes under PC2. PC1 and PC3 can be subjected as yield factor and different selection schemes to bring rapid improvement of dependent variables. However, Sanni et al., (2012) and Nachimuthu et al., (2014) reported some contradictory results than our findings that PC3 related to earliness and yield contributed traits.

In PC1 vs PC2 score plot, rice lines in first quarter had high differences for characters contributing positively to PC1 and PC2. PKBB 1502 showed more diversity in this quarter than PKBB 1503 and PKBB 1507 and they are contributing more towards yield causal traits. The lines of quarter 2 showed much more variability for contributing traits having negative for PC1 and positive for PC2. PKBB 1504 exhibited more variation in this quarter than PKBB 1505. They are underlying more towards earliness characters. Lines at third quarter revealed that contributing traits were negative for PC1 and PC2.

Similarly, the fourth quarter had variability causative; negatively to PC2 and positively to PC1. PKBB 1501 exhibited more variation in the fourth quarter than PKBB 1506 and PKBB 1510. The lines located at far point of a

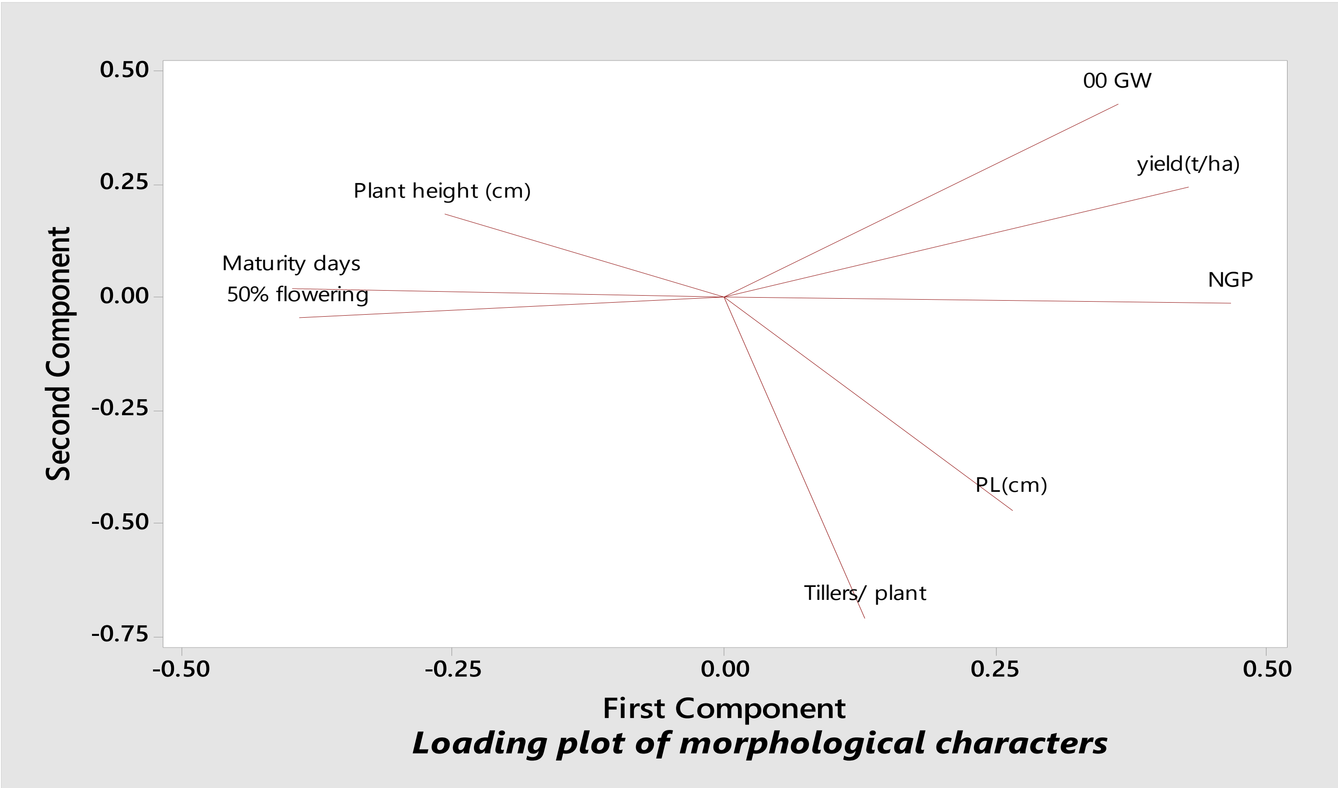

Figure 1. Loading plot of morphological characters between PC1 and PC2.

Table 3. Interpretation of rotated component matrix for the traits having value ≥ 0.1 in each PCs.

PC1	PC2	PC3	PC4	PC5
PL(cm)	100 GW	50% Flowering	PH	NGP
NGP	Yield	Days to Maturity	PL	
100 GW		NGP	NGP	
Yield		Yield		

quarter showed more variability compared to other lines present in the same quarter (Figure 2).

In order to determine the association between different measured characters of promising rice lines in differential display, it there need to subject them under biplot analysis. In this study, biplot of 10 advance rice lines was plotted along with their measured traits and biplot scattered all genotypes in their respective suitable response of traits (Figure 3). The biplot between PC1 and PC2 was ploted by using the variability among different earliness and yield contributing traits to identify relationship between these. In biplot, PKBB1502 and PKBB 1501 had longest vector towards yield contributing traits but was away from the late maturing vector. It showed that these lines were high yielding and earlier maturing.

The PK BB 1503 and PKBB 1507 were closely located to 100 GW and NGP vector, respectively. These vectors revealed that both lines had high value of yield contributing traits. But the PK1503 had longer vector as compared to PK1507 and showed more yielding potential than PKBB1507. On the other side, PKBB 1504, PKBB 1505, PKBB 1508 and PKBB 1509 had positive values toward days to maturity (late maturing) and negative towards the yield contributing vector which depicted that these lines are late maturing and lower yielding. The line PKBB 1510 had negative vector towards the late maturing vector and positive toward tillers/plant. Similarly, PKBB 1506 laid very closely to the panicle length vector. These results suggested that these rice genotypes are earlier maturing, have more tillering ability and are average yielding (Figure 3).

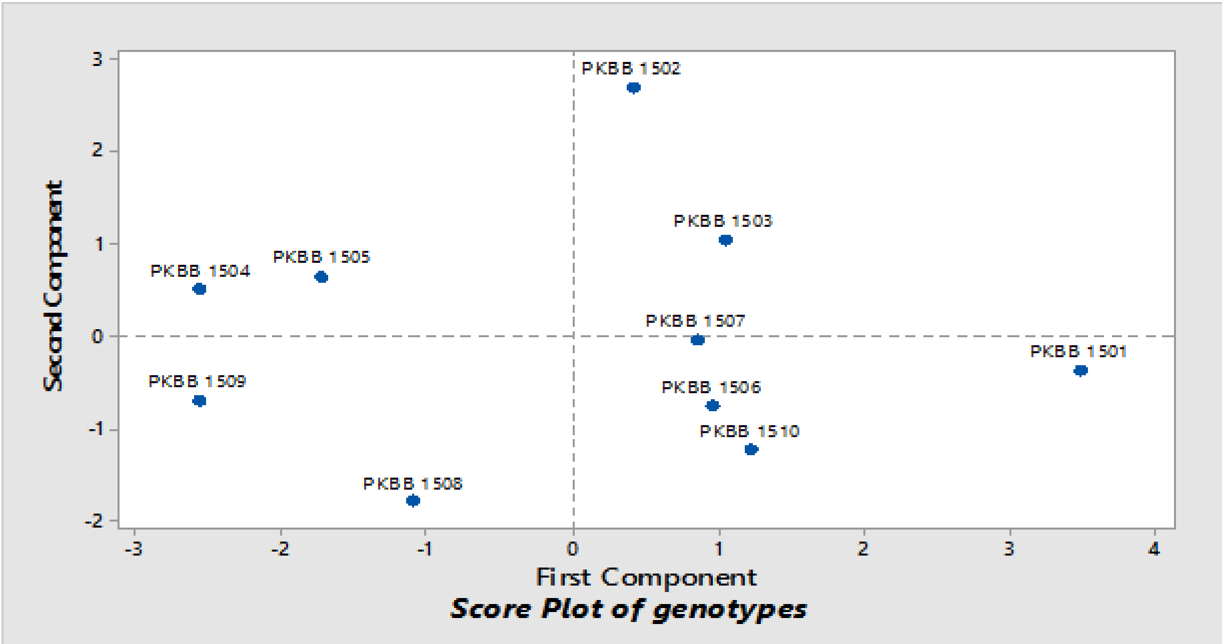

Figure 2. Distribution of rice advance lines across two principal components (PC1 vs PC2).

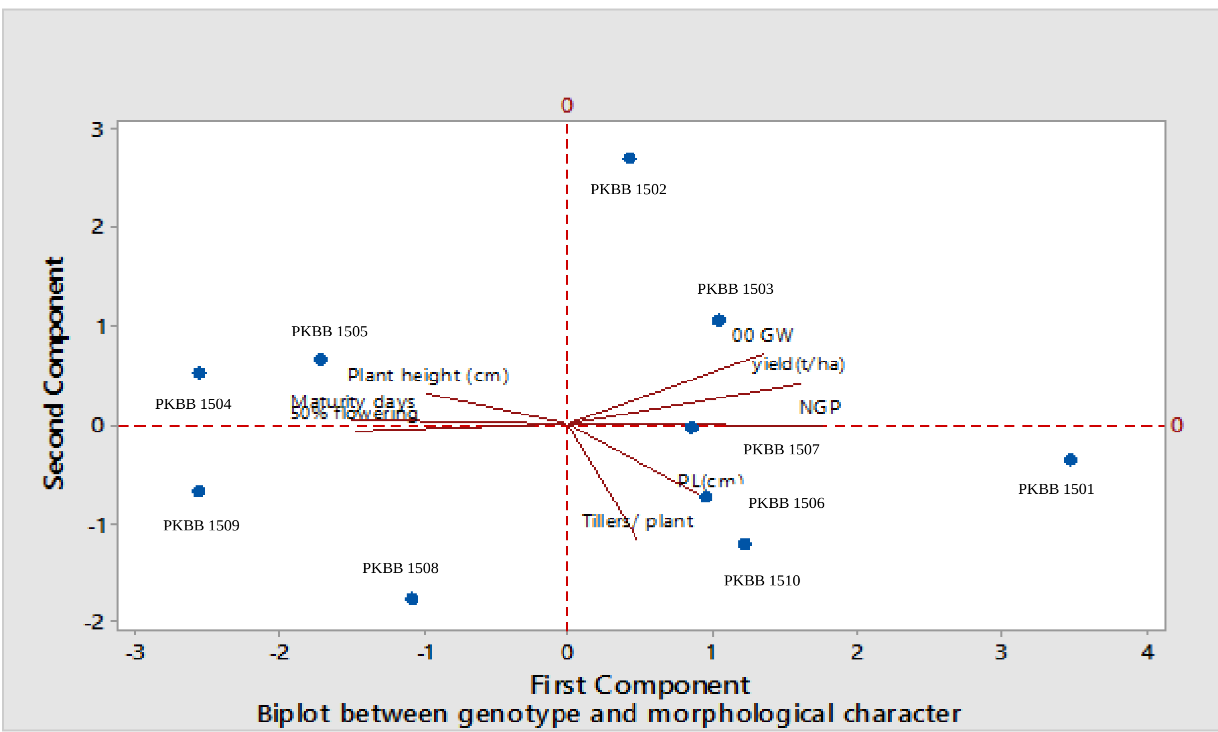

Figure 3. Graphical representation of rice advance lines and morphological characters between PC1 and PC2.

Conclusion

Our study will help plant breeders to evaluate advance uniform lines using multivariate analysis to characterize and evaluate the advance breeding materials accurately. The evaluated advance lines can also be used for the development of new lines for other important traits of interest such as tillering ability and disease resistance. The lines that are grouped under the same PC may share some underlying biological relationship, and their associations are often useful for generating hypothesis for better understanding of behaviour of complex traits that would allow breeders to maximize their knowledge. Thus, the prominent traits coming together in different principal components are causal towards clarifying the variability and have the trend to remain together. This may be kept into consideration during utilization of these characters in hybridization program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of brewery effluent on the anatomical and morphological structure of *Talinum triangulare* (Jacq) Willd

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The study was carried out to determine effect of brewery effluent on the anatomical and morphological characters of *Talinum triangulare*. Seeds from matured *T. triangulare* were germinated and transplanted into plastic containers. The seeds were irrigated with 10, 20, 30 and 40% effluent concentrations respectively. A control experiment irrigated with normal water was also set aside. Data were collected on weekly basis until after the thirteenth week when the experiment was terminated. The plant showed significant reduction in the leaf area, seed number, shoot height, this morphological response is also associated with a reduction in various anatomical structure that were studied. The significant reduction was more obvious on plants irrigated with the 30 and 40% effluent concentrations. Stem tissue such as epidermis, cortical cell distance and vessel diameter showed significant reduction. The reduction in the morphological and anatomical character might be an adaptive mechanisms employed by the plant in order to cope with the heavy concentration of the effluent. This study showed that brewery effluents have toxic effect on the *T. triangulare* and the effects were more pronounced on those irrigated with 30 and 40% effluent concentrations. The physical and morphological characters observed in plants are the repercussion of the various endogenous characters among which are the anatomical characters.

Key words: Brewery effluent, vegetables, irrigation, plant cells.

INTRODUCTION

Brewery effluent is waste generated by breweries industries in the process of production of a particular product. Some breweries effluents are known to contain high concentration of lead, mercury which has been the source of pollution into the plant and the society. The use of industrial effluents process for irrigation of food crops especially leafy and fruit vegetable has increase in the recent year in urban areas due to shortage of clean water

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(Arora et al*.,* 2008). The use has also been promoted by urban farmers due to the belief that such effluents contain high nutrients that can promote rapid vegetative growth of their crops which reduce or eliminate the cost of the fertilization either in organic or inorganic form. Fatoba et al. (2011) reported that vegetable are produced throughout the year due to the availability of industrial effluent to irrigate them. However, there should be caution in the use of effluent for irrigation of plants that are tender and herbaceous like vegetables. Uaboi-Egbenni et al*.* (2009) reported that in Nigeria, most of the urban farmers divert effluents to farm land to irrigate their vegetable farm to meet up the risen demand of vegetable in our society. Ramasubramanian (1993) observed the germination percentage and development of *Phaseolus mungo* growth rate in sand culture decrease with an increase in the concentration of some effluents obtained from some industries. The release of toxic materials contaminates the earth environment and interferes with human health. This also affects the quality of life or the natural functioning of the ecosystem including in both living organisms and their non-living environment. The release of such an effluent into the plant body is also a source of pollution into the plant (Uaboi-Egbenni et al*.*, 2009). Although, industrial processes are desirable, at the same time, the serious and irreversible damage done to the environment through their apparently innocuous discharges of effluents are unquantifiable. Until now, effluents are discharged into rivers, estuaries, lagoons (Ajmal, 1984). Industrial effluents have a great inhibitory effect on the germination and development of many plants species, the percentage of surviving plant decrease with an increase in treatment concentration (Rajni and Chauchan, 1996).

Talinum triangulare is commonly known as water leaf which belongs to the family Portulacaceae, it grows from a short taproot and can be up to 2.5 m in height. *T. triangulare* is an herbaceous annual and perennial plant with a broad, worldwide distribution. The demand for waterleaf is high in Nigeria especially in western part of this country, and it is therefore a major source of income for farmers. Its high demand is attributed to its nutritional value and importance as a softener when cooking the common fibrous leafy vegetables such as Afang (*Gnetum africana*), Atama (*Heinsia crinata*), and Editan (*Lasienthera bulchozianum*). It is also cooked with green Amaranthus (*Amaranthus curentus*) and fluted pumpkin (*Telfairia occidentalis*).

Due to the high demand for this vegetable in Nigeria, Urban farming of *T. triangulare* through effluent irrigation has grown tremendously. Several researchers have reported the effect of such effluent irrigation on the nutrient and heavy metal content of vegetables (Arora et al., 2008; Fatoba et al., 2011). This research work was designed to assess the morphological and anatomical structure of *T. triangulare* in response to the irrigation of brewery effluent.

MATERIALS AND METHODS

The seeds from matured *T. triangulare* plants were collected from the forest behind the Department of Botany Obafemi Awolowo University, Ile-Ife (7° 28´ 0´´ N, 4º 34´ 0´´ E). Identification of this plant was done in the Herbarium (IFE) of the Department of Botany Obafemi Awolowo University; Ile-Ife and voucher specimens were deposited. The effluent used was collected from the International Breweries Ilesa, Osun State (Nigeria) (7° 37´ 0´´N, 4° 44´ 0´´E). The soil was collected behind the Department of Botany, Obafemi Awolowo University, Ile-Ife. This experiment was conducted in the screen house to protect the plants from direct rainfall contaminations, trampling and to avoid being destroyed by rodents. Seeds *of T. triangulare* were planted in two big bowls in front of Botany Department, Obafemi Awolowo University, Ile-Ife. After germination, three *T. triangulare* plants were transplanted into each of the plastic container provided which had been filled with soil and arranged in a complete randomized design to avoid competition. The effluent was serially diluted to give representative concentration of 10, 20, 30 and 40%. Three replicates of each of the treatment were used and the fifteen plastic containers were labeled based on their means of irrigation such as control, 10, 20, 30 and 40% effluent concentration. A central experiment was also set aside in the plastic container which was irrigated with normal water continue from the seventh week after planting until thirteenth week when the experiment was terminated. Quantitative characters recorded on weekly basis are: plants shoot height, leaf length, leaf breadth, leaf area, number of branches and number of fruits**.** The mean values of these quantitative characters were recorded. The leaf area was calculated according to the formula of Hoyt and Bradfied (1962) shown as:

$$LA = LL \times LB \times 0.75$$

Where, LA is the leaf area; LL is the leaf length, LB is the leaf breadth, and 0.75 is the correction factor for the shape of the leaf.

For the purpose of studying the epidermal structures, sizeable portion of mature leaves for each of the concentration were cut from standard median portion. Epidermal peels were made by free hand method, the abaxial and adaxial epidermis were preserve in 50% ethyl alcohol until when require, the epidermal surface were then stained in 1% safranin 'O' for about 5 min and mounted on clean slide in 25% glycerol for microscopic examination (Dutta, 2003). Ten slides from each of the upper and the lower epidermis were prepared per concentration and studied. Out of the slides prepared, ten were selected per concentration for size measurements. Also, frequencies counting per field of view were also made from the slides. Finally, different counts were made from different portion of each slide.

Stomata index was calculated according to Dilcher (1974):

$$SI = \left(\frac{S}{E+S}\right) \times 100$$

Where S.I is the stomata size, S is the stomata per unit area and E is the number of epidermal plus subsidiary cells in the same unit area. The transverse section of the stem were made by using microtome and stained with aniline blue for 5 min and mounted in glycerine on clean glass slide with the edge cover slip for microscope observation. Epidermis, cortical distance, vessel distribution and the layer of both collenchyma and parenchyma cell were studied and their sizes measured with ocular micrometer. Photomicrographs of the section were taken. Transverse sections of the root were cut using a rotring sledge microtome. Permanent slides were prepared by staining sections in aniline blue for about 5 min and later counter stain with 1% aqueous solution of safranin

Table 1. Morphological characters of *T. triangulare* studied.

Characters	Control	10%	20%	30%	40%
Number of the leaf	15.83±9.70[a]	16.17±8.54[a]	13.67±65.04[a]	12.00±2.65[a]	8.33±4.13[a]
Length of the leaf (µm)	6.24±2.30[b]	5.42±4.20[d]	6.40±2.65[d]	4.23±2.45[d]	4.73±4.15[b]
Width of the leaf (µm)	2.67±0.66[b]	2.49±0.15[d]	2.26±1.46[d]	1.78±0.44[e]	2.14±0.13[c]
Leaf area (µm²)	17.16±32.73[a]	10.18±2.97[b]	10.11±7.49[b]	5.89±2.11[b]	7.64±1.05[a]
Number of branches	8.00±17.62[b]	7.33±14.69[d]	5.00±8.91[d]	2.83±5.38[d]	1.33±2.17[c]
Shoot height (µm)	7.20±3.13[b]	6.52±2.15[d]	7.39±2.23[b]	4.94±0.60[b]	5.81±3.20[b]
Seed number	16.50±31.09[a]	10.83±14.26[b]	6.83±10.15[b]	4.50±3.45[b]	2.67±2.54[a]

Means with the same letter(s) in each row are not significantly different at 5% level of significance.

"O" for 4 to 5 min. This was then mounted in 25% glycerine on clean glass slides with the edges of the cover slip for microscopic studies. Arrangements of cortical cell, epidermis, vascular bundles distribution were observed. Photomicrographs of slides were taken with Amscope MT microscope camera version 3.001 attached to a light microscope. Illustrations of the foliar epidermal features and transverse sections of both stem and root were done by Camera Lucida under × 25 objective power of Leitz DIALUX research microscope. Mean of all the parameter were subjected to analysis of variance to calculate the least significant difference (LSD) with the use of statistical package for Social science (SPSS) software IBM SPSS Version 21 and the level of testing significance was set at 0.05%. Duncan's multiple range tests were used to analyze the data obtained.

RESULTS

There is no significant difference in the number of leaf of *T. triangulare* when the various concentrations of the effluent were compared with the control (Table 1). There is a significant difference between the control and the various effluent concentrations in the length and width of leaf of *T. triangulare*. The number of branches, shoot height and seed number similarly decreased significantly with an increase the effluent concentrations (Table 1).

The number of stomata on the abaxial surface of the leaf of *T. triangulare* decreases significantly at 10% effluent concentration (Figures 1 and 2), there was also a significant increase at 20% effluent concentration, then a decrease at 30% effluent concentration and a sporadic increase at 40% effluent concentration (Table 2). On the adaxial surface, there was a significant increase at 20% effluent concentration in the stomata number and then a significant decrease in the stomata number from 30 to 40% effluent concentrations (Figures 1 and 2). The size of guard cell significantly increased at 40% effluent concentration. The length of epidermal cell at both abaxial and adaxial surfaces significantly decreased at 40% effluent concentration (Table 2).

The vessel diameter reduced significantly at 10% effluent concentration, however at both 20 and 30% effluent concentrations, there was no significant difference except at 40% effluent concentration where there was a reduction in the vessel diameter (Table 3 and Figure 3). There was a significant increase in the number of vascular bundle at both 30 and 40% effluent concentrations (Table 3). The epidermal thickness of the stem decreased significantly as the effluent concentration increases. The thickness of both parenchyma and collenchyma cells similarly decreased significantly as the effluent concentration increases (Table 3). In the root of *T. triangulare* studied, the epidermal layer, cortical layer and vessel diameter decreased significantly as the effluent concentration increases (Table 4 and Figure 4).

DISCUSSION

The brewery effluent reduces the leaf area of the plant significantly. Stevovic et al. (2010) reported that leaves from plant growing in polluted area were significantly reduced than leaves from an unpolluted area. Similarly, Rafia et al. (2009) reported that the reduction found in the leaf area of *Phaseolus mungo and Lens culinaris* may be considered as an adaptive advantage that enables leaves to develop and function in habitats marked by strong variations of heavy metals such as lead toxicity with solar radiation, air temperature and humidity. The increase in the leaf area reported under 40% effluent concentrations could be as result of the adaptive measures employed by some characters in the plant in order to withstand the effluent concentrations. Morphological features and physiological responses are linked to adaptive characteristics of plants in stressed environments (Dzomeku, 2012).

The continuous and consistent reduction in the seed number of *T. triangulare* is an indication that the effluent are in no way favourable to their growth. This might subsequently affects the availability of this vegetable in the market for consumers. Also, significant reduction has been reported to occur in all morphological parameters such as leaves length as the concentration of the effluent increases (Iqbal, 1985; Jahan and Zafar, 1992). Various morphological attributes of plant have been reported to be important features used by plant to withstand unfavorable condition.

Many diverse changes in anatomical characters of

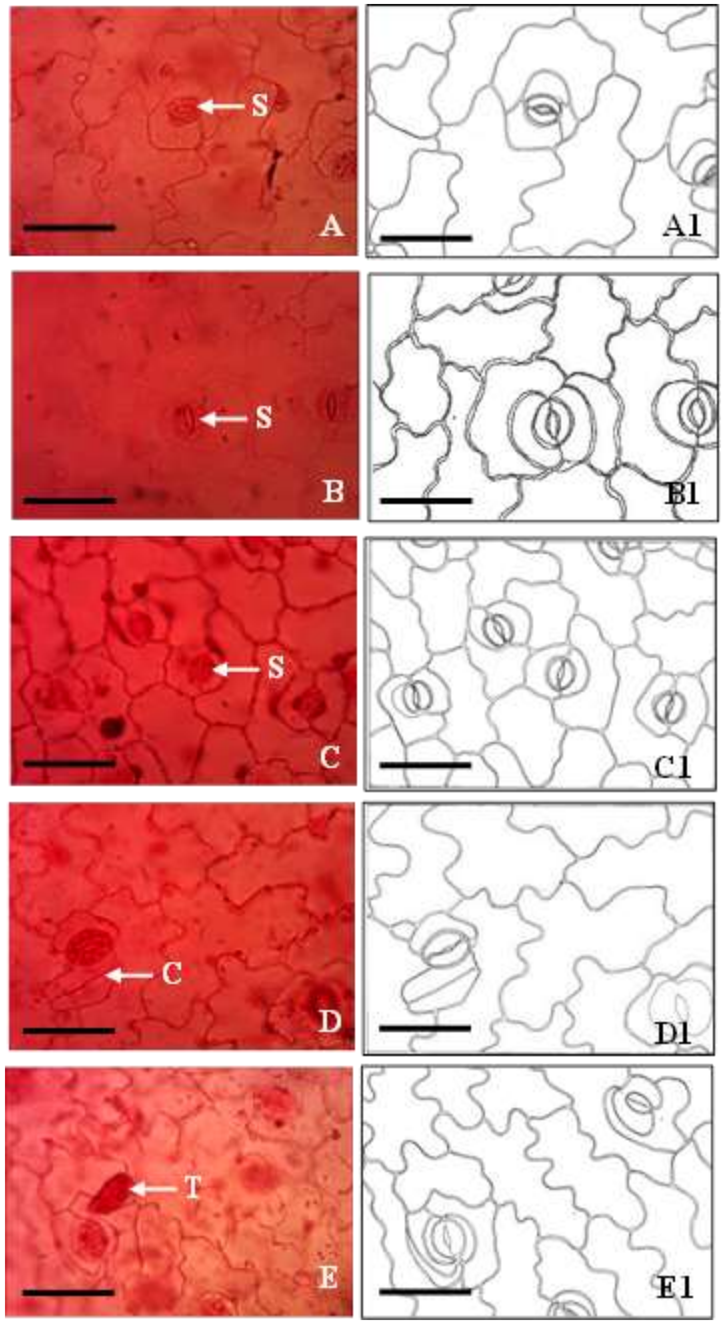

Figure 1. Leaf Epidermal Surface on Abaxial Surface of *Talinum triangulare* studied. A and A1= Control; B and B1 = 10%; C and C1 = 20%; D and D1= 30%; E and E1= 40%. S = Stomata; C = Crystal; T = Tannin. Scale = 44.74 µm.

different plant have been previously reported by a number of authors (Khudsar and Igbal, 2001; Papadakis et al., 2004; Akinlabi et al., 2014; Ekpemerechi et al., 2014). There was a remarkable reduction in the number of stomata from the control to 10% effluent concentration. A significant reduction in stomata was reported in *Cenchrus ciliaris and Cynodon dactylon* in response to Cadmium stress. It has been reported that transpiration may decrease because of lower stomata index and size per unit of leaf area (Molas, 1997). The decrease in stomata size may be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis (Verma et al., 2006). These modifications are important response to the

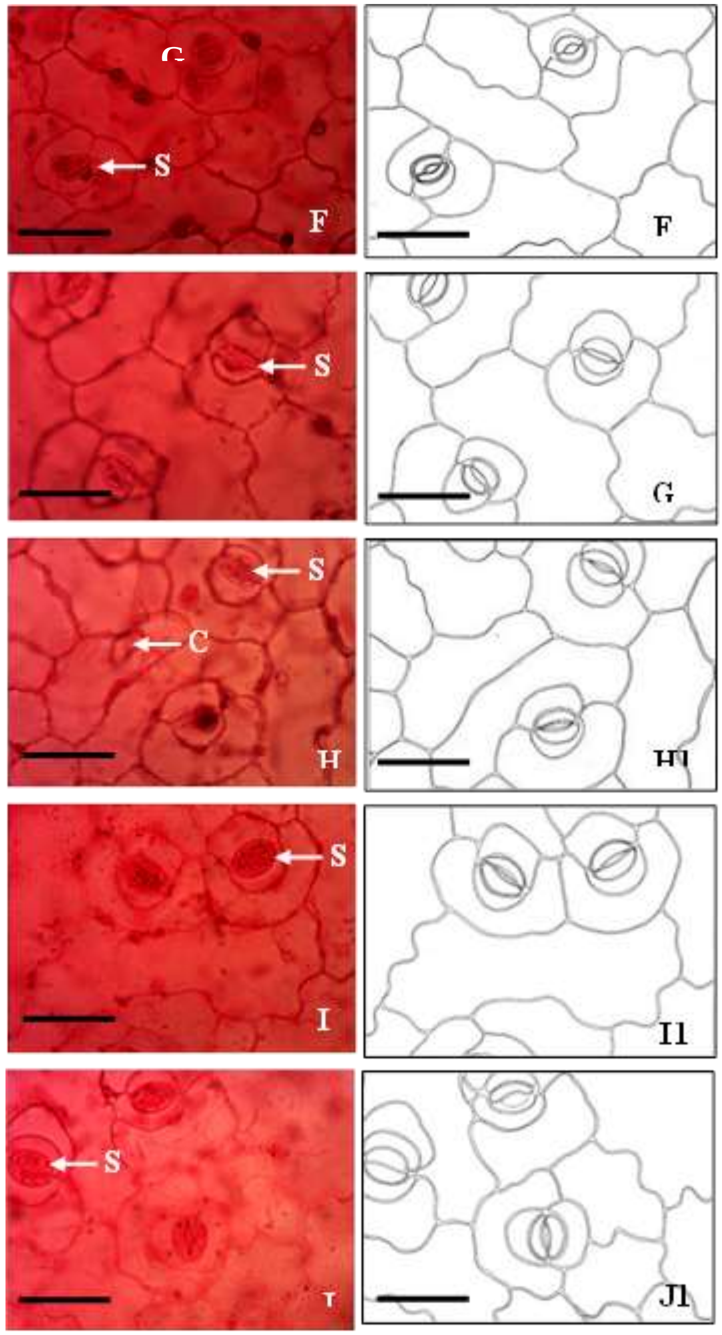

Figure 2. Leaf Epidermal Surface on Adaxial Surface of *T. triangulare* studied. F and F1 = Control; G and G1 = 10%; H and H1 = 20%; I and I1 = 30%; J and J1 = 40%. S = Stomata; C = Crystal. Scale = 44.74 µm.

environmental stress which affected plant used in controlling the absorption of pollutants (Gostin, 2009). Decrease in stomata size on both the adaxial and the abaxial surfaces in all the treatment aside the control is an indication of *T. triangulare* survival strategy in the presence of pollutants from breweries effluent. Reduced stomata help in increasing the rate of photosynthesis without excessive transpiration. This type stomata size modification is an indication of the presence of heavy metal toxicity which could prove that brewery effluents may have contained toxic heavy metals. Due to the important of the adaxial surface in photosynthesis and gas exchange, the impact of the toxicity of the effluent was most felt on the leaf surface.

Table 2. Leaf anatomical characters on the abaxial and adaxial surface of *T. triangulare* studied.

Character	Percentage concentration of the effluent				
	Control	10%	20%	30%	40%
Stomata number on abaxial surface	13.05±2.10[b]	5.75±0.98[c]	12.10±1.74[a]	8.20±1.03[b]	14.75±2.91[a]
Stomata number on adaxial surface	7.10±0.85[c]	7.15±0.82[b]	7.65±0.46[b]	6.60±0.88[c]	4.65±1.02[e]
Length of guard cell on abaxial surface (µm)	0.88±0.12[i]	1.15±0.07[i]	0.97±0.09[k]	1.14±0.08[i]	0.940.08[j]
Length of guard cell on adaxial surface (µm)	0.96±0.18[i]	1.12±0.09[i]	1.07±0.11[j]	1.21±0.06[i]	1.24±0.12[h]
Width of guard cell on abaxial surface (µm)	0.29±0.05[i]	0.28±0.07[k]	0.31±0.05[l]	0.37±0.04[k]	0.33±0.06[j]
Width of guard cell on adaxial surface (µm)	0.24±0.05[i]	0.28±0.07[k]	0.27±0.05[l]	0.370.04[k]	0.36±0.06[j]
Length of Epidermal cell on abaxial surface (µm)	4.25±1.03[e]	4.30±0.43[d]	4.05±0.54[e]	3.91±0.37[e]	2.38±0.53[g]
Length of Epidermal cell on adaxial surface (µm)	4.08±0.63[e]	3.85±0.50[e]	3.36±0.61[f]	2.96±0.44[f]	2.68±0.38[f]

Means with the same letter(s) in each row are not significantly different at 5% level of significance.

Table 3. Stem anatomical characters of *T. triangulare* studied.

Character	Percentage concentration of the effluent				
	Control	10%	20%	30%	40%
Vessel diameter (µm)	0.85±0.40[i]	0.72±0.72[k]	0.74±0.19[k]	0.55±0.16[k]	0.45±0.26[j]
Number of vascular bundles	2.50±1.08[f]	3.05±1.08[f]	2.80±5.00[g]	3.55±1.58[e]	3.57±1.32[e]
Collenchyma thickness (µm)	6.06±3.21[d]	6.21±0.82[c]	3.83±2.14[e]	3.61±0.67[e]	3.20±3.10[f]
Parenchyma thickness (µm)	14.30±5.30[a]	11.87±2.73[a]	12.30±3.31[a]	9.24±5.74[a]	9.03±2.09[b]
Epidermal thickness (µm)	1.52±0.09[g]	1.53±0.17[i]	1.07±0.13[j]	1.10±0.18[i]	1.05±0.12[h]

Means with the same letter(s) in each row are not significantly different at 5% level of significance.

The length of the epidermal cell decreases as the concentration of the effluent increases, a significant increase was recorded at 40% effluent concentration. Epidermal cell number increase significantly as the effluent concentration increases. This could be an adaptive measure to neutralize the adverse effect of the effluent and the growth of *T. triangulare.* The result presented here is supported by earlier work of Gostin (2009) that showed a significant increase in epidermis in the leaves of plant growing in the polluted environment as compared to the leaves collected from non-polluted area. Also, Gomes et al. (2011) reported increased epidermal thickness in *Brachiaria decumbens* due to Cadmium stress.

There is a significant decrease in the vessel diameter as the effluent concentration increases, this constantly reduces the flow of assimilates to the plant parts. This is one of the reasons for the stunted growth in *T. triangulare* as the effluent concentration increases. The result of this work is supported by Kasim (2005) who reported that xylem vessel of *Vicia faba* showed significant reduction in their diameter in response to higher effluent concentration relative to their corresponding control. This result is also in conformity with the observation of Ghouse and Yunus (1972) and Khudsar et al. (2000), who reported that soil pollution, can cause a decrease in vessel abundance in several herbs and shrub. The increase in the concentration of effluents affects the internal organs of *T. triangulare* negatively. Among these internal organs are the vascular bundles which collapsed and ruptured as a result of increase in effluent concentration. Flow of assimilates, mineral nutrients and water will subsequently be reduced. Reduction in number of conducting elements has been reported in literature as being an adaptive measure to secure water flow. Proliferation of parenchyma cells in the stem of *T. triangulare* was promoted at very low concentrations of the brewery effluent. The effluents toxic effect resulted in drastic reduction of the number of parenchyma and collenchyma cells at 40% effluent concentration. This showed that at low concentration, *T. triangulare* was able to maintain the productions of parenchyma which constitute adaptive mechanism to regulate ion concentrations entering the stem. But, at high concentrations which seem to be toxic, the number of parenchyma and collenchyma cells was reduced in order to prevent excess ions from the effluents entering the xylem and reduce toxicity from effluent conduction to the aerial parts. The increase in concentration of effluent causes increase in the activity of phellogen to produce numerous intercellular spaces leading to the formation of lenticels.

Root as first organ of plant in different stages of development utilizes different mechanisms to cope with

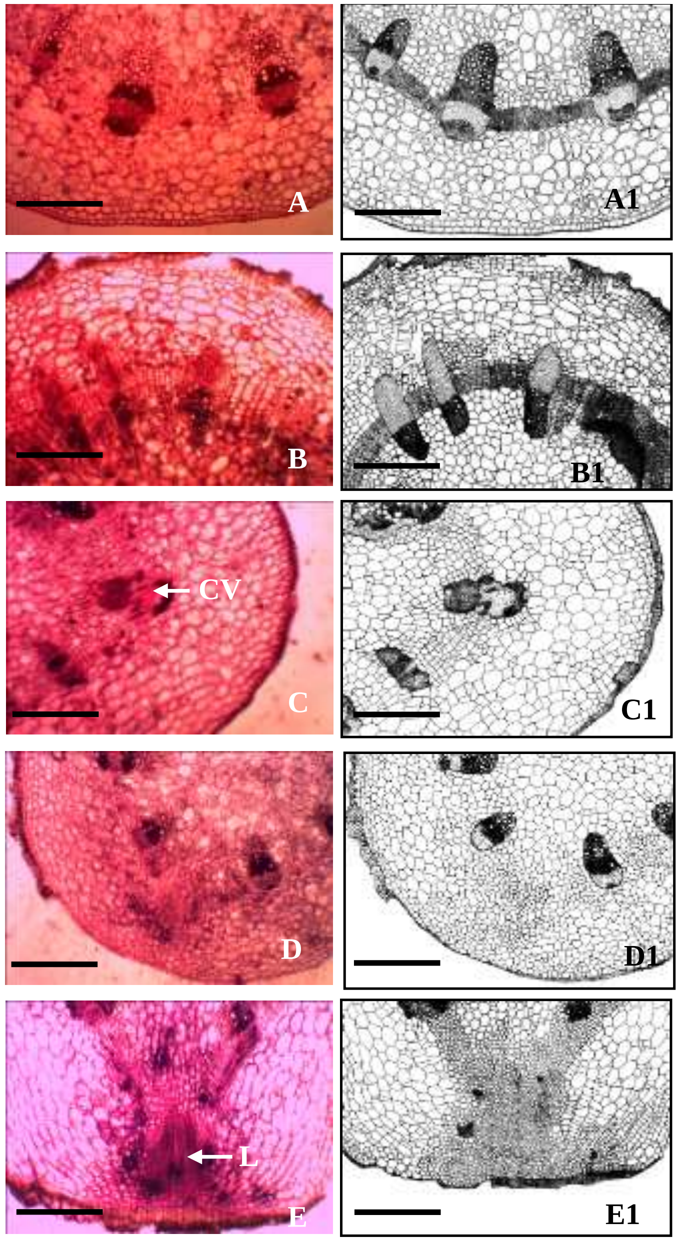

Figure 3. Transverse section of stem of *T. triangulare* studied: A and A1 = Control; B and B1 = 10%; C and C1 = 20%; D and D1 = 30%; E and E1 = 40%. L = lenticels; CV = collapsed vascular bundle. Scale = 44.74 µm.

Table 4. Root anatomical characters of *T. triangulare* studied.

Character	Percentage concentration of the effluent				
	Control	10%	20%	30%	40%
Epidermal layer (µm)	3.13±0.39^{g}	3.16±0.16^{i}	0.79±0.06^{k}	0.57±0.07^{k}	0.45±0.05^{j}
Cortical layer (µm)	7.04±1.05^{c}	5.64±0.42^{c}	5.95±0.53^{c}	5.31±0.31^{d}	4.96±0.77^{c}
Vessel diameter (µm)	0.66±0.06^{i}	0.53±0.06^{k}	0.54±0.05^{k}	0.45±0.07^{k}	0.35±0.10^{j}
Number of druses	6.95±2.20^{c}	7.45±1.41^{b}	4.95±1.44^{d}	6.35±0.97^{c}	5.10±1.16^{c}

Means with the same letter(s) in each row are not significantly different at 5% level of significance.

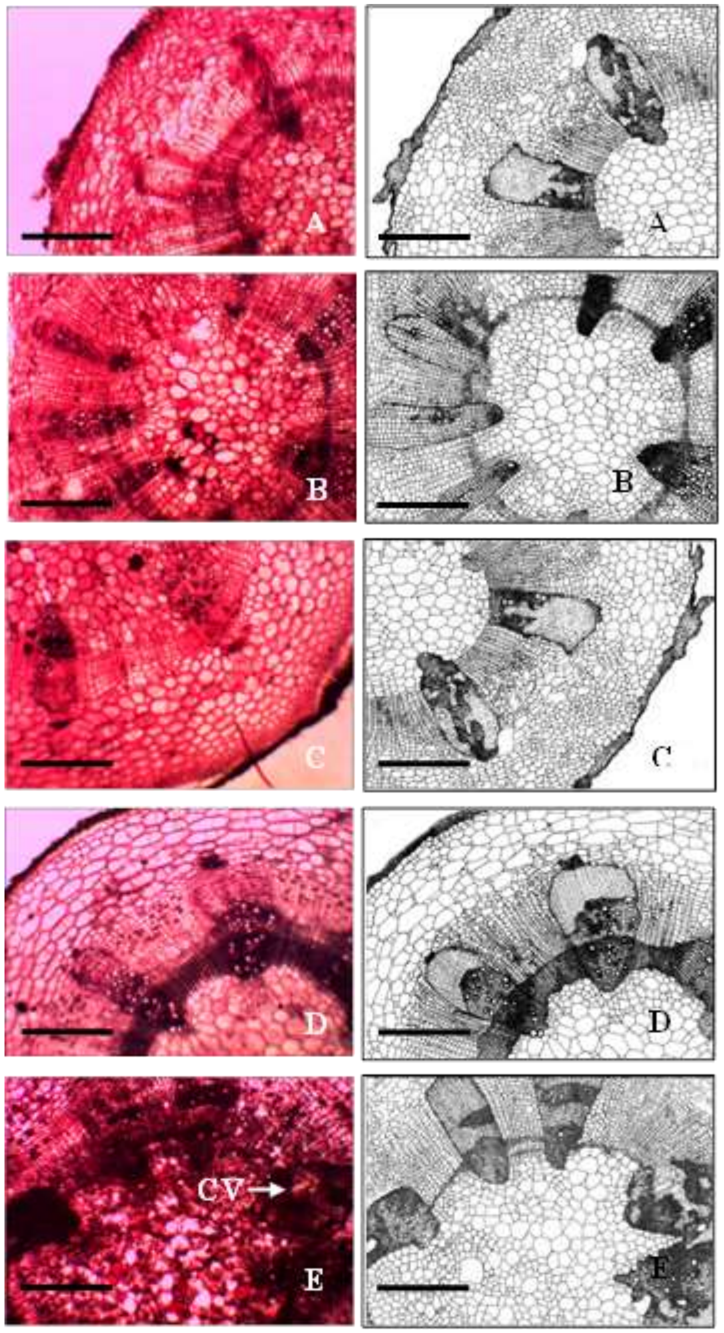

Figure 4. Transverse section of rootof *T. triangulare* studied: A and A1 = control; B and B1 = 10%; C and C1 = 20%; D and D1 = 30%; E and E1 = 40%. CV = collapsed vascular bundle; Scale = 44.74 µm.

the presence of any pollutant in the soil. However, in this study, cortical layer suffer significant reduction in the highest percentage effluent concentration when compared to the control. Literatures have reported a remarkable decrease in cell size which might be the result of a decrease in elasticity of cell walls of the root (Sieghardt, 1984; Barcelo et al., 1986). This was supported by the work of Kasim (2005) who find out significant reduction in the cortical cell of root of *Sorghum bicolor* induced by some heavy metal which are also likely to be present in the effluent used for this study.

Conclusion

The physical and morphological characters observed in plants are the repercussion of the various endogenous characters among which are the anatomical characters. The response of anatomical characters to the various effluent concentrations in *T. triangulare* depicts the observed exomorphological features. The vegetable should not be grown in the area where breweries are sited and also, in an environment where there is shortage of water. About 10% concentration of this effluent can be used to irrigate this vegetable. However, it must be noted that brewery effluents have a great adverse effect on both the anatomical and morphological structure of the *T. triangulare* studied. The effect of effluent is more pronounced on *T. triangulare* irrigated with 30 and 40% effluent concentrations.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Genotypic response to weeding regimes of upland rice on woodland savannah sub-ecological area of Lake Albert crescent zone of Uganda

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Weeds represent one of the major biological constraints to upland rice production in low input agricultural systems. The effects of weeding regimes and rice cultivars on weed growth and rice yield were investigated over three seasons. Four weeding regimes [0 (no weeding control), 1, 2, and 3] and three popular rice varieties (NARIC 2, a local cultivar, and NERICA 4), were tested in 4x3 factorial experiment in a Randomised Complete block with three replicates. The most important weed species recorded were; *Biden pilosa, Commelina benghalensis* L., *Euphorbia hirta* L., *Micrococca mercurialis* Benth., *Galisonga parviflora* Cav, *Sida rhombifolia* L., *Triumfeta* spp*, Guizotia scabra, Celocia trigyna, Cyprus rotundus, Panicum Maximum Jacq,* and *Imperata cylindrica* L. Across cultivars, the best weeding regimes for weed control and rice yields were single weeding and weeding twice. Differences among interaction effects between variety and weeding regime were not significant for most traits, except ripening ratio and grain yield in experiment one and experiment two. Across weeding regimes, NERICA 4 out yielded the other varieties in all the three experiments. However, a single well timed hand hoe weeding, together with the use of a cultivar with good adaptation to unfavourable rice growing conditions, such as NERICA 4, would increase land and labour productivity of upland rice-based systems in Uganda.

Key words: Genotype, rice, upland, weeding regimes, yield.

INTRODUCTION

Rice currently ranks as the second most important cereal in Uganda after maize (Anyanga, 2015). Over the last 10 years, production of this crop has more than doubled owing to the expansion of the rice production areas to upland ecology. In addition, the introduction and promotion of upland rice varieties dubbed NERICA (2005 to 2015) has resulted into large scale production of the crop. Indeed, upland rice covers close to 70% of the total area under rice cultivation in the country (Mohamed, 2012). In spite of these developments, the country is still considered a net rice importer, and will perhaps continue unless domestic production increases remarkably to counter the demand by the growing urban population (World Bank, 1993; Hyuha, 2006; Mohamed, 2012). NERICA's potential yield in sub-Saharan Africa is 5 t/ha; with fertiliser application. However, farmer field conditions in Uganda give a yield of 1.5 t/ha according to several reports (Imanywoha, 2001; Otsuka and Kalirajan 2006). This clearly undermines the status of rice as an important food security and income crop in Uganda. Several reports have highlighted the major causes of this yield gap (Odogola et al., 2006; Hyuha et al., 2007; Kaizzi et al., 2014). Among these, failure to manage weeds and low soil fertility are central to the problem.

Uninhibited weed growth among crops is estimated to cause yield losses in the range of 48 to 100% (Akobundu, 1980; Becker and Johnson, 2001; Johnson et al., 2004; Toure et al., 2013). Specifically, weeds compete severely with rice plants for space, nutrients, air, water and light adversely affecting plant height, leaf architecture, tillering habit, shading ability, growth pattern and crop duration (Miah et al., 1990). In addition, weeds depress the normal yield of grains per panicle and grain weight (Bari et al., 1995). Therefore, a higher rice yield should be a motivation for maintaining a weed free rice crop environment.

Rice is categorised as a weak weed competitive crop. However, several studies have reported the existence of genetic variation in weed competitiveness among rice cultivars (Fofana and Rauber, 200; Haefele et al., 2004; Rodenburg et al., 2009; Saito et al., 2010). Weed competiveness comprises weed tolerance, the ability to maintain high yields despite weed competition, and weed suppressive ability, the ability to suppress weed growth and reduce weed seed production (Zhao et al., 2006a). The number of weed competitive cultivars with high adaptation to African agro-ecosystems reported to date is limited (Wopereis et al., 2008; Rodenburg and Johnson, 2009).

To reduce the effects of weed pressure on crop yields, most upland rice farmers rely largely on pre- and postharvest fires, preparation, and hand or hoe weeding of fields. Labour-intensive hand weeding is often preferred by farmers because the use of herbicides use is costly, and low literacy rates among farmers in Uganda, like the rest of SSA, further limits herbicide use (Rodenburg and Johnson, 2009). As such, labour availability for weed control becomes limited as weeds multiply with cropping season, resulting into high levels of yield reduction (Saito and Futakuchi, 2014). However, the most commonly accepted approach to manage weeds is to follow an integrated weed management strategy comprising the combined use of two or more available and effective technologies (Sanyal et al. 2008). Often rice farmers in the uplands of Uganda weed two to three times (by hand or hoe) during the rice growing season, depending on the weed pressure (Alou et al., 2012; Anyanga, 2015). Such weeding interventions should, however, be well timed to optimize weed suppression, grain yield (Dzomeku et al. 2007; Ekeleme et al. 2009), and the time available for the farmer to attend to other non-farm activities (Alou et al., 2012). Therefore, the identification of superior weed competitive rice genotypes would be an attractive, cost effective and safe approach for sustaining rice productivity, particularly for resource-constrained farmers. Weed competitive genotypes would be part of an integrated weed management strategy. The ideal weed competitive genotypes are high yielding under both weedy and weed-free conditions. Therefore, the purpose of this study was to (i) investigate the effect of weeding regimes across popular upland rice varieties and (ii) determine the most weed competitive upland rice variety in the Lake Albert crescent zone of Uganda.

MATERIALS AND METHODS

Description of the study site characteristics

Three upland rice experiments were conducted over 3 years (2012 to 2014) at Bulindi Zonal Agricultural Research and Development Institute (BUZARDI) located in the Lake Albertine Crescent Zone of

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Uganda. The three experiments were conducted in different fields within the research station. This station lies on average longitudes 312954 N and latitudes 12950 E. This institute receives a bimodal rainfall (March to June and August to December) and average rainfall was 1000 mm per year. This experimental site is located in Hoima district which is one of the leading Upland rice producing districts in Uganda (Lodin, 2005). The vegetation in the area is dominantly savannah with short and tall grasses and shrubs with widespread bush burning practiced (Lodin, 2005). Soils in the area are classified as acidic ferralsols (FAO System, 2004) with low exchangeable bases and organic matter. They are dominantly clay (heavy textured), acidic and low particularly in available phosphorus and total nitrogen.

Soil analysis data based on Alou et al. (2012) found that the soils in this experimental station are typically clay loam with sand content of less than 45%. The mean soil pH is below the critical value of 5.2 and below the range of 5.5 to 6.5 considered favorable for plant nutrition. In addition, the mean values of available phosphorus, total nitrogen and exchangeable bases were found to be low.

Experimental design, crop management and data collection

For the study of genotypic response to weeding regimes, a 4 x 3 factorial experiment was established based on randomized complete block design with three replications, and weeding regime and rice variety as factors at four and three levels, respectively. The four levels of weeding regimes were; 1 hoe weeding at 21 days after sowing (DAG), 2 hand hoe weeding at 21 DAG and 42 DAG, and three hand hoe weeding at 21, 42 and 63 DAG, and a no weeding control, while the three levels of rice variety as popular upland rice varieties were; NERICA 4, NARIC2 and Local variety). The plots were of dimensions 5.0 m long × 2.1 m wide. No fertilizers and/or pesticides was applied in this trial. A seeding rate of 50 kg/ha was used to estimate the seed requirement for the trial plots. Seed were dibbled manually in hills 12.5 cm between hills and 30 cm between rows. Sowing was done by the second week of August in each year while harvesting was done by end of November of each year.

Data collection

Data were collected on number of panicles, number of spikelets, spikelet sterility, 1000-grain weight and yield at harvest.

Data analysis

The data collected were subjected to analysis of variance to determine whether the mean squares due to weeding regimes were significant using Genstat version 14 (Payne et al., 2011). An analysis of variance was performed for each trait in each season using ANOVA in Genstat (Payne et al., 2011). Each season (experiment) was analysed separately because experiment × weeding regime × variety interaction was significant. The ANOVA of each experiment was followed by pairwise comparisons using the least significant differences (LSD), when the F-test for the treatment effect was found significant ($P \leq 0.05$).

Broad-sense heritability (H) was used to evaluate the stability of performance for rice yield components *via* estimation of repeatability across replications. The higher the value of H, the greater the genetic stability. H was estimated for rice yield components for each weed treatment for each season, and calculated from variance components, as:

$$H = \frac{\sigma^2 v}{(\sigma^2 v + \sigma e^2 / r)}$$

Where, $\sigma^2 v$ and σe^2 are variety and within – experiment error variances, respectively, and r is the number of replicates. Variance components were estimated using Genstat version 14 (Payne et al., 2011). The estimate of H was biased upward by confounding of variety and variety by experiment variances, but beneficial in approximately comparing the precision for different weed treatments (Zhao et al., 2006b).

RESULTS

Dominant weed species associated with upland rice fields in the Lake Albert crescent zone of Uganda

Several weed species were encountered in this study. These were composed of both broad leaf and grass species. However, their relative abundances depended on the weeding regime applied and previous land use (Nagasawa et al., unpublished). Table 1 shows the major weed species encountered in the fields.

The effect of weeding regimes on selected agronomic traits in rice varieties in the Lake Albert crescent zone of Uganda

In Experiment 1 (second season of 2012 or 2012B), significant differences among weeding regimes were observed for number of panicles per m^{-2}, number of spikelets per panicle and ripening percentage ($P<0.05$ to $P<0.001$) (Table 2). Varietal differences were not significantly ($P>0.05$) different, except number of spikelets per panicle. The differences among interaction effects of weeding regime × variety were not significant ($P > 0.05$) for most traits (Table 2). The number of panicle per $metre^{-2}$ (ranged from 149 to 310), number of spikelets per panicle (ranged from 33 to 67), and ripening ratio (ranged from 12.5 to 44.5%) had 254, 52 and 23% as

Table 1. Major weed species identified from upland rice fields in the Lake Albert crescent zone of Uganda.

Scientific names	Common/English name	Special attribute
Broad leaf weeds		
Biden pilosa	Black jack	Very abundant in crop fields, and highly competitive producing a lot of seed.
Commelina benghalensis L.	Day flower, Bengal spiderwort, Wandering jew	An alternate host of plant pathogens and nematodes
Euphorbia hirta L.	Garden spurge, milk weed, asthma-plant	An early colonizer of bare lands, Common weed in upland rice fields and has a wide native range
Micrococca mercurialis Benth	-	Has high native range, used as a vegetable in some parts of Uganda
Galisonga parviflora Cav.	Gallant soldier	Prolific weed with a short life cycle
Sida rhombifolia L.	Queensland hemp	Has high native range
Triumfeta spp		Notorious weed with high native range and reproductive potential
Guizotia scabra	-	Notorious weed in farmlands where it is unknown. Can withstand flooding.
Celocia trigyna	Wool flower	In some parts of Africa, its eaten as vegetable, it also has anthelmintic properties in humans, elsewhere, it's a weed. Competitive with crops for water and soil nutrients
Grass species		
Cyprus rotundus	Purple nutsedge	Highly competitive with crops for both soil nutrients and moisture.
Panicum Maximum Jacq.	Guinea grass	Domesticated as a forage, otherwise becomes weed in crop fields.
Imperata cylindrical (L.)	Spear grass	Highly dominant and competitive weed species

Sources of species description: (Terry and Michieka, 1987; Promotion of Rice Development Project, 2012).

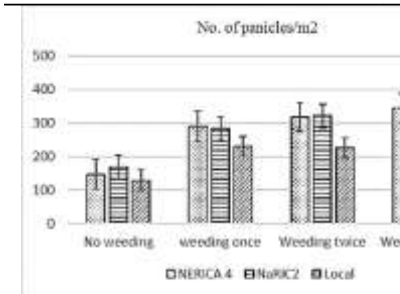
Table 2. Mean squares for the combined analysis of effect of weeding regime on the agronomic performance of farmer preferred upland rice germplasm for second season of 2012 in the Lake Albert crescent zone of Uganda.

Source of variation	DF	1000 grain wt	Plant height	No. of panicles per m^2	No of spikelet per panicle	Ripening percentage (%)	Yield
Weeding regime	3	0.13543**	0.002418ns	0.210276**	0.16704***	0.8779**	0.4165ns
Variety	2	0.04623ns	0.003000ns	0.042475*	0.02919ns	0.0486ns	0.1638ns
Weeding regime × Variety	6	0.07183ns	0.001191ns	0.003310ns	0.01214ns	0.0405ns	0.1013ns
Residual	22	0.08445	0.001223	0.009256	0.02036	0.1427	0.2195
Total	35						

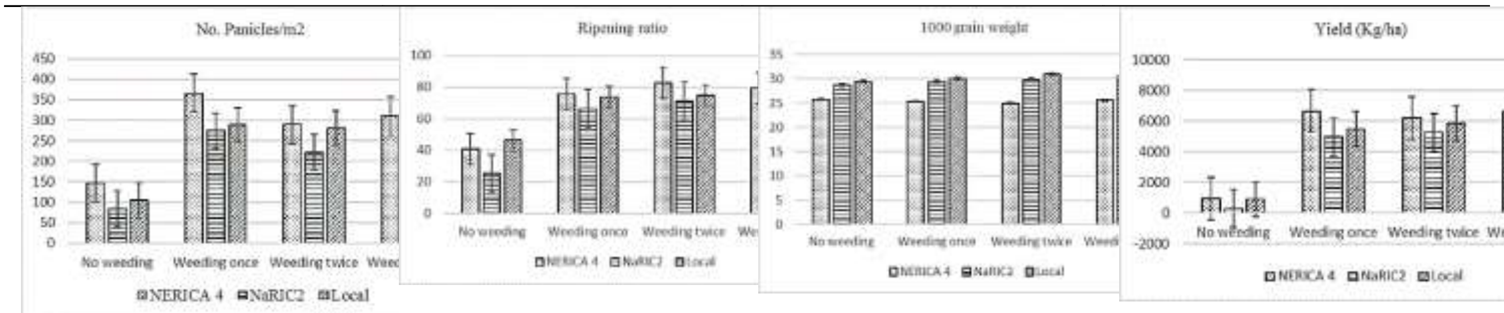
Ns = not significant; * = significant at P<0.05; ** = significant at P<0.01; *** = Significant at P<0.001; DF = degrees of freedom.

average trait scores for each weeding regime respectively (Table 5). Across varieties, number of panicles per metre^{-2} (ranged from 34 to 83) with an average trait scores of 61 for variety NERICA 4; ranged from 34 to 62 an average traits score of 62 for NaRIC 2; ranged from 30 to 66 had an average traits score of 48 for Local variety (Figure 1).

In Experiment 2 (second season of 2013 or 2013B), Experiment1



Experiment 2



Experiment 3

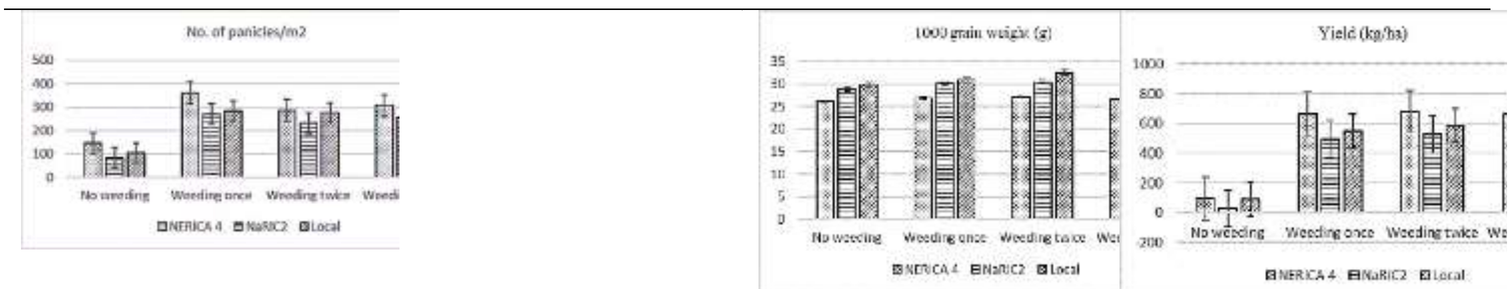


Figure 1. The effect of weeding regime on selected agronomic traits by variety for second seasons 2012, 2013 and 2014 in the Lake Albert crescent zone.

Table 3. Mean squares for the combined analysis of effect of weeding regime on the agronomic performance of farmer preferred upland rice germplasm for second season of 2013 in the Lake Albert crescent zone of Uganda.

Source of variation	DF	1000 grain weight	Plant height	Number of panicles per m^2	Number of spikelet per panicle	Ripening percentage (%)	Yield
Weeding regime	3	1.242ns	437.15***	67826.0***	3991.7***	3213.76***	2.15302***
Variety	2	81.819***	110.43ns	15167.0***	354.3ns	261.81*	0.12741***
Weeding regime × Variety	6	0.978ns	29.65ns	1242.0ns	252.7ns	111.69*	0.04847**
Residual	22	1.343	41.64	1127.0	220.6	52.44	0.01220
Total	35						

Ns = not significant; * = significant at P<0.05; ** = significant at P<0.01; *** = Significant at P<0.00; DF = degrees of freedom.

significant differences among weeding regimes were observed for plant height, number of panicles per m^{-2}, number of spikelets per panicles per metre squared, ripening percentage and yield (P<0.05 to P<0.001). Significant differences in variety responses were observed for 1000 grain weight, number of panicles per m^{-2}, ripening percentage and yield (P<0.05 to P<0.001) (Table 3). The differences among interaction of weeding regime × variety interaction was significant for only ripening percentage and yield (P<0.05 to P<0.01) (Table 3). The number of panicle per m^{-2} (ranged from 112 to 310), number of spikelets per panicle (ranged from 56 to 105), ripening percentage (ranged from 37.5 to 76.9), plant height (ranged from 78.4 to 95.3 cm) and yield (ranged from 714 to 5786 kg/ha) had 239, 87, 66%, 88 cm, 4449 kg/ha as the average trait scores for each weeding regime respectively (Table 6).

Across rice varieties, number of panicles per metre^{-2} (ranged 275 from to 361) with an average trait score of 275 for NERICA 4; ranged 83 from to 271 and an average traits score of 211 for NaRIC2; ranged from 103 to 284 with an average trait score of 225 for Local. The 1000 grain weight scores ranged from 26.13 to 27 g with a variety average score of 26.7 g for NERICA 4; ranged from 28.87 to 31.1 g with an variety average of 30.1 g for NaRIC 2, ranged from 29.93 to 32.53 g with a variety average of 31.1 g for Local variety. The ripening percentage scores ranged from 40.9 to 82.9 g with a variety average score of 69.2 g for NERICA 4, ranged from 25.4 to 79.4 g with a variety average of 63.2 g for NaRIC 2; ranged from 46.3 to 74.5 g with a variety average of 66.6 g for local variety (Figure 1).

In Experiment 3 (second season of 2014B or 2014B), significant differences among weeding regimes were observed for all traits measured (P<0.05 to P<0.001) (Table 4). Varietal differences were significantly different for 1000 grain weight, number of panicles per metre^{-2}, and yield (P<0.01 to P<0.001). The differences among interaction of weeding regime x variety was not significant for all traits (P>0.05) (Table 4). The number of panicles per m^{-2} ranged from 111 to 306, number of spikelets per panicle ranged from 56 to 105, 1000 grain weight ranged from 28.31 to 30.01 g, plant height ranged from 66.9 to 86.9 cm, ripening percentage ranged from 37.5 to 76.9% and yield had 237, 86, 29, 81, 66.3%, and 438 kg/ha as average trait scores for each weeding regime respectively (Table 7).

Across varieties, number of panicles per m^{-2} ranged from 145 to 308 with an average trait scores of 275 for NERICA 4; ranged from 83 to 271 with an average traits score of 211 for NaRIC 2; ranged from 103 to 284 g with an average trait score of 224 for local variety. The 1000 grain weight ranged from 26.3 to 27.0 with an average trait score of 26.7 g for NERICA 4; ranged from 28.87 to 31.07 with an average traits score of 30.1 g for NaRIC 2; ranged from 29.93 to 32.53 g with an average trait score of 31.1 g for local variety. The yield (ranged from 96 to 666) with an average trait scores of 527 kg/ha for NERICA 4; ranged from 29 to 542 kg/ha an average traits score of 3398.5 kg/ha for NaRIC 2; ranged from 90 to 587 kg/ha with an average trait score of 423 kg/ha for local variety as (Figure 1).

Overall, there were increases in the trait responses with each weeding regime and the extent of increases varied by trait and also by season (Tables 5, 6, and 7). In addition, varietal responses also varied with traits and seasons (Figure 1).

Results of broad sense heritability estimation for three upland varieties across weeding treatments are presented in Table 8. Heritability was lower where weeding was done compared to plots where no weeding was conducted for ripening percentage and plant height in all experiments, and for plant height only in Experiment 1.

Table 4. Mean squares for the effect of combined analysis of weeding regime on the agronomic performance of farmer preferred upland rice germplasm for second season of 2014 in the Lake Albert crescent zone of Uganda.

Source of variation	DF	1000 grain weight	Plant height	Number of panicles per m^2	Number of spikelet per panicle	Ripening percentage (%)	Yield
Weeding regime	3	0.0009783*	0.027638**	0.379434**	3929.5**	0.239639**	2.15269**
Variety	2	0.014444388**	0.000249ns	0.060685**	384.6ns	0.013891ns	0.13560**
Weeding regime × Variety	6	0.0001640ns	0.001894ns	0.006913ns	284.9ns	0.016127*	0.04573*
Residual	22	0.0002708	0.001841	0.005060	267.9	0.004634	0.01313
Total	35						

Ns = not significant; * = significant at P<0.05; ** = significant at P<0.01; *** = Significant at P<0.001; DF = degrees of freedom.

The heritability for 1000 grain weight, number of panicles per metre squared, number of spikelets per panicle was similar across weed treatments in all experiments, except where weeding was done once in Experiment 2, which had a lower heritability. In contrast, heritability for ripening percentage, plant height and yield varied across weeding treatments.

DISCUSSION

Weeds are a major biological constraint to upland rice production, especially in low input systems, where resource constrained farmers cannot afford herbicides and therefore rely on manual labour for weed control (Becker and Johnson, 2001; Rodenburg and Johnson, 2009; Saito et al. 2010). In this study, the effect of weed management was investigated on the yield of the associated crop although it could have far reaching consequences on weed species composition and abundances. Effects of management variables on weed flora have been studied for annual crop production systems. Crop rotation and reduced tillage were found to be more important than the amounts of fertilizer and herbicide applied in restraining seed production for both grassy and broadleaf weeds (Kagode et al., 1999). In many parts of Uganda, weed management is not stringent, and is characterised with shallow tillage of weedy fields immediately before sowing, followed by occasional weed removal during the cropping season. As such, weed composition is more related to soil characteristics than cropping system (Ugen and Wortmann, 1997). Towards crop maturation, weeds are normally not removed and after crop harvest, weeds re-establish and continue to grow during the off season period resulting in a dynamic weed flora with an abundant supply of seeds and other propagules (Ugen and Wortmann, 1997).

This study investigated the effect of weeding regimes on selected agronomic traits in farmer preferred upland rice varieties. Generally, there was an increase in yield progressing from no weeding to first weeding, to second and third weeding, although the increases in yield varied with season and the trait considered. This observation agrees with that of Toure et al (2013) and JICA (2010) and confirms the benefit of weeding in rice production. Furthermore, traits such as number of panicle per metre squared, plant height and yield showed more variation compared others such as ripening percentage and 1000 grain weight. This observation corroborates that of Hogue et al (2013).

Given the fact that the small holder upland rice farmers rely on manual labour for weed control which also becomes scarce, it is important to consider the actual number of weeding regimes that would maintain a high rice yield. This study showed that there was almost no difference between the second and the third weeding regimes across the varieties and the traits measured. This observation agrees with that of Akobundu and Ahissou (1985), Alou et al. (2012) and Toure et al. (2011). Therefore, at least one well-timed weeding regime is sufficient to get a good rice yield under upland field conditions of Lake Albert crescent zone of Uganda.

At variety level, the observation of increase in rice yield at each weeding regime level was still evident. However, there was variation in varietal response to each weeding regime. This observation is similar that of several studies Fofana and Rauber (2000); Haefele et al., 2004; Rodenburg et al., 2009; Saito et al., 2010). Moreover, the variations observed across the range of varieties used in this study varied with each trait and season. Traits such as number of panicles per metre squared, ripening percentage, and yield showed the highest variation.

The interaction of weeding regime and variety

Table 5. Means for the effect of weeding regime on the agronomic performance of farmer preferred upland rice germplasm for second season of 2012 in the Lake Albert crescent zone of Uganda.

Weeding regime	Traits			
	Number of panicles/m^2	Number of spikelets per panicle	Ripening percentage	1000 grain weight (g)
No weeding	149a	32.8a	44.5a	28.2a
Weeding once	269^{b}	$47.4a^{b}$	12.7a	$24.4a^{b}$
Weeding twice	289^{b}	60.6^{bc}	12.5a	$19.9a^{b}$
Weeding thrice	310^{b}	66.7^{c}	22.2^{b}	23.7^{b}
Mean	254	52.0	23.0	24.1
LSD (0.05)	52.3	18.5	13.6	5.2
CV (%)	21.4	37.1	61.6	8.3

*Values within the column followed by the same letter are not significant at P<0.05.

Table 6. Means for the effect of weeding regime on the agronomic performance of farmer preferred upland rice germplasm for second season of 2013 in the Lake Albert crescent zone of Uganda.

Weeding regime	Traits				
	Number of panicles/m^2	Number of spikelets per panicle	Ripening percentage (%)	Plant height (cm)	Yield (kg/ha)
No weeding	112^{a}	56^{a}	37.5^{a}	78.4^{a}	714
Weeding once	310^{b}	91^{b}	72.0^{b}	95.3^{b}	5703
Weeding twice	264^{bc}	105^{b}	76.0^{b}	89.2^{bc}	5786
Weeding thrice	269^{c}	94^{b}	77.0^{b}	88.2^{c}	5593
Mean	239	87	65.6	87.8	4449
LSD (0.05)	44	14.8	8.5	6.4	1056
CV (%)	19.1	17.7	13.5	7.5	24.7

*Values within the column followed by the same letter are not significant at P<0.05.

Table 7. Means for the effect of weeding regime on the agronomic performance of farmer preferred upland rice germplasm for second season of 2014 in the Lake Albert crescent zone of Uganda.

Weeding regime	Traits				
	Number of panicles/m^2	Number of spikelets per panicle	Ripening percentage (%)	Plant height (cm)	Yield (kg/ha)
No weeding	111^{a}	56^{a}	37.5^{a}	66.9^{a}	71^{a}
Weeding once	306^{b}	88^{b}	74.6^{b}	86.1^{b}	570^{b}
Weeding twice	266^{b}	105^{bc}	76.1^{b}	86.9^{b}	598^{b}
Weeding thrice	266^{b}	95^{c}	76.9^{b}	85.5^{b}	559^{b}
Mean	237	86	66.3	81.4	449.5
LSD (0.05)	43.1	16.1	8.27	7.1	116.3
CV(%)	18.9	19.4	13.0	9.0	26.9

*Values within the column followed by the same letter are not significant at P<0.05.

Table 8. Heritability of three rice varieties grown in three trials across 4 weeding treatments.

Trait	Exp.1	Exp. 2	Exp. 3
1000 grain weight			
T1 = no weeding	0.82	0.95	0.95
T2 = Weeding once	0.83	0.99	1.00
T3 = Weeding twice	0.52	0.99	0.99
T4 = Weeding thrice	0.88	0.98	0.98
Number of panicles per m $^{-2}$			
T1 = no weeding	0.68	0.97	0.97
T2 = Weeding once	0.96	0.93	0.93
T3 = Weeding twice	0.87	0.97	0.93
T4 = Weeding thrice	0.82	0.97	0.97
Number of spikelets per panicle			
T1 = no weeding	0.18	0.77	0.77
T2 = Weeding once	0.88	0.66	0.73
T3 = Weeding twice	0.86	0.77	0.77
T4 = Weeding thrice	0.24	0.96	0.96
Ripening percentage			
T1 = no weeding	0.52	0.98	0.98
T2 = Weeding once	0.24	0.71	0.85
T3 = Weeding twice	0.24	0.95	0.95
T4 = Weeding thrice	0.67	0.93	0.93
Plant height (cm)			
T1 = no weeding	0.83	0.82	0.73
T2 = Weeding once	0.56	0.91	0.90
T3 = Weeding twice	0.09	0.82	0.44
T4 = Weeding thrice	0.78	0.68	0.91
Yield (kg/ha)			
T1 = no weeding	0.24	0.95	0.95
T2 = Weeding once	0.19	0.76	0.76
T3 = Weeding twice	0.16	0.61	0.64
T4 = Weeding thrice	0.69	0.94	0.94

Exp.1= Experiment 1; Exp. 2 = Experiment 2 and Exp.3 = Experiment 3; T1-T4 = treatments 1 to 4.

was not significant for most traits in all experiments, except traits such as ripening ratio and yield in experiment two and three. Yield is quantitative trait which is highly influenced by environment. In the context of our study, the different weeding regimes and seasons caused unique interactions with variety. Modal et al. (2013) observed a similar trend using three *Aus* rice varieties BR 26, BRRI dhan27, BRRI dhan48 and Pariza; and five weeding treatments viz. no weeding, one hand weeding at 20 DAS (Days after sowing), two hand weeding at 20 DAS and 30 DAS, three hand weeding at 20, 30 and 40 DAS and weed free condition planted using broadcasting method.

It should be noted that our results are unique to the population of upland rice varieties evaluated, and the rice production ecology (in this case, upland ecology). As such, they may not be applicable to other rice production ecologies or water stress conditions, and rice varieties. Therefore, validating our findings, including the identification of clear distinction between the effect of soil characteristics and management (weeding) might be needed before making recommendations based on them.

Conclusion

In conclusion, this study has found that there is a profound effect of weeding regime on upland rice yield in the study region. In addition, the study has also established that different varieties respond differently to the weeding regimes implemented by the farmer. Overall, the farmer may need to apply at least one hand hoe weeding activity on a highly responsive and well adapted variety, such as NERICA 4 to achieve an acceptable yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Assessment of the allelopathic effects of seeds and seedlings of rotational crops and ryegrass

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Allelopathic effects were evaluated for a series of rotational crops, namely barley, canola, wheat, lupine, medic, lucerne (alfalfa) and ryegrass in laboratory studies. This followed on a field assessment of these crops to identify inter-species phytotoxic effects. Two different experiments to determine phytotoxicity effects of seed and seedling leachates were conducted under controlled conditions. The first experiment was set up in the laboratory to observe the mutual effect of seed leachates from the plant series. The second experiment was conducted in the laboratory to study the effect of seedling leachates from all the plants in the series on germination and early development of all other species. The phytotoxic activity observed for lupine and medic under controlled conditions, corresponds to results obtained in the field and confirms that these leguminous crops could be considered as living or terminated mulches for weed suppression. Crop mixtures containing legumes may provide weed suppression which would reduce dependency on herbicides that are associated with the development of weed resistance.

Key words: Cumulative germination, inhibition, stimulation, phytotoxic leachates.

INTRODUCTION

Plants can defend themselves against a wide array of enemies, from microbes to large animals, yet there is great variability in the effectiveness of such defences, both within and between species (Todesco et al., 2010). When competing against neighbouring plants, defence is by way of chemical interference, which was described by Hoffman et al. (1996) as a significant co-evolutionary force in plant communities when chemicals released from a plant or its litter affects other plants. This phenomenon, better known as allelopathy, encompasses both detrimental and beneficial interactions between plants through chemicals released by the donor (Xuan and Tsuzuki, 2002) and may be much more important as a mechanism in recipient than in origin plant communities where it maintains climax vegetation (Hierro and Callaway, 2003).

According to Kato-Noguchi (2000), chemicals with allelopathic activity are present in many plants and in many organs, including leaves, flowers, fruits and, buds. These allelochemicals are of varied chemical nature,

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e.g., phenolics, terpenes, alkaloids, flavonoids, etc. (Gupta 2005). In agricultural ecosystems it is one of the important mechanisms of interference, affecting crop performance (Batish et al. 2002). Allelochemicals appear to affect all aspects of crop development including germination, radicle and, plumule (coleoptile in monocots) growth, seedling growth, metabolism, plant growth, flowering and, fructification. According to Kong et al. (2008) allelopathy usually occurs at early growth stages of a plant species when allelochemicals are released from the root system. Overall plants exude up to 20% of fixed carbon and 15% of nitrogen (Venturi and Keel 2016) which includes an array of simple molecules, such as sugars, organic acids and secondary metabolites, as well as complex polymers, such as mucilage (Sasse et al., 2018).

The persistent use of herbicides in crop fields has created shifts in weed populations and the evolvement of severe herbicide resistance. Due to this phenomenon, there is interest in the exploitation of allelopathic activity as it is possible to utilise it as a cost-effective alternative to external inputs and thus contribute to sustainable agriculture (Wu et al., 1999). Kong et al. (2008) reported that rice seedlings release allelochemicals from its roots to paddy soils at early growth stages to inhibit neighbouring weeds. Since plant roots exude an enormous variety of potentially valuable low molecular weight compounds into the rhisosphere, individual components of rice root exudates, such as allelochemicals, can modify the soil microbial community. Although every plant produces exudates, the amount and composition of root exudates varies (Sasse et al., 2018). In addition, plant exudation is defined by the genotype of the host, it changes with plant developmental stage and it is modulated by abiotic stresses. Smith et al. (2001) emphasized that typical field studies cannot separate the effects of competition from allelopathy since they happen simultaneously between roots and shoots.

Loddo et al. (2014) regarded germination as a crucial moment in the life cycle of annual weeds, as plants can only rely upon seed resources to complete emergence and the early growth phase. However, the degree of weed seed germination inhibition and growth suppression which can be attributed to crop allelopathy is valuable and highly important (Asghari and Tewari, 2007). For instance, aqueous shoot extracts of buckwheat (*Fagopyrum esculentum* Moënch) stimulated Powell amaranth (*Amaranthus powellii* S. Wats.) germination slightly, but inhibited radicle growth (Kumar et al., 2009). Aqueous soil extracts from buckwheat-amended soil inhibited germination of Powell amaranth whilst extracts from soils that was not amended, showed no effect. For most plant species, shoot extracts were more effective than root extracts in inhibiting seed germination and growth of downy broom. Kumar et al. (2009) reported that shoot extracts of two goldenrod species (*Euthamia graminifolia* L. Nutt. and *Solidago canadensis* L.) had inhibitory effects on both germination and growth of radish (*Raphanus sativus* L.) and lettuce (*L. sativa* L.). By contrast, root extracts had no inhibitory effects on germination of these two species, but suppressed root growth.

Smith et al. (2001) cautioned that artificial environments must be devised that remove any possibility of competition while allowing chemical exchange to take place in studies on root leachates. In addition, Loddo et al. (2014) inferred that there is a general demand for more effective, efficient and sustainable integrated weed management (IWM) strategies to decrease dependency on herbicide use, but at the same time, guarantee adequate weed control and satisfactory yields to comply with worldwide increasing food demand. Acquiring thorough knowledge about weed biology and ecology represents a fundamental step in the development of IWM strategies that can meet these important challenges (Loddo et al., 2014).

It was reported by Belel and Belel (2015) that certain weed species tend to influence the performance of cultivated crops by exerting positive or negative effects on their germination and subsequent growth. Even though perennial ryegrass has been noted in studies for its ability to exhibit allelopathic potential in field and laboratory settings (Weston, 1990), none has considered the mutual role of leachates, which has been proven to contain allelochemicals, from seeds and seedlings of ryegrass and rotational crops grown in the Western Cape Province of South Africa. Therefore, the primary objective of this research was to evaluate the mutual effects of leachates exuded from both seeds and seedlings of ryegrass and selected rotational crop species for effective ryegrass management and less reliance on herbicides.

MATERIALS AND METHODS

Laboratory experiments were conducted to determine the mutual impact of seed and seedling leachates from seven seed types. One month prior to commencement of the experiment, mature, commercial quality seeds of each type were sourced from Agricol© seed company (https://www.agricol.co.za/) and stored at room temperature (18-22°C). The research approach for seed and seedling leachates was similar in concept to that followed by Kato-Noguchi (2000) and Ma et al. (2012) for assessing whether crop seeds and seedlings release phytotoxins that affect both the germination and development of radicles of selected rotational crops.

Seeds used in the laboratory were representative of the rotational crops grown in the Western Cape's grain production area. Seed viability for all seed types was above 95% according to tetrazolium chloride tests (International Seed Testing Association, 1985). These included barley (*Hordeum vulgare* L. v. Clipper), canola (*Brassica napus* L. v. ATR Hyden), wheat (*Triticum aestivum* v. SST 88), lupine (*Lupinus albus* L. v. Tanjil), lucerne (alfalfa) (*Medicago sativa* L. v. SA standard), medic (*Medicago truncatula* Gaertn. v. Parabinga) and ryegrass pasture type (*Lolium multiflorum* Lam. v. Energa). Ryegrass pasture type seed was used to ensure germination consistency among seed, as well as a stable seed source.

Figure 1. Illustration of the lay-out of Petri dishes in the incubator for germination studies on the effects of leachates from seeds of selected rotational crops and ryegrass.

Seed leachates

The first experiment was set up in the laboratory to observe the mutual effects of seed leachates from the plant series (Figure 1). Ten seeds of each plant type were placed in Petri-dishes in combinations with ten seeds of each of the other species in the series; thus 20 seeds in total for each treatment combination. Seeds were placed on brown germination paper which lined 9.5 cm diameter Petri-dishes and moistened with 5 ml distilled water. The lay-out was done according to a randomised block design with ten replicates, equalling 100 seeds per species. Control Petri-dishes contained only one seed type (not in combinations). Petri-dishes were sealed with Parafilm® to maintain moisture levels and incubated at an alternating temperature range of 25/15°C which corresponds with conditions at crop planting in late autumn. The photoperiod was set at a 12 h/12 h day/night cycle, while the light phase from white fluorescent lamps coincided with the higher temperature. Germination was determined after 7 and 14 days of incubation by counting the number of germinated seeds and measuring the length of the radicle. A seed was regarded as germinated when the radicle was at least 2 mm long and was subsequently removed from the Petri-dish before it was resealed with Parafilm® and returned to the incubator. Upon termination of the experiment at 14 days, viability of non-germinated seeds was tested with a 0.4% tetrazolium chloride solution. Seeds showing a pink to reddish colour after 4 h were considered viable (International Seed Testing Association, 1985). Germination percentages were based on the total number of viable seeds placed in Petri-dishes for each seed type.

Seedling leachates

The second experiment was conducted in the laboratory to determine the mutual effect of seedling leachates from all the plants in the series on germination and early development (Figure 2). One hundred seeds of each plant type in the series were germinated in Petri-dishes as described above for the seed leachate experiment. The seedlings were allowed to develop until they reached a length of roughly 50 mm, after which seedlings from each species were placed in a 4 cm porcelain Büchner funnel and washed for 10 s with 5 ml distilled water to yield a leachate. This leachate was funnelled into 9.5 cm diameter Petri-dishes lined with brown germination paper onto which 10 seeds from each plant type had been evenly placed according to a randomised block design with ten replicates, equalling 100 seeds per species. Control treatments were treated with distilled water only. Henceforth, the experiment was conducted exactly as described above, from the sealing of Petri-dishes with Parafilm® for the seed leachate experiment. All data were statistically analysed (ANOVA) with the statistical program SAS, but this was preceded by exploring outliers and tests for normality and homogeneity of variances. Least significant differences were used to identify significant differences between means at the 5% level of probability.

RESULTS

Overall for all seed types, barley and lupine seed leachates showed the most activity on the plant series tested. Results showed that barley or lupine seed leachates significantly inhibited both radicle lengths and cumulative germination of canola, wheat, lucerne, medic and ryegrass.

Barley

Upon termination of the experiment at 14 days, seed

Figure 2. Illustration of the set-up for funnelling leachates from a Büchner funnel to a Petri-dish for germination studies on the effects of leachates from seedlings of selected rotational crops and ryegrass.

leachates from both wheat and medic had significantly reduced cumulative germination of barley which attained 69 and 72% respectively, compared to the control (Table 1). No significant interactions were observed for seedling leachates.

Canola

Radicle length of canola was significantly reduced by seed leachates from barley (44%), lupine (24%) and lucerne (46%) respectively, compared to the control (Table 2). Lupine seed leachate had strong inhibitory effects on cumulative germination of canola which was reduced by 65% compared to the control.

Wheat

Radicle elongation of wheat was significantly reduced by phytotoxic seed leachates from barley (30%), wheat (34%) and lupine (18%) compared to the control (Table 3). Also, cumulative germination of wheat was significantly inhibited by lupine seed leachate (31%). After treatment with seedling leachate of canola, strong inhibitory effects of radicle length of wheat (62%) was observed compared to the control.

Lupine

Seed leachate of barley inhibited both lupine radicle elongation and cumulative germination significantly by 33 and 25% respectively compared to the control (Table 4). After treatment with lucerne seedling leachate, the cumulative germination of lupine, was significantly less (61%) than the control.

Lucerne (alfalfa)

Lucerne radicle length was significantly inhibited by seed

Table 1. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Hordeum vulgare* L. v. Clipper.

Plant type	Seed leachate		Seedling leachate	
	Barley radicle length (mm)	**Cumulative germination % at 14 days**	**Barley radicle length (mm)**	**Cumulative germination % at 14 days**
Barley	26.4[a]	77[ab]	35[a]	73[a]
Canola	25.2[a]	97[a]	30.5[a]	90[a]
Wheat	23.5[a]	67 [b]	27.3[a]	77[a]
Lupine	13[a]	73[ab]	36.7[a]	97[a]
Lucerne	12.2[a]	90[ab]	29.9[a]	83[a]
Medic	13[a]	70 [b]	32.7[a]	73[a]
Ryegrass	25.7[a]	80[ab]	33[a]	90[a]
Control	21.6[a]	97[a]	40.4[a]	100[a]
LSD (P≤0.05)	NS	25	NS	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

Table 2. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Brassica napus* L. v. ATR Hyden.

Plant type	Seed leachate		Seedling leachate	
	Canola radicle length (mm)	**Cumulative germination % at 14 days**	**Canola radicle length (mm)**	**Cumulative germination % at 14 days**
Barley	10.5 [c]	73[ab]	26[a]	87[a]
Canola	22.4[a]	97[a]	22[a]	83[a]
Wheat	12.4[abc]	70[ab]	26[a]	90[a]
Lupine	5.8 [c]	60 [b]	22.8[a]	87[a]
Lucerne	10.8 [bc]	100[a]	19.2[a]	80[a]
Medic	22.3[ab]	93[a]	21.7[a]	93[a]
Ryegrass	23.4[a]	90[ab]	22.3[a]	87[a]
Control	23.7[a]	93[a]	19.5[a]	73[a]
LSD (P≤0.05)	11.5	33	NS	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

leachates from both barley (15%) and lupine (100%) compared to the control (Table 5). Lupine seed leachate also completely inhibited lucerne cumulative germination (100%). By contrast, both canola (98%) and ryegrass (93%) seed leachates showed strong stimulatory effects on lucerne seedlings with regard to radicle elongation (Table 5). This growth promoting activity of ryegrass was also evident in its seedling leachate which significantly stimulated (80%) the radicle length of lucerne (alfalfa) seedlings compared to the control.

Medic

The radicle length of medic was significantly inhibited by seed leachates from both barley (42%) and lupine (40%) compared to the control (Table 6). This phytotoxic activity of lupine was also observed for seedling leachate which strongly inhibited both radicle elongation of medic (64%) and cumulative germination of medic (82%) compared to the control (Table 6).

Ryegrass

The radicle length of ryegrass was significantly inhibited by phytotoxic seed leachates from barley (34%), wheat (43%) and lupine (4%) compared to the control (Table 7). This growth-inhibiting effect from barley (52%) and lupine (18%) seed leachates, was also evident in cumulative

Table 3. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Triticum aestivum* v. SST 88.

Plant type	Seed leachate		Seedling leachate	
	Wheat radicle length (mm)	Cumulative germination % at 14 days	Wheat radicle length (mm)	Cumulative germination % at 14 days
Barley	8.5 [bc]	53 [bc]	33[abc]	80[a]
Canola	19.5[ab]	70[ab]	25.7 [c]	83[a]
Wheat	9.5[bc]	93[a]	44.6[a]	77[a]
Lupine	5[c]	27[c]	31 [bc]	73[a]
Lucerne	20.2[ab]	83[ab]	40.6[ab]	83[a]
Medic	15.6[abc]	83[ab]	35.8[abc]	87[a]
Ryegrass	24.8[a]	93[a]	41.2[ab]	70[a]
Control	27.9[a]	87[ab]	41.4[ab]	87[a]
LSD (P≤0.05)	12.5	38	13.5	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

Table 4. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Lupinus albus* L. v. Tanjil.

Plant type	Seed leachate		Seedling leachate	
	Lupine radicle length (mm)	Cumulative germination % at 14 days	Lupine radicle length (mm)	Cumulative germination % at 14 days
Barley	2.8[b]	13[b]	21.9[a]	80[ab]
Canola	6.8[ab]	53[a]	15.2[a]	90[a]
Wheat	8.9[ab]	40[ab]	26.3[a]	87[a]
Lupine	11.9[a]	70[a]	23[a]	90[a]
Lucerne	9.6[ab]	43[ab]	12.9[a]	57 [b]
Medic	13.5[a]	63[a]	16.5[a]	77[ab]
Ryegrass	9.1[ab]	47[ab]	24[a]	77[ab]
Control	8.4[ab]	53[a]	27.7[a]	93[a]
LSD (P≤0.05)	9	40	NS	25

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

germination percentages of ryegrass. Lupine seedling leachate significantly inhibited ryegrass radicle elongation by 68% compared to the control (Table 7).

DISCUSSION

The goals of this study were to evaluate the mutual effects of leachates exuded from both seeds and seedlings of ryegrass and selected rotational crop species. It was also envisaged that results would offer perspectives on possible phytotoxic reactions. In general, results from the current study are consistent with a field assessment by Ferreira and Reinhardt (2010) which showed suppression of ryegrass weed type by selected leguminous crops. Furthermore, results confirm an observation by Baghestani et al. (1999) that phytotoxic effects on seed germination depend on the donor and receiver plants.

Barley

Cumulative germination of barley was inhibited by both wheat and medic seed leachates. In the reciprocal response reaction, barley seed leachates reduced canola, wheat, lucerne, medic and ryegrass radicle lengths as well as lupine and ryegrass cumulative

Table 5. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Medicago sativa* L. v. SA standard.

Plant type	Seed leachate		Seedling leachate	
	Lucerne radicle length (mm)	Cumulative germination % at 14 days	Lucerne radicle length (mm)	Cumulative germination % at 14 days
Barley	3.7 [bc]	33[c]	18.3[abc]	63[a]
Canola	20.8[ab]	87[a]	21.4[ab]	80[a]
Wheat	11.9[abc]	47 [bc]	20.1[abc]	70[a]
Lupine	0 [c]	0 d	14.7 [bc]	63[a]
Lucerne	17.1[abc]	73[ab]	12.3[c]	63[a]
Medic	20.0[ab]	57[abc]	22.8[ab]	70[a]
Ryegrass	20.6[ab]	83[a]	26.4[a]	57[a]
Control	25.2[a]	43 [bc]	14.7 [bc]	73[a]
LSD (P≤0.05)	17.4	31	8.7	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

Table 6. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Medicago truncatula* Gaertn. v. Parabinga.

Plant type	Seed leachate		Seedling leachate	
	Medics radicle length (mm)	Cumulative germination % at 14 days	Medics radicle length (mm)	Cumulative germination % at 14 days
Barley	13.4[b]	57[a]	17.0[ab]	73[ab]
Canola	31.7[a]	73[a]	27.8[a]	70[ab]
Wheat	19[ab]	77[a]	25.8[ab]	73[ab]
Lupine	12.6[b]	50[a]	15.6 [b]	63 [b]
Lucerne	17[ab]	60[a]	18.5[ab]	73[ab]
Medic	19.7[ab]	93[a]	19.2[ab]	77[a]
Ryegrass	31.8[a]	70[a]	26.8[a]	70[ab]
Control	31.6[a]	77[a]	24.5[ab]	77[a]
LSD (P≤0.05)	16.1	NS	10.9	13

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

germination percentages. These results are in accordance with those of Xuan et al. (2005), who also reported plant inhibition by lucerne. However, Ben-Hammouda et al. (2001) reported that the allelopathic potential of barley increased near physiological maturity. According to Baghestani et al. (1999) vanillic and o-coumaric acids along with scopoletin may be responsible for the allelopathic effects of barley and wheat. However, Bouhaouel et al. (2016) reported that barley produces a complex mixture of allelochemicals that seem to be highly diversified. Moreover, barley genotypes exhibited differential allelopathic activity against weeds.

Canola

Canola radicle length was inhibited by barley, lupine and lucerne seed leachates. Lupine seed leachate also reduced canola cumulative germination. In the reciprocal response reaction, canola seed leachate promoted lucerne radicle length, but by contrast reduced wheat radicle length. The effects of lupine, lucerne and medic on barley, canola and wheat are generally similar to those reported by Xuan and Tsuzuki (2002). Many reports have indicated that lucerne (*M. sativa* L.) plants contain water-soluble allelochemicals that are released into the soil environment from fresh leaf, stem and crown tissues, as well as from dry hay, old roots and seeds. However, when Mutlu and Atici (2009) evaluated the allelopathic potential of root and shoot extracts from *Nepeta meyeri* they found a general phytotoxic effect at all concentrations on seed germination of barley, while at lower concentrations wheat and canola seedling growth

Table 7. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Lolium multiflorum* Lam. v. Energa

Plant type	Seed leachate		Seedling leachate	
	Rye grass radicle length (mm)	Cumulative germination % at 14 days	Rye grass radicle length (mm)	Cumulative germination % at 14 days
Barley	12.4[cd]	50[bc]	35.6[ab]	83[a]
Canola	33.8[ab]	87[a]	34.7[ab]	83[a]
Wheat	15.8[bcd]	73[ab]	47.4[a]	87[a]
Lupine	1.5[d]	17 [c]	31.6 [b]	87[a]
Lucerne	25.1[abc]	90[a]	36.2[ab]	83[a]
Medic	24.0[abc]	97[a]	39.1[ab]	80[a]
Ryegrass	28.2[abc]	90[a]	46.4[a]	93[a]
Control	36.8[a]	97[a]	46.1[a]	90[a]
LSD (P≤0.05)	19.5	34	13.9	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

was increased. This an example of the hormesis effect in that the same allelochemicals could have resulted in different growth responses, either positive or negative, from the species considered here. Furthermore, the span between stimulation and inhibition for allelochemicals can be small and hormetic effects (Calabrese, 2007) may occur in a natural setting if doses released are low (Belz, 2008). Under field conditions this equates to higher and lower doses as plant density varies. In addition, plant genotype was considered by Asaduzzaman et al. (2014) who measured the allelopathic activity of canola on the reduction in ryegrass root and shoot growth. These differed significantly between canola genotypes in their ability to inhibit ryegrass root length.

Wheat

The radicle length of wheat was decreased when germinated with barley, wheat and lupine seed leachate and also canola seedling leachate. Lupine seed leachate also reduced wheat cumulative germination. In the reciprocal response reaction, wheat seed leachate inhibited ryegrass radicle length, but showed autotoxicity by reducing wheat radicle length. Current results on wheat radicle inhibition by wheat seed leachate, corresponds with results on varietal autotoxicity reported by Wu et al. (2007) who studied wheat varieties. Allelopathic studies by Bakhshayeshan-Agdam et al. (2015) indicated that the germination response among the crops tested for wheat and common bean were the most resistant to redroot pigweed (*Amaranthus retroflexus* L.) leachate treatments.

Lupine

Barley seed leachate reduced both lupine radicle length and cumulative germination. In addition, cumulative germination of lupine was also inhibited by lucerne seedling leachate. In the reciprocal response reaction, lupine seed leachate inhibited both radicle length and cumulative germination of canola, wheat and lucerne. A report by Belel and Belel (2015) on a different leguminous crop, namely cowpea (*Vigna unguiculata* (L.) Walp) showed the toxic effect of nutgrass (*Cyperus tuberosus* Rottb.).

Lucerne

While lucerne radicle length was reduced by barley seed leachate, lupine seed leachate showed complete inhibition of both its radicle growth and cumulative germination. By contrast, both canola and ryegrass seed leachates stimulated the growth of lucerne seedlings with regard to radicle length. This activity of ryegrass was also evident in its seedling leachate which increased radicle length of lucerne seedlings. In the reciprocal response reaction, lucerne seedling leachate showed inhibition of both canola radicle length and lupine cumulative germination.

Bakhshayeshan-Agdam et al. (2015) reported slight germination in the presence of redroot pigweed (*Amaranthus retroflexus* L.) allelochemicals, but seedling growth was completely inhibited. It was concluded that of the four crops tested, lucerne germination was the most sensitive to the leachate treatments. Studies by Gholami et al. (2014) indicated that lucerne produces allelopathic triterpene saponins and flavonoids which might be the major cause of yield reduction in subsequent crops. Chung et al. (2000) showed that chlorogenic acid occurs in relatively large amounts in lucerne aqueous extracts as compared to salisylic acid, and bioassays suggest that chlorogenic acid is involved in lucerne autotoxicity.

Medic

The radicle length of medic was inhibited not only by barley and lupine seed leachates, but also by lupine seedling leachate. Additionally, activity of lupine seedling leachate was also evident in reduced medic cumulative germination. In the response reaction, medic seed leachate showed reduced barley cumulative germination.

Ryegrass

The radicle length of ryegrass was inhibited by seed leachates from barley, wheat and lupine. This growth-inhibiting effect by both barley and lupine seed and seedling leachates was also evident in ryegrass cumulative germination percentages. Further proof of this activity by lupine seedling leachate was also evident in ryegrass radicle length. In the reciprocal response reaction, both ryegrass seed and seedling leachates showed a stimulatory reaction on lucerne radicle elongation.

These findings on wheat are in accordance with those by Wu et al. (2000), who evaluated 92 wheat cultivars for their allelopathic activity on the inhibition of root growth of annual ryegrass. They found significant differences between wheat cultivars in their allelopathic potential at the seedling stage on the inhibition of root elongation of annual ryegrass, with percentage inhibition ranging from 24 to 91%. Although no growth-inhibiting effect from canola seed or seedling leachates on ryegrass was observed in this study, the varietal responses by wheat (Wu et al. 2000), was also confirmed for canola in a report by Asaduzzaman et al. (2014) as it showed considerable genetic variation among canola genotypes for their allelopathic effects on ryegrass. It was concluded that highly allelopathic canola genotypes could potentially suppress ryegrass in integrated weed management programmes.

Allelopathic compounds affect several metabolic and physiological processes in seeds that are known to retard germination and impair seedling growth (Mathiassen et al., 2006). Moreover, Shui et al. (2010) reported that for both perennial ryegrass (*L. perenne* L.) and lucerne (*M. sativa* L.) seedling growth was more sensitive than seed germination as an indicator of allelopathic effect. Contrary to this, results from this study indicate more sensitivity by seed germination than seedling growth and depend on the specific acceptor species and donor leachate.

Given the synchronous germination of ryegrass weed type and rotational crops in the field, this data confirms earlier findings by Ferreira and Reinhardt (2010). Ryegrass typically germinates in autumn and its growth habit and life cycle coincide with that of most rotational crops tested in this study. Chemical control of ryegrass has become very problematic due to the increasing incidence of herbicide resistance. Moreover, in some countries legislations have required the agricultural industry to become less dependent on pesticide use by following integrated weed management principles (Barzman et al., 2015). This would surely aid the move to more sustainable systems by reducing, or in future, even eliminate the use of herbicides by innovative approaches (Peigné et al., 2015). Since results from the present study suggest that wheat, barley and lupine strongly inhibited ryegrass radicle elongation with the two latter named crops showing the most activity, one possible option in the field for weed suppression might entail high barley or lupine seeding densities. Though, from practical experience in the field, this would not be a feasible weed management option. This is due to the fact that mono cropping requires many inputs in terms of agrochemicals. In addition, final lupine plant population in the field are seldom above 50 plants m^{-2} which is due to the soil and climatic factors of the Western Cape, South Africa. Under these field conditions adequate ryegrass suppression would theoretically only be achieved at a final lupine plant population of 100 plants m^{-2}. Since this is not feasible, the only realistic option would be a smother crop mixture with a monocotyledonous species such as barley, black oats or rye in combination with lupine as the leguminous component.

Conclusion

For effective ryegrass management with less reliance on herbicides, results from the present study showed that leguminous crops should play a more prominent role in crop rotation systems. Leguminous crops should not only be considered as rotational crops, but also used in intercropping where living mulches are used for weed suppression. However, under field conditions, such a practice is likely to be exposed to the vagaries of environmental factors, as well as likely being crop cultivar and weed-specific. Nevertheless, by promoting diversity in weed management tools, this would ensure sustainability and reduce the possibility of the development of weed resistance**.**

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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