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Journal of Plant Breeding and Crop Science

July-September 2020
ISSN 2006-9758
DOI: 10.5897/JPBCS
www.academicjournals.org

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Vol. 12(3), pp. 184-191, July-September 2020
DOI: 10.5897/JPBCS2019.0804
Article Number: 709704F64097
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Genetic progress for tuber yield and related traits of potato (*Solanum tuberosum* L.) in Ethiopia

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Received 7 February, 2019; Accepted 14 June, 2019

Little work has been done to evaluate the progress made in potato improvement in Ethiopia during the past three decades and information about traits most contributing to progress in yield is scarce. Hence, this experiment was conducted to estimate rate of genetic improvement made over time in yield and to determine traits most contributing to progress in tuber yield. Twenty varieties of potato released in Ethiopia between 1987 and 2013 and one farmers` cultivar were evaluated at Holetta and Adaberga, central highlands of Ethiopia in 2017 main cropping season. The experiment was arranged in a randomized complete block design with three replications. Analysis of variance for tuber yield and tuber related traits showed the existence of highly significant (P < 0.01) differences between varieties for all traits. There was a total tuber yield increment of 137.39 and 0.851% over farmers` cultivar and the oldest variety, respectively. The relative rate of gain was 1.15 and 1.42% year^{-1} for total tuber yield and marketable tuber yield, respectively. The annual rate of genetic progress was found to be 0.3177 and 0.3401 ton year^{-1} for total tuber yield and marketable tuber yield, respectively.

Key words: Genetic progress, tuber yield, yields related traits, *Solanum tuberosum* L., regression.

INTRODUCTION

Potato (*Solanum tuberosum* L.*)* is one of the vital tuber crops in terms of food security for a growing population and increased hunger rates (CIP, 2018). Since its introduction to Ethiopia by a German immigrant in 1858 Wilhelm Schimper, the crop was cultivated by few number of farmers as a garden crop. The expansion and its adoption by Ethiopian farmers were gradual and stagnant for several decades (Baye and Gebremedhin, 2013). Strategic potato research in Ethiopia began in 1975 with the understanding of the constraints challenging its production and productivity (Baye and Gebremedhin, 2013). The development and dissemination of more than 36 improved varieties, coupled with other technological packages, contributed greatly to the improvement and rapid expansion in potato production (MANR, 2016). The major objective of potato breeding has been to develop potato cultivars that have maximum yield potential, adaptable to wide agro-ecologies and resistant to late blight that has been the most devastating disease throughout the dominant potato producing highlands of

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Table 1. Description of the study sites.

Location	Latitude	Longitude	Altitude (m.a.s.l.)	Mean annual rain fall (mm)	Mean annual temperature (°C)
Holetta Research Centre	09° 00' N	38° 29' E	2400	1100	14.15
Adaberga Research station	09° 16' N	38° 23' E	2500	1225	18.0

Source: HARC (2015).

the country (Wassu, 2016). However, considerable number of released varieties became vulnerable to potato devastating disease because of the pathogen (*Phytophthora infestans)* ability to rapidly overcome resistant genes even though improvements on late blight resistance were made by breeders (Getachew et al., 2016; Wassu, 2017). This indicates the study on genetic progress with identifying the most important traits for yield as well as resistance of the crop for late blight was the missing component except little attempt made by Haramaya University (Wassu, 2017). Hence periodic assessment of genetic progress for these important traits could supplement the breeding program of the crop in the country. Furthermore, estimation of genetic progress of a breeding program and periodic assessment of advance (Wassu, 2017; CGIAR, 2016) in the genetic gain of a crop is vital to understand changes produced by breeding, to assess the past efforts made in genetic yield potential and to put forward future breeding strategy.

MATERIALS AND METHODS

The field trial was carried out under rain fed condition at two locations, Holetta Agricultural Research Center and Adea Berga Research station (Table 1) during the main cropping season of 2017 using 20 released varieties and 1 farmers` cultivar of potato (Table 2). The experiment was arranged in a randomized complete block design with three replications. The experimental plot size was 4 rows each 3 m long and 3.6 m wide; plant spacing was 30 cm between plants and 75 cm between rows. Fertilizer used in the rate of 108.44 kg N, 92.43 kg P and 16.59 kg S per hectare in the form of Urea (143 kg/ha) and blended fertilizer (NPS) (237 kg/ha) as per the recommendation for the study area (MALR, 2017). Other agronomic practices and data collection was conducted based on the recommendations of Holetta Research Centre (Lemaga et al., 1992).

Data analysis

All data were subjected to separate analysis of variance (ANOVA) of individual locations and a combined ANOVA over locations was done using the procedure of SAS software version 9.3 (SAS Institute, 2010) and a general linear model (GLM) for tuber yield related traits (Gomez and Gomez, 1984). The combined analysis of variance over locations were computed after homogeneity test of error variances using F-test as stated by Gomez and Gomez (1984).

Regression analysis was performed to calculate the genetic gain of yield and other yield related traits. The average annual rate of genetic gain for each trait was estimated by regressing of the mean value of each trait against the corresponding year of release of each variety (Singh and Chaudhary, 2007).

The regression equation was, Y = a + bx, assuming the linear relationship where Y = the mean value of the dependent variable, x = the mean value of the independent variable, a = the constant value and b = the regression coefficient.

$$\text{Annual rate of gain } (b) = \frac{CovXY}{\text{Var}X}$$

Where, *X* = the year of variety release, *Y* = the mean value of each character for each variety, *Cov* = Covariance and *Var* = Variance.

Regression analysis was conducted taking tuber yield and other traits dependent and year of release as independent variable. The annual rate of genetic gain achieved over the last 30 years of potato improvement was determined as the ratio of genetic gain to the corresponding mean value of the oldest variety and expressed as percentage.

Relative annual rate of gain = Cov (X, Y)/Var (X)

Where, X is the year of variety release, Y is the mean value of each trait for each variety; Cov (X,Y) is the covariance of X and Y, and Var (X) is variance of X (year of variety release). Percent genetic gain per year for each variety was calculated as:

Percent Genetic Gain Year^{-1} = [(XG-XAL-624)/XAL-624]/YG-YAL-624 × 100

Where, X is the mean value of observations for a given trait, Y is the year of release of each variety (G), AL-624 is the oldest variety of potato released in 1987.

The increment over farmers' cultivars for each trait was calculated as:

Percent increment of Variety (%) = XG - XFC/XFC × 100

Where, XG is the mean value of each variety for each trait, XFC is the mean value of the farmers' cultivar (Nech Abeba) in Central highlands of Ethiopia.

In this experiment, Alemaya 624 was considered as the oldest variety since it was the first potato variety released in the country in 1987.

Step-wise regression analysis was computed using the SAS software (version 9.3) (SAS Institute, 2010) to identify the most contributing traits to the variability that exists in the yield related traits were computed (Gomez and Gomez, 1984).

RESULTS

Improvement made on tuber yield and yield related traits

Yield data of each mean values from combined analysis

Table 2. List of experimental materials included in the study.

No.	Variety	Accession code	Year of release	Breeding centre	Recommended altitude (m.a.s.l)
1	Dagim	CIP-396004.337	2013	ADARC	2000-2800
2	Bubu	CIP-384321.3	2011	HU	1700-2000
3	Belete	CIP-393371.58	2009	HARC	1600-2800
4	Gudene	CIP-386423.13	2006	HARC	1600-2800
5	Challa	CIP 387412-2	2005	HU	1700-2000
6	Mara chare	CIP 389701-3	2005	AwARC	1700-2700
7	Shenkolla	KP- 90134.5	2005	AwARC	1700-2700
8	Gabissa	CIP 3870-96-11	2005	HU	1700-2000
9	Gera	KP-90134.2	2003	ShARC	2700-3200
10	Jalene	CIP-384321.19	2002	HARC	1600-2800
11	Gorebella	CIP-382173.12	2002	ShARC	1700-2400
12	Guassa	CIP-384321.9	2002	ADARC	2000-2800
13	Zengena	CIP-380479.6	2001	AwARC	2000-2800
14	Zemen	AL-105	2001	HU	1700-2000
15	Bedassa	AL-114	2001	HU	2400-3350
16	Chiro	AL-111	1998	HU	2700-3200
17	Wechecha	KROEZE 72-2951	1997	HARC	1700-2800
18	Menagesha	CIP-374080.5	1993	HARC	Above 2400
19	Awash	CIP-378501.3	1991	HARC	1500-2000
20	Alemaya 624	AL-624	1987	HU	1700-2400
21	Nech Abeba		...	...	Central highlands

*HU = Haramaya University, HARC = Holetta Agricultural Research Centre, AwARC = Awassa Agricultural Research Centre, ShARC = Sheno Agricultural Research Centre, ADARC = Adet Agricultural Research Centre.
Source: MANR (2016)

of variance were used to regress the breeding progress of varieties for their tuber yield and other important traits. Potato varieties had huge total and marketable tuber yield difference. Trends in genetic progress, percent genetic gain over location and annual relative rate of genetic progress were calculated and presented in Tables 3 to 5.

Total tuber yield of variety Belete showed an increment of 137.39 and 0.851% over Nech Abeba (farmers` cultivar) and the oldest variety (AL-624), respectively (Table 5). The trend also showed total tuber yield increment over the oldest variety was 1.08% (Awash), 0.79% (Gudene and Gabissa) and 0.78% (Bedassa). On the other hand, the recent variety Dagim had a yield progress of -0.596% over the oldest variety. Hence, the yield increment was not constant throughout the breeding period and some varieties had yield performance below the oldest variety AL-624.

In terms of potato tuber yield, the genetic progress made over the last two to three decades since the first improved potato variety AL-624 was released comparing with the maximum yielding variety Belete was 0.009 t ha^{-1}. The relative annual rate of gain was 1.15 and 1.42% $year^{-1}$ for total tuber yield and marketable tuber yield, respectively. Both the average tuber number and tuber weight showed annual relative gain of 0.01 and 0.79% $year^{-1}$. Similarly, the annual rate of genetic progress in the present study was found to be 0.3177 ton $year^{-1}$ (20.27%) and 0.3401 ton $year^{-1}$ (24.55%) per hectare for total and marketable tuber yield, respectively (Figure 1a). On the other hand, non-significant and positive regression value was recorded for average tuber number and average tuber weight against year of variety release (Table 4).

Improvements on tuber quality traits

Year of variety release had positive and significant regression value with tuber dry matter content, starch content and total starch yield, but positive and statically non-significant positive value for tuber specific gravity (Table 4) indicating that potato breeders made improvements on tuber quality traits through breeding for the last two to three decades. Among quality traits studied, total starch yield had maximum relative gain 1.70% $year^{-1}$ followed by starch and dry matter content (0.80 and 0.44% $year^{-1}$, respectively). For both tuber dry matter and starch content in percent, the annual rate of genetic progress in the present study was found to be

Table 3. Estimates of the mean annual relative genetic gains and correlation coefficients of all traits with total tuber yield r (TTY).

Trait	Over all mean	Annual RGG (% year^{-1})	Correlation coefficients r (TTY)
Days to 50% flowering(days)	59.63	-0.04	0.066
Days to physiological maturity (days)	99.14	0.38	-0.077
Number of leaves per hill	40.79	0.33	0.387
Plant height (cm)	59.36	0.46	0.657**
Stem number per plant	4.43	0.71	0.531*
Average tuber number per hill	11.26	0.01	0.599**
Average tuber weight (gtuber^{-1})	51.55	0.79	0.475*
Total tuber yield (t ha^{-1})	25.26	1.15	-
Marketable tuber yield (t ha^{-1})	21.39	1.42	0.985**
Unmarketable tuber yield (t ha^{-1})	3.87	-0.53	0.268
Specific gravity (g cm^{-3})	1.09	0.04	0.445*
Dry matter content (%)	21.89	0.44	0.541*
Starch content (g/100 g^{-1})	14.26	0.80	0.537*
Total starch yield (t ha^{-1})	3.60	1.70	0.937**

RGG = Rate of genetic gain, r (TTY) = correlation coefficient for total tuber yield

Table 4. Estimates of mean values, coefficient of determination (R^2), regression coefficient (b), intercept and correlation coefficient with year of release (ryor) of various yield and yield related traits from linear regression of the mean values of each traits for the variety.

Trait	Mean	R^2	b	Intercept	ryor
Days to 50% flowering(days)	59.63	0.0038	-0.0222	104.60	-0.061
Days to physiological maturity (days)	99.14	0.3384	0.3507	-602.56	0.582**
Leaf number per hill	40.79	0.0477	0.1544	-268.20	0.219
Plant height (cm)	59.36	0.0558	0.2900	-520.84	0.241
Stem number per plant	4.43	0.0492	0.0320	-59.21	0.222
Average tuber number per hill	11.26	0.0001	0.0008	9.62	0.003
Average tuber weight (gtuber^{-1})	51.55	0.1260	0.4160	-779.99	0.355
Total tuber yield (t ha^{-1})	25.26	0.2027	0.3177	-610.25	0.450*
Marketable tuber yield (t ha^{-1})	21.39	0.2455	0.3401	-659.03	0.496*
Unmarketable tuber yield (t ha^{-1})	3.87	0.0257	-0.0195	42.97	-0.160
Specific gravity (g cm^{-3})	1.09	0.1690	0.0004	0.21	0.411
Dry matter content (%)	21.89	0.2054	0.0981	-174.40	0.453*
Starch content (%)	14.26	0.1950	0.1168	-219.45	0.442*
Total starch yield (t ha^{-1})	3.60	0.2474	0.0677	-131.87	0.497*

ryor= correlation coefficient with year of release.

0.1326 (R^2=0.2063) and 0.1633 (R^2=0.2077), respectively (Figure 1b and c). Total starch yield also showed positive significant value with year of variety release, whereas tuber specific gravity had positive and statistically non-significant regression value against year of variety release (Table 4).

Genetic progress on growth and Phenological traits

Year of variety release had positive and significant regression value with days to physiological maturity. Non-significant negative regression value was recorded for days to 50% flowering indicating the breeding made some weak improvements for this trait. Days to physiological maturity showed a significant positive regression value with a certain amount of increase against the year of variety release without affecting yield of potato (Table 4). However, days to 50% flowering had negative and statistically non-significant correlation value with yield of potato varieties indicating that high yielding varieties have a retarded flowering. On the other hand, all

Table 5. Trends in genetic progresses in total tuber yield of potato released from 1987 to 2013.

Variety	Year of release	Mean TTY (t ha^{-1})	Yield increment over			
			Nech Abeba		AL-624	
			t ha^{-1}	%	t ha^{-1}	%
Nech Abeba	Pre-1975	13.8	--	--	---	---
Alemaya-624	1987	27.7	1.00	99.96	--	--
Awash	1991	28.9	1.09	108.59	0.011	1.079
Menagesha	1993	16.1	0.16	16.01	-0.070	-6.997
Wechecha	1997	17.2	0.24	24.07	-0.038	-3.795
Chiro	1998	28.1	1.03	102.70	0.001	0.125
Bedassa	2001	30.7	0.74	74.39	-0.009	-0.913
Zemen	2001	25.8	1.22	121.72	0.008	0.778
Zengena	2001	15.9	0.86	86.44	-0.005	-0.483
Mean	2001	24.13	0.15	15.02	-0.030	-3.034
Guassa	2002	17.8	0.74	73.57	-0.009	-0.880
Gorebella	2002	30.4	0.28	28.44	-0.024	-2.384
Jalene	2002	23.8	1.20	120.00	0.007	0.668
Mean	2002	24.11	0.72	72.26	-0.009	-0.924
Gera	2003	29.6	1.14	113.61	0.004	0.427
Gabissa	2005	31.6	0.95	94.96	-0.001	-0.139
Shenkolla	2005	29.6	1.28	128.29	0.008	0.787
Marachere	2005	20.4	1.14	113.69	0.004	0.382
Challa	2005	26.3	0.48	47.68	-0.015	-1.452
Mean	2005	27.5	0.90	90.18	-0.003	-0.272
Gudene	2006	31.8	1.30	130.09	0.008	0.793
Belete	2009	32.8	1.37	137.39	0.009	0.850
Bubu	2011	28.9	1.09	109.09	0.002	0.190
Dagim	2013	23.4	0.69	68.97	-0.006	-0.596

*TTY = total tuber yield (t ha^{-1}), t ha^{-1} = ton per hectare, AL_624 = Alemaya 624.

the phenological and growth traits except days to physiological maturity demonstrated statically non-significant values with the year of variety release (Table 4). Days to 50% flowering and days to physiological maturity showed annual relative gains of -0.04 and 0.38% year^{-1}. The other growth traits like number of leaves per hill, plant height and stem number per hill showed relative gains of 0.33, 0.46, and 0.71% year^{-1}, respectively. The genetic progress for growth traits recorded was 0.29 cm for plant height, 0.15 for number of leaves per plant and 0.03 for stem number per hill.

The results of stepwise regression analysis between total tuber yield and other traits were presented in Table 6. The traits (predictors) that were retained in the model after regression analysis with the dependent traits were significant ($P \leq 0.05$ and $P \leq 0.01$) and regarded as the most important traits for predicting the tuber yield. Based on stepwise regression analysis, the best combination of characters that contributed to genetic progresses in tuber yield included were average tuber number per hill, stem number per hill and marketable tuber yield. About 98.2% of the variations on total tuber yield were explained by stem number per hill followed by average tuber number per hill and marketable tuber yield t ha^{-1} contributing to 97.8 and 97.2%, respectively. Therefore, it can be considered that changes in the above three traits had possibly contribution to the changes in total tuber yield in the last two to three decades of potato breeding in Ethiopia.

DISCUSSION

Improvements made on tuber yield and other yield related traits

Strategic potato research in Ethiopia began in 1975 amid to solve the constraints challenging the production and productivity of potato (Baye and Gebremedhin, 2013). The development and dissemination of many improved varieties, coupled with other technological alternative, contributed greatly to the improvement and rapid expansion in potato production. More than 36 improved potato varieties have been recommended from different

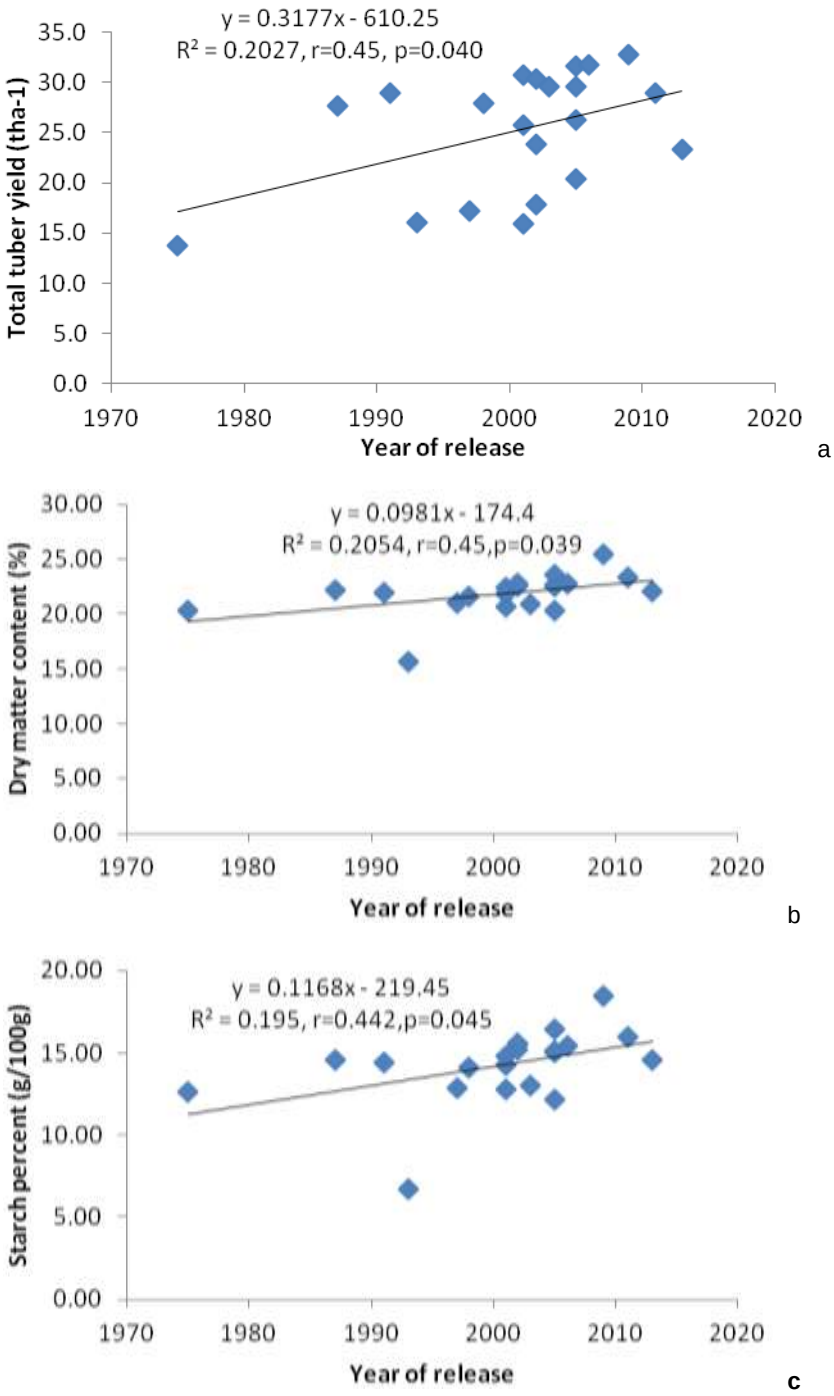

Figure 1. Plot of total tuber yield (t ha^{-1}), dry matter content (%) and starch content (g100 g^{-1}), (a, b and c) of varieties against year of release, respectively.

research institutions and private seed companies since 1987. The analysis of variance revealed the presence of highly significant ($P \leq 0.01$) differences between varieties for all traits. There was a considerable variation of yield among improved varieties and farmers` variety (13.8 to 32.8 t ha^{-1}). The improved variety Belete released in 2009 had the maximum yield and maximum mean values for most tuber's quality traits (specific gravity, dry matter content starch content, and total starch yield), whereas improved variety Menagesha released in1993 had lowest values for yield and other tuber quality traits. Total tuber yield of variety Belete showed yield increment of 137.39 and 0.851% over Nech Abeba (farmers` variety) and the oldest variety (AL-624), respectively. The relative rate of

Table 6. Summary of stepwise regression analysis of mean total tuber yield as dependent variable on independent variables.

Total tuber yield				
Independent variable	**Intercept**	**Regression coefficient (b)**	**R^2**	**VIF**
Marketable tuber yield		0.986**	0.972	1.46
Average tuber number	0.377	0.204**	0.978	1.25
Stem number per hill		0.337**	0.982	1.35

**Highly significant difference ($p \leq 0.01$), VIF = variance inflation factor.

annual gain was 1.15 and 1.42% year^{-1} for total tuber yield and marketable tuber yield, respectively. The mean total and marketable tuber yields increased over location at the rate of 0.3177 and 0.3401 t ha^{-1}, respectively.

The inconsistency in yield increment among varieties respect with their year of release could be due to variety AL-624 performed well across a wide range of agro ecologie. Attributing on farm productivity to genetic improvement is also more problematic in a vegetatively propagated crop like potatoes than in sexually propagated cereal. The absence of effective seed systems means that a productivity effect from a variety is confounded with the effect of cleaner or physiologically more correct seed (Sarker et al., 2018). The other important reason could be seed degeneration of the varieties due to their different seed sources and recycling the same seed source for subsequent production seasons (Sarker et al., 2018). Seed potato degeneration, the reduction in yield or quality caused by an accumulation of pathogens and pests in planting material due to successive cycles of vegetative propagation had a great influence on the performance of varieties (Thomas-Sharma et al., 2016).

Similar finding was recorded by Wassu (2017) and some recently released varieties showed lower yield performance than the oldest variety (AL-624) in Eastern Ethiopia. The correlation analysis of the potato for total and marketable tuber yield and respective year of variety release had also positive and significant association. Whereas unmarketable tuber yield had negative non-significant correlation value with year of variety release indicating that potato breeders in Ethiopia made some considerable improvement on seed tuber size.

Wassu (2017) recorded the highest estimates for annual genetic gain of total tuber yield (4.05%) in the locality of Hirna with the potato variety Gera and the lowest at Arbarakete for the potato variety Gorebella (-3.02%). Similarly, the variety Chiro at Haramaya had the lowest (-3.43%) and Gera variety at Hirna had the highest (4.94%) genetic gain for marketable tuber yield relative to the oldest variety AL-624. Similar genetic gains were reported by many researchers due to improvements in varieties and agronomic practices (Tamene et al., 2015; CGIAR, 2016). Studies on crop improvement for important agronomic and quality traits were undertaken by different scholars using gene engineering and other breeding methods (Douches et al., 2015; Massa et al., 2015; Liu, 2017).

Conclusion

The study showed that the high annual rates of gain have been achieved in tuber yields and tuber quality related traits through three decades of potato improvement efforts made in the nation though the gain was inconsistent over years of release of varieties and across the centres that developed the varieties. This might be due to different seed generations, variable sources of seeds from different growing and storage conditions and variations in seed physiological status. Therefore, further study is recommended with tissue culture planting materials with no seed degeneration difference among the varieties to isolate the variety effect, tuber seeds of checks has to meet the same health and physiological standards of prospective varieties over major growing areas including all released varieties and farmers cultivars of each growing area, and considering disease reaction and other important agronomic aspects of potato varieties to design appropriate potato improvement strategy in the country.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 12(3), pp. 192-199, July-September 2020
DOI: 10.5897/JPBCS2020.0886
Article Number: 505B14C64109
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

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Journal of Plant Breeding and Crop Science

Full Length Research Paper

Sorghum mutation breeding for tolerance to water deficit under climate change

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Received 29 February, 2020; Accepted 1June, 2020

This study aimed at creating genetic variability by induced mutagenesis in farmers' preferred sorghum variety (ICSV1049) to breed mutant lines for water deficit tolerance. Sorghum seeds were irradiated by gamma rays and sown as one panicle-to-one progeny method. Putative lines M5 (143) and parent were screened under water deficit stress. Data analysis showed that leaf senescence (LS) was positively correlated to relative water content (RWC), panicle weight (PaWt), grain weight (GrWt) and chlorophyll content 13 days after water deficit application (SPAD II). Semi-dwarf trait (SDwf) with plants height (Ht)<100 cm were observed among 3.38% of lines, while 13.5% exhibited early maturity (<90 days). The leaves of 87.3% of lines were semi-erectile. Averaged overall lines, mutation has reduced date to flowering (DaFl), date to grain maturity (DaMa) and LS at 9.2, 4.1 and 8.1% compared to the parent, respectively. However, SPAD I (chlorophyll content first day of water deficit application), SPAD II, RWC, GrWt, PaWt and Ht were increased at 30.8, 40.5, 36.5, 22.2, 37.5 and 9.3%, respectively. Based on the results, seven mutant lines exhibited tolerance to water deficit.

Key words: Mutagenesis, genetic variability, drought-tolerance.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the major cereal crops in the world. It is the fifth most cultivated dry cereal after wheat, maize, rice and barley and the second most cultivated in Africa after maize (Ng'uni et al., 2011). It is a staple food crop for millions of African farmers living in the semi-arid tropics (Dora et al., 2014). However, sorghum cultivation is affected by drought, a situation which could become severe in sub-Saharan Africa in the context of climate change. Water deficit caused by drought is the most severe environmental limitation to sorghum grain yield during the entire crop production period (Sánchez-Blanco et al., 2002). Due to population growth (3%) in Africa, the core challenge for agriculture in Africa would be to increase food production under changing climatic conditions.

Drought events can occur at any stage of sorghum

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growth but three stages are identified as critical phases sensitive to water deficit (Menezes et al., 2015). The growth stage 1 (GS1) corresponds to the vegetative phase, the growth stage 2 (GS2) corresponds to the pre-flowering phase with panicle initiation at flowering and the growth stage 3 (GS3) corresponds to the post-floral phase with filling and physiological maturity of the grains. Stay-green in sorghum is one of the reliable traits related to drought tolerance. Traits associated with pre- or post-floral water deficit resistance in sorghum also involve relative water content (RWC) and leaf senescence (Sakhi et al., 2014). The most sustainable ways to mitigate adverse effects of drought on sorghum production are field irrigation and provision of drought-tolerant varieties to farmers. Unfortunately, farmers in developing countries cannot afford irrigation facilities. Therefore, the development of drought-tolerant sorghum varieties is the most promising option to assist African farmers in adapting to drought. The strategy for development of new crop genotypes for drought tolerance could be to create variation within the gene pool. Genetic variability in traditional sorghum varieties is very low in Burkina Faso, around 4.5% of the genetic variability between agro-ecological zones and 5.8% between villages in the same zone (Kondombo-Barro, 2010). Mutation induction has been proven to be an effective method to increase genetic variability in crops. Induced mutagenesis in crop varieties preferred by farmers is a promising strategy to improve agronomic traits such as tolerance to water stress. Genetic variability created through mutagenesis is important for sustainable agriculture (Griggs et al., 2013). According to International Atomic Energy Agency (IAEA) database (http://mvgs.iaea.org), there are more than 3,300 officially released mutant varieties of 170 different species in more than 60 countries around the world that not only increase biodiversity but also provide material for plant breeding (Jankowicz-Cieslak et al., 2017). Mutation induction can be carried out using chemical or physical mutagens (Shahab et al., 2018). Some of the agronomic traits generated as result of mutation induction are: increasing 3-Deoxyanthocyanidin accumulation in sorghum leaves (Petti et al., 2014), dwarfism, early flowering, high protein digestibility and high lysine content which have been widely used in sorghum breeding (Oria et al., 2000). The aim of this study was to develop drought-tolerant mutants in a farmer-preferred sorghum variety (ICSV1049) for adaptation to water deficit that limits cereal production in sub-Saharan Africa.

MATERIALS AND METHODS

Study sites and genetic materials

A survey on adoption and dissemination of sorghum varieties from participatory breeding in Burkina Faso was conducted in partnership between the Institute for the Environment and Agricultural Research (INERA) and the Centre for International Cooperation in Agronomic Research for Development (CIRAD). Based on results from the survey, sorghum varieties preferred by farmers were identified (Sanou et al., 2014), including variety ICSV1049. Some traits of this variety include: 115-120 days to maturity, plant height ranging from 1.80 to 2.10 m, white grain colour and semi erectile leaf. The dry seeds of this variety were irradiated with gamma rays (^{60}Co) at doses of 200, 300 and 400 Gy at the Center for the Application of Isotope and Radiation Technology, National Nuclear Energy Agency (BATAN), Jl. Cinere Pasar Jumat, Jakarta, Indonesia. The trials were conducted in two localities, namely the eastern region (Kouaré Research Station) (11° 95' 03'' N and 0° 30' 58'' E) where mutant lines screening up to M4 generation was carried out and a selection of potential drought-tolerant mutants was achieved after terminal stress. Screening of M5 mutant lines was conducted in the central region (Kamboinsé Research Station) (12°28 N, 1° 32 W) for artificial water deficit application to minimize the environmental effect on the genotypes.

Both research stations are located in the North-Sudanian agroecological zone (latitude 11°30”–13°) with rainfall between 750-1000 mm per year. The soils are mostly revived tropical ferruginous types on Kamboinsé Station and sandy-loam, tropical, and ferruginous on Kouaré Station.

Generation of mutant progenies and selection of potential drought-tolerant M4 mutant lines

The irradiated seeds and control were sown and M1 panicles were harvested and planted as M2 panicle-to-one progeny. Forty of the M2 seeds from each M1 panicle were planted as a head row. Three panicles from each row were bagged before anthesis. To prevent redundancy of mutations, only one fertile plant from each M2 head row were selected to produce M3 seeds according to Xin et al. (2008). The M3 families were repeatedly evaluated for phenotypes distinctive from wild-type ICSV1049. Thus, the phenotypes were organized into tillering types, plant height, leaves vigor, panicle shapes and seeds size from ascend stage to grains physiological maturity. 394 lines were selected on the basis of the phenotypes described earlier and confirmed in the next generation. A field-trial was conducted during the cropping season in 2017 at Kouaré Research Station to evaluate 394 putative mutant lines at the M4 generation and their parent for tolerance to the end of season drought. The planting was done on 18th August so that the bloom stage coincided with the end of rainfall. Each line was planted on row 2.7 m length, 0.3 m between planting hills and 0.7 m between rows. The experimental design was an alpha lattice design plot using 15 blocks and each block contained 20 genotypes with two replications.

The field was weeded three times. Mineral fertilizer of 100 kg ha^{-1} of NPK (12-24-12) was applied to the plots at sowing and 50 kg ha^{-1} of urea was applied at the booting growth stage. The amount of rainfall recorded from planting to the harvest (18th August to 15th December, 2017) was 201.5 mm corresponding to 10 rain events or 23.4% of total rainfall (860 mm) recorded in 2017 on Kouaré Station.

The selection of drought-tolerant lines was made in under field conditions based on productivity per line including phenotypic traits such as tiller number, panicle filling, grains quality, number of leaves per plant and leaf vigour. A total of 143 mutant progenies M5 were selected for screening under water deficit in controlled conditions.

Screening of M5 mutant lines under soil water deficit

The potential drought tolerant lines which exhibited different

Table 1. Frequency of induced traits observed in sorghum M5 mutant lines.

Phenotype description	Abbreviation	Number of mutant lines	Frequency (%)
Semi-erectile leaves (normal)	Nm	103	87.28
Late maturity (>90 days)	LMa	102	86.44
Single stalked	Sst	92	77.96
Multiple tillers	Mtl	26	22.03
Early maturity (<90 days)	EMa	16	13.55
Erect leaves	ErL	15	12.71
Semi-dwarf (<100 cm)	SDwf	4	3.38

Table 2. Effect of mutation on the reduction or increasing of parameters.

Parameter	Means of parameters								
	SPAD I	SPAD II	DaFl	DaMa	RWC	LS	GrWt	PaWt	Ht
ML	44.1	25.3	69	93	46.7	86.3	23.1	55	126.1
Wt	33.7	18	76	97	34.2	94	18.9	40	115.3
Gr/Re (%) P<0.0001	30.8	40.5	9.2	4.1	36.5	8.1	22.2	37.5	9.3

ML = mutant line, Wt= wild type, Ht= plant height (cm), DaFl= flowering date (days), DaMa= maturity date (days), RWC= relative water content (%), LS= leaf senescence (%), GrWt= grain weight (g) and PaWt= panicle weight (g), Gr (%): percentage of increased traits, Re (%) = percentage of reduced traits.

morphological traits of the parent were selected and confirmed by screening under water deficit in controlled conditions during the dry season of 2018. The experiment was conducted at Kamboinsé research station (143 mutant lines and one control were screened). The experimental design used was an alpha lattice square with 12 blocks and 12 genotypes per block using three replications. Each genotype was sown on row of 1.5 m length. The spacing of planting hills within single and between rows, plot fertilization and weeding were carried out as described previously. After planting, watering was performed every three days with tap water installed around the field. Sorghum seedlings were thinned at 14 days after sowing to get one plant per hill.

Water deficit stress was applied to sorghum plants by cessation of irrigation 65 days after the sowing (DAS) until harvest.

Data collection and statistical analyses

From each surviving plant, the following parameters were measured: (1) panicle weight per plant (PaWt); (2) grain weight per plant (GrWt), (3) mature plant height (Ht); (4) days to flowering (DaFl); (5) days to Maturity (DaMa); (6) the chlorophyll content 65 days after sowing (DAS) (SPAD I) and 13 days after the stress (DASt) or 78 DAS (SPAD II) using the chlorophyll meter (SPAD 502 Plus); (7) leaf senescence (LS) scored 14 DASt using a scoring scale (Sakhi et al., 2014); (8) relative leaf water content (RWC %) 7 DASt (Saddam et al., 2014). The phenotypes were grouped into tillers number, dwarf plants, early maturity, late maturity, single stalked, erectile leaves compared to the parent characters. Growth percentage/reduction percentage (Gr/Re) was calculated as:

Gr or Re (%) = ((Wt value - ML value) × 100)/Wt value.

Statistical analyses were carried out using Statistical Analysis System (SAS, 9.1, 2 Institute, Cary, NC). Analysis of variance (ANOVA) was used to determine significance based on P-value. Means were separated using Newman Keuls Multiple Range test and differences between sorghum lines traits were considered significant levels of 5% ($P < 0.05$). The correlation coefficient between traits and genotypes clustering were analysed using R x 64 3.5.2 software.

RESULTS

Induced traits in M5 mutant lines

Selection of sorghum mutants is based on phenotypes observed by comparison of putative mutants with the parent variety (ICSV1049). Upon exposition of 143 M5 lines to soil water deficit, 118 lines and the parent survived and the semi-erect leaves, late maturity and single stalked were the most frequently observed phenotypes (Table 1). Some agronomic traits such as tiller number, plant height, leaf aspect and grain maturity were affected by gamma radiation (Table 1). As expected, variation was higher in the M5 population screened under water deficit compared to control population.

ANOVA showed significant differences ($P < 0.0001$) between mutant lines (ML) and wild type (Wt) for all measured parameters. Averaged overall lines, date to flowering (DaFl), date to grain maturity (DaMa) and leaf senescence (LS) were reduced at 9.2, 4.1 and 8.1% compared to the parent, respectively. However, SPAD I, SPAD II, RWC, GrWt, PaWt and Ht were increased at 30.8, 40.5, 36.5, 22.2, 37.5 and 9.3%, respectively due to mutation effect (Table 2).

Table 3. Pearson correlation between measured traits of Sorghum M5 population screened at Kamboinsé, 2018.

Correlation	Ht	SPAD I	SPAD II	DaFl	DaMa	RWC	GrWt	PaWt	LS
Ht	1								
SPAD I	-0.15	1							
SPAD II	0.04	0.04	1						
DaFl	0.28*	-0.51**	0.17*	1					
DaMa	0.18*	-0.5**	0.18*	0.75**	1				
RWC	0.008	-0.13*	0.08	0.03	-0.002	1			
GrWt	0.04	0.31*	0.16*	-0.20*	-0.28*	0.25*	1		
PaWt	0.01	0.41*	0.21*	-0.27*	-0.32*	0.25*	0.76**	1	
LS	0.08	0.16*	0.24*	-0.09	-0.06	0.57**	0.36*	0.39*	1

Ht = Plant height (cm), SPAD I = chlorophyll content at first day of water deficit application (µmol/mg), SPAD II = chlorophyll content at 13 days after water deficit application (µmol/mg), DaFl = flowering delay (days), DaMa = physiological maturity delay (days), RWC = relative water content (%), GrW t= grains weight (g), PaWt = panicle weight (g), LS = leaf senescence (%). ** = highly significant difference at 5% threshold, * = significant difference at 1% threshold.

Table 4. Clustering of mutant lines and control according to LS.

Number of genotypes with means range (%)					
C1	C2	C3	C4	P value	CV (%)
105 (64.3-100)	6 (52.6-61.7)	5 (25.2-46.4)	3 (11.3-20)	<0.0001	11.74

CV: Coefficient of variation.

Correlations between measured parameters

Panicle weight (g) had high significance and positive correlation with grain weight (Table 3). Leaf senescence and relative water content were significantly and positively correlated (R^2=0.57%). Panicle and grain weight were also correlated with leaf senescence, SPAD I, SPAD II and RWC. The correlation between weight of panicle, weight of grains and leaf senescence is relevant to the leaf senescence effect on grain yield. However, there was a negative correlation between LS, DaFl and DaMa (Table 3).

RWC is negatively correlated to SPAD I and positively correlated with leaf senescence while plant height was positively correlated to all the characters except SPAD I which indicates that these traits do not evolve in the same direction as the SPAD I. There is a negative correlation between DaFl, MaDa and grain yield (Table 3).

Clustering and selecting of best mutant lines for tolerance to water deficit

Analysis of variance of LS showed that there was a significant difference (P < 0.0001) between the mutant lines. Therefore, genotypes were grouped with statistically identical leaf senescence values. Thus, sorghum mutants were classified into 4 clusters (Table 4). Mutant lines with average leaf senescence between 64.3 and 100% (C1) were 105 including the parent. The next cluster made up of 6 lines with average leaf senescence between 52.6 and 61.7% (C2) followed by cluster (C3) with 5 lines and an average leaf senescence around 25.2 to 46.4%. The last cluster (C4) consisting of 3 lines had an average leaf senescence around 11.3 to 20%. The lowest percentages of LS were recorded in C3 (LS average~36%) and C4 (LS average~16.38%) corresponding to the scale 4 and 2, respectively. The highest average SPAD I value was recorded with mutant lines found in cluster C3 (44.6 µmol/mg) and C1 (44.2 µmol/mg) compared to the others clusters. However, the coefficient of variation (<15%) indicates that there is low variability of chlorophyll content at the beginning of the application of soil water deficit. The highest values of SPAD II were recorded at 33.8 µmol/mg in C4 and there was high variability in chlorophyll content (SPAD II) after the application of soil water deficit (CV>15%). The highest grain and panicle weights were recorded with clusters C3 and C4 clusters and the lowest were recorded with C1 and C2. There was a significant difference (P<0.0001) between the traits within the cluster

Table 5. Means for different traits related to leaf senescence clustering.

Trait	C1			C2			C3			C4		
	Means	CV %	P-value	Means	CV %	P-value	Means	CV %	P-value	Means	CV %	P-value
Ht (cm)	126.6	10.8	<0.0001	137	10.8	0.29	115.5	9.7	0.02	104.2	17.5	0.54
SPAD I (µmol/mg)	44.2	13.5	<0.0001	40.6	12.2	0.13	44.6	14.4	0.04	42.1	15	0.39
SPAD II (µmol/mg)	24.7	26.3	<0.0001	29.8	20.3	0.02	26.4	31.2	0.16	33.8	19.5	0.26
DaFl (days)	69	7	<0.0001	71	6.2	0.28	66	7.7	0.18	70	8.3	0.84
DaMa (days)	93	2.4	<0.0001	94	2.9	0.83	95	4.4	0.06	94	5.5	0.95
RWC (%)	43.5	23.5	<0.0001	62.8	18.4	0.26	69.3	13.1	0.19	76.2	7.5	0.01
GrWt (g)	21.6	32.9	<0.0001	28.2	16.8	0.03	35.8	12.9	0.01	38.4	10.4	0.2
PaWt (g)	50.2	34.5	<0.0001	68.1	25.7	0.006	104.6	19.1	0.001	97.06	23.8	0.45

Ht= Plant height, SPAD I and SPAD II, chlorophyll content at initial day of water deficit application and 13 days after water deficit application, DaFl= flowering delay, DaMa= physiological maturity delay, RWC= relative water content, GrWt= grains weight, PaWt= panicle weight, LS= leaf senescence, CV = coefficient of variation.

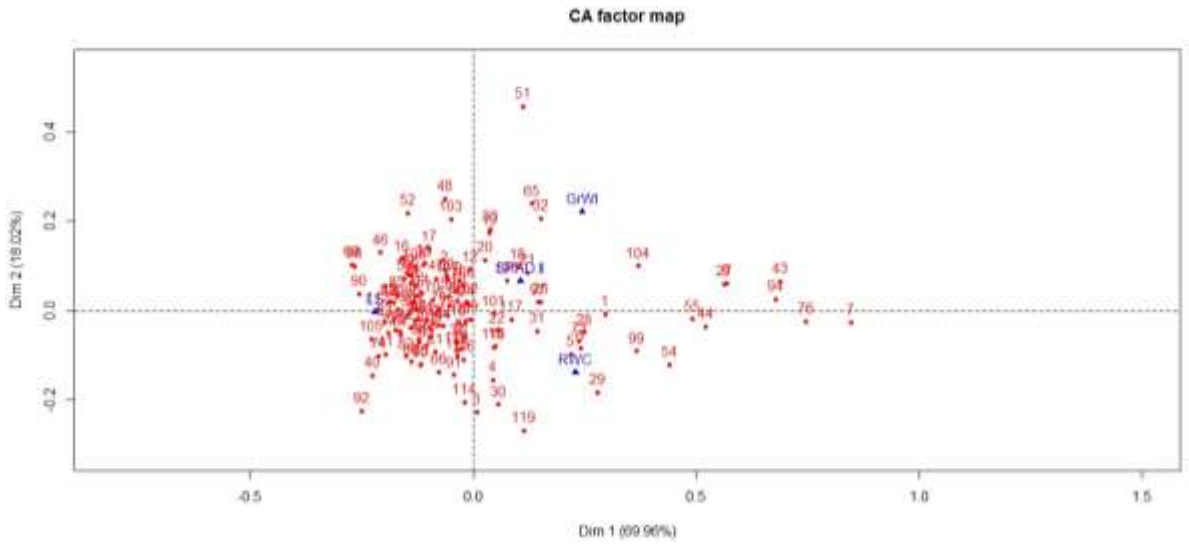

Figure 1. Detection of mutant lines with water deficit tolerance traits. The numbers represent the mutant lines.

C1. But no significant difference was observed within the other clusters (P>0.05) except C3 where PaWt exhibited a significant statistical difference (P<0.05) (Table 5). Tables 4 and 5 indicated that sorghum tolerant mutants to water deficit could be selected inside C3 and C4.

According to LS, RWC, SPADII and GrWt which are the best parameters of tolerance to water deficit, seven promising water deficit tolerant mutants were selected. This selecting was based on the mutants with high values of RWC, SPAD II, GrWt and low values of LS (Figure 1). Based on the analysis outputs, the best performing mutants under water deficit conditions were ICM5_6, ICM5_104, ICM5_76, ICM5_3, ICM5_30, ICM5_15 and ICM5_105. They are distinguished from other mutants and parent by high relative water content (between 61 - 83%), high SPAD II (21 – 37 µmol/mg) and GrWt (28-54 g) with the lowest LS (10.6-39.9%) while RWC, SPAD II, GrWt and LS of the parent were 34%, 18 µmol/mg, 18.9 g and 94%, respectively (Figure 2).

DISCUSSION

Mutation induction is a powerful tool that plant breeders use to create genetic variability. That variability can be exploited to select desired traits. Mutagens can affect all parts of the plants by either decreasing plants height or increasing it relative to the parent. Their effect can shorten or extend the plant cycle. In plants exposed to mutagens, morphological abnormalities and reduced

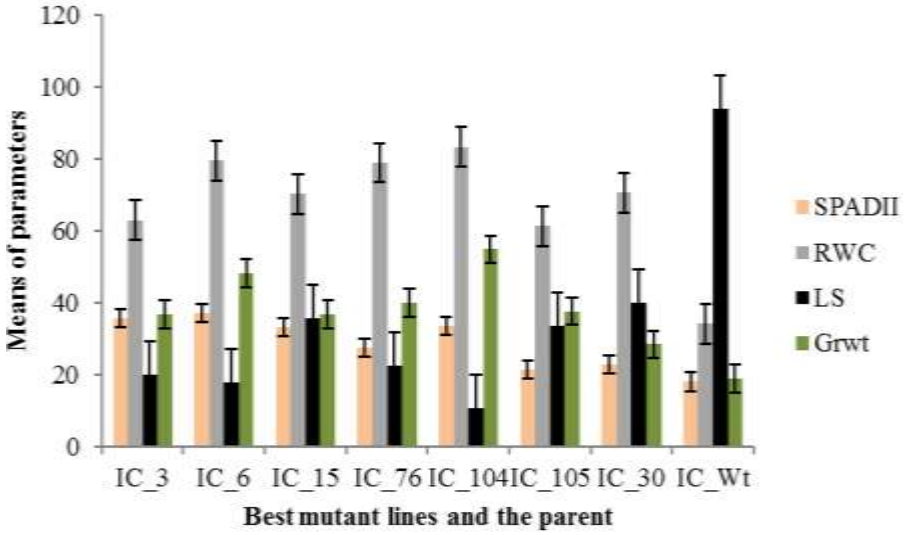

Figure 2. Water deficit tolerant mutant lines.

growth have been observed and attributed to oxidative stress (Singh, 2003) or deleterious mutational effects (Valluru et al., 2019). The mutagenesis affected some agronomic traits in millet such as reduction in plant height compared to the control (Ambli and Mullainathan, 2014). The results of this study showed that the overall height of plant mutant and grain yield were higher than that of the parent. These results support findings by previous studies (Burow et al., 2014) in which mutation increased sorghum plant height and grain yield. However, reduction in plant height was observed in mutagenized rice (Talebi et al., 2012), rapeseed and mustard (Javed et al., 2003). The present results together with those cited confirm that induced mutation using gamma rays can play an important role in the genetic variability induction within plant architecture.

The positive correlation between weights of panicle and grains to SPAD I, SPAD II and RWC suggests that these traits can be simultaneously selected and may be used as selection criteria for tolerance to soil water deficit. Negative correlation between RWC and SPAD I suggest that it would be difficult to select drought tolerant plants at the beginning of water deficit application based on these two parameters. The positive correlation between RWC and LS indicates that mutants which accumulate sufficient water in their leaves are those which have a slower leaf age. Therefore, genetic improvement of RWC also implies genetic improvement of LS. RWC is a useful trait for plants to mitigate the effects of drought at the reproductive stage. Negative correlation between delay flowering, delay maturity and grain yield implies that early mutants have no significant difference in yield compared to late mutants. These results disagree with those obtained by previous studies (Menezes et al., 2015) reporting that there is a positive correlation between productivity and maturity in sorghum grain under water deficit. Water deficit tolerance is the capacity of plants to support water deficit while keeping suitable physiological activities to safeguard cellular and metabolic integrity at tissue and cellular level (Xiong et al., 2006). Plant leaf senescence is considered as a post-flowering drought stress symptom (Burke et al., 2013). Green plants such as sorghum have two options for maintaining a high tissue water status during periods of soil moisture deficit, either by decreasing water loss due to transpiration or by increasing water uptake (Devnarain et al., 2016). Leaf senescence reduces seriously the source-sink translocation from leaves to grain (Krupa et al., 2017). In the present study, drought scoring based on LS was significantly higher for the wild type accession. Based on some reports (Ji et al., 2010) the results of this study on PaWt and GrWt revealed that soil water deficit affects grain number and weight. The decrease in quantitative traits such as yield of some mutant lines may be attributed to the physiological disruption or chromosomal deterioration caused to plant cells by the mutagen gamma ray (Thilagavathi and Mullainathan, 2011). Relative water content designates the metabolic activity in tissues and used as the most meaningful index for dehydration tolerance. RWC ranged between 85 and 95% and a critical reduction of less than 50% could cause tissue death (Vinodhana and Ganesamurthy, 2010). From the results of this study, some sorghum mutant lines were able to maintain RWC above 60% for 14 days during soil water deficit. Previous studies showed that maintenance of a relatively high RWC during mild drought is indicative of drought tolerance (Colom and Vazzana, 2003).

Sorbitol treatments to simulate drought-induced osmotic stress in sorghum cell suspension cultures showed that sorbitol raised an overall increase in secretion of 92 proteins that were differentially expressed in response to sorbitol-induced osmotic stress (Ngara et al., 2018). So, additional molecular studies on developed sorghum mutant lines would allow identifying protein or genes of interest via biotechnological or marker assisted breeding strategies with the prospect to combine them in one line for more performance.

Conclusion

Late drought is the most limiting factor in sorghum production. The results showed that induced mutation is a suitable tool to create genetic variability for selecting drought tolerant mutants. Further evaluation of those mutants to confirm their tolerance and stability under water deficit conditions would be useful. So, multi-local tests on other experimental sites will be conducted in the coming years to evaluate the agronomic performance of the best lines, taking into account genotype-environment interaction. In addition to leaf senescence and relative water content already recommended in phenotyping for drought-tolerance, the chlorophyll content 13 days after water stress application should also be considered as phenotypic trait in similar studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 12(3), pp. 200-218, July-September 2020
DOI: 10.5897/JPBCS2020.0893
Article Number: D398E1864434
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

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Journal of Plant Breeding and Crop Science

Full Length Research Paper

Assessing the effectiveness of nonwoven fabric pollination tents for improved grass breeding

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Received 29 April, 2020; Accepted 2 July, 2020

The practices of using isolation and distance in the seed production of open pollinated crops are fundamental concepts to ensure seed purity. We uniquely examined the effectiveness of replacing isolation plots for seed production and grass breeding with different sizes of novel nonwoven synthetic fabric pollination control tents (PCTs). Two fabrics, DWB10 and DWB24, were used along with multiple genotypes of tall fescue at Ardmore, Burneyville and Gene Autry locations in Oklahoma, USA during 2018 and 2019. Treatment effects were consistently significant in both years, but location differences were more pronounced in 2019. Interactions of treatments with locations or genotypes were not predominant. The two tent fabrics, generally, performed equally well for various traits in both years. Tent performance for both fabrics was particularly superior over control for various traits in 2019 (e.g., DWB10 tent showed a 36% increase for seed yield (SY) over the control). Introduction of fans in tents for increasing pollen flow in 2019 was not advantageous as it reduced the SY by 23%. The average temperature within tents was higher with lower average humidity than the control producing a microclimate for good yield and disease free seeds. The final germination (%) of seeds from tents and controls at 21 days was high and not much different with a minimum overall germination of 89% at Burneyville in 2018. There was no evidence of pollen contamination from tetraploid ryegrass pollen in any of the tent fabrics. Bad weather in 2018 affected the sturdiness of tents, but modifications in 2019 corrected all such mishaps. Further improvements in the structures, design and cover have since been made for field exploitation of technology in grass improvement and seed multiplication.

Key words: Ryegrass, fescue grass, pollination control tents, nonwoven fabrics.

INTRODUCTION

The *Festuca* (fescue) genus (2n=6x=42) is closely related to diploid (2n=14) ryegrass (*Lolium*) with plant taxonomists having moved several species from the genus *Festuca*, including the grasses tall fescue and meadow fescue, to the genus *Lolium*. The wide range of uses for fescues and ryegrasses vary from ornamental and turf to highly nutritious pasture for haying and grazing livestock (Darbyshire and Pavlick, 2012). These grasses

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can also be used in soil erosion control programs. There are a large range of grass cultivars derived from these genera leading to the production of substantial amounts of certified seed annually.

The majority of grasses are cross-pollinated by wind and are largely self-incompatible thus preventing their self-pollination. Individual plants in populations are highly heterozygous being hybrids of hypothetical parents. However, natural populations have substantial additive genetic variation for selection to be effective for most agronomic traits (Vogel et al., 1989). Individual plant selections, usually in thickly seeded stands or swards as forages or turf grasses, can be impractical and as such, evaluation and selection are often practiced in space-planted nurseries or small sward plots. The most effective breeding programs in forage grasses limit hand emasculation or crossing, instead utilizing recurrent selection improvement systems, which have the added benefit of retaining genetic variation in the populations. This system is continued with the random mating of selected individuals in isolated polycross nurseries to produce progenies for the next cycle of selection (Brown et al., 2014). The selected polycross progeny are then used in the development of synthetic populations. In the UK, hand emasculated pair-wise crosses between two species *Lolium multiflorum* and *Lolium perenne* are being used to make synthetic varieties from the interspecific hybrid, *Lolium boucheanum*. Grass breeders are now more interested in these types of hybrids, exploiting the higher heterosis of F1 hybrid varieties between two or more compatible interspecific combinations vs. more narrow based synthetic varieties. This approach, though more productive, requires modifications in the ways interspecific varieties are composed and subsequently multiplied.

Wind pollination is related to the size of the grass pollen being distributed since neither very large nor very small grains are wind pollinated. Grass pollen is divided into two types based on grain size; wild grass types range from 25 to 35 µ (with exceptions of 35 or 40 microns) and the cultivated grasses range from 35 to 50 µ with the modal peak at 40 µ (Erdtman, 1943). According to Wodehouse (1935), *Lolium* pollen grain size ranges from 22 to 33 µ. Geisler (1945) reported a range of 24-39 µ with modal peak of 31 µ for a group of six grass species including *Poa* and *Festuca*.

Pollination bags, isolation plots, isolation chambers and pollination control tents (PCTs) are some of the methods used in the controlled crossing of grasses. Pollination bags are only useful on a limited scale. Isolation chambers are expensive to build and run in order to maintain a controlled microclimate, and isolation plots demand very long distances between plots of the same species (305 m for Breeder Seed) thus limiting the number of entries to be multiplied. However, research on the effectiveness of PCTs is limited. This study addresses this gap by evaluating novel PCT technologies in a grass breeding program using nonwoven, re-usable synthetic tent fabrics.

The aim of this study is to examine the possibility of substituting the use of isolation and distance in small field crossing or seed production nurseries with new PCTs and extending our knowledge about the microclimate within such structures in order to obtain a healthy and high seed set while at the same time providing pollen proofing. Consequently, it lays the foundation for new research in plant breeding, investigating novel options that are potent enough to increase the efficiency of breeding operations in all crops by enabling many crosses to be made simultaneously or by increasing the number of seed multiplications of promising populations. The major objectives of the study were: (1) evaluating PCT structures for robustness, durability and strength of cover fabric materials under field conditions, (2) testing the pollen proofing ability of fabric materials, (3) comparing the microclimate within PCTs with outside control conditions, and (4) assessing the comparative seed output of healthy seeds and plant performance for biological traits.

MATERIALS AND METHODS

Experimental sites

Three environmental sites, located in southern Oklahoma, USA on Noble Research Institute, LLC farms, which varied in humidity, temperature, windiness, minor elevation difference and soil type were chosen for PCT testing by placing one set of PCTs at each site. The first site was in Ardmore on the research park farm (34.10° N, 97.10° W; elevation 266 m) on Heiden clay (fine, montmorillonitic, thermic Udic Chromusterts). The second site was in Gene Autry on the Dupy farm (34.17°N, 96.58° W; elevation 223 m) on Dale silt loam (fine-silty, mixed, superactive, thermic Pachic Haplustoll). The final site was located in Burneyville on the Red River farm (33.53°N, 97.15° W; elevation 221 m) on Eufaula loamy sand (siliceous, thermic Psammentic Paleustalfs). The distance of sites ranges from 5 to 56 km from the Noble Research Institute's main campus.

PCT types

DWB010 and DWB24 pollination tents with different fabric materials were used in the present study and were obtained from PBS International, UK. These materials were used in order to allow better air permeability, as they are more open compared to the regular control counterpart Duraweb® (Hayes and Virk, 2016). However, their architecture and fibre shape hinders pollen grain transmission by creating a more difficult passage through the fabric. The following are the major features of the fabrics.

DWB10

Nonwoven spun-bound polyester; thickness (mm) 0.33; mass per unit area/weight (gm^{-2}) 100; air permeability ($l/m^2/s$) 550; light transmission (% 350 - 800 nm wavelength) 35.5; maximum pore size (microns) 152; fibre cross section is simple. It has waxier surface than DWB24.

Figure 1. Large pollination control tent (PCT) set up (left) 2019 and Research Associate Dusty Pittman setting the frame and doing some final weeding and plant care before the cover is placed over the PVC frame of small tent in 2019. A soaker hose was placed inside the tent frame for watering the plants without disturbing the cover during pollination process.

DWB24

Nonwoven spun-bound polyester; thickness (mm) 0.40; mass per unit area/weight (gm^{-2}) 110; air permeability ($l/m^2/s$) 1470; light transmission (% 350 - 800 nm wavelength) 39; maximum pore size (microns) 214; fibre cross section is complex.
The following types of PCTs were used:

(a) Small PCTs tested in 2018 and 2019
- Three small PCTs, DWB10, size 1.5 m × 3 m × 2 m
- Three small PCTs, DWB24, size 1.5 m × 3 m × 2 m)

(b) Large PCTs tested in 2019
- One large PCT, DWB10, size 6 m × 6 m × 2 m
- One large PCT, DWB24, size 3 m × 12 m × 2 m).

Design

The frames of PCTs were made of PVC piping and were secured to the ground by placing two sand bags (22.6 kg) on each side on the bottom pipe of the frame. The structures were rigid once assembled. Additional dirt was placed around all sides of the PCT when it rained because the dirt tended to settle or was washed away, exposing the skirt edges. Soaker hoses were supplied to each tent for supplementary irrigation, if needed. Covers of both types of fabric fitted snuggly on the appropriate frames (Figure 1).

Smaller PCTs used in 2018 were stored for re-use in 2019. Both types of fabric were washed using a solution of Clorox® bleach (10%) and distilled water before re-use to clean and remove any contaminants. Duct tape was used on any fabric seams of the PCTs (both fabrics) to make minor repairs.

Small PCTs

During 2018 and 2019, two smaller PCTs, DWB10 and DWB24 were placed at each of three sites. Within each PCT, 15 tall fescue plants (*Lolium arundinaceum* (Scherb.) Darbyshire) representing three genotypes and cloned five times each were transplanted and grown.

A control isolation group (open pollinated) was planted at each location containing 15 tall fescue plants (same 3 genotypes used in tents and cloned 5 times each = 15 plants) at a minimum of 305 m away, which is the minimum distance between breeder or foundation seed increase of tall fescue as recommended by the Seed Certification Service in Oregon, USA (Oregon Seed Certification Service Handbook, 2018).

In the 2019 trial, an additional control was added at each location. This 'open control' of 15 tall fescue plants (open pollinated) was located at least 305 m from the PCTs and the other control group of tall fescue plants.

Perennial ryegrass plants (*Lolium perenne* L.; 2n=4x=28) were planted around each PCT in 2018 (4 per side) to act as "pollen donors" for testing contamination in the PCTs, if any. Both species, inside and outside the PCTs, are out crossing and can hybridize (that is, *Festulolium*) allowing for detection of any chromosomal recombination between the tetraploid ryegrass and the hexaploid tall fescue resulting from pollen contamination. It was determined that approximately 15 plants around the PCT would generate enough pollen pressure with particular concentration to the southwest direction due to prevailing winds. All plants were grown and vernalized to induce flowering in early summer.

In 2018, PCTs were set up on 18th May at Ardmore and Gene Autry, and on 21st May at Burneyville. All plants were at the $E_3 – R_0$ growth stage (Moore et al., 1991) when transplanted in the field on 9-11th April in 2018 and the 22nd April 2019 (Figure 1). All tents at all locations were removed on 25th June 2018. In 2019, small PCTs were erected on 4th June and taken down on 5th July at all three locations.

Increasing pollen flow in small PCTs in 2019

Overall, seed yields in 2018 were low across all treatments and especially inside the PCTs. It was thought that airflow within PCTs may be restricted compared with the natural environment. It was hypothesized that increasing the air circulation and hence the mobility of pollen within the PCT would aid cross-pollination and improve seed yield. Therefore, portable electric fans powered by solar panels were placed in all treatments, that is, small PCTs, control, and open control groups at Ardmore and Gene Autry locations in 2019 experiments. Fans were also placed in the middle of the open control at two locations (Table 1). However, no fans were placed in any treatment at the Burneyville site. Fans were easy to setup and there was no issue with them or the solar panels in the PCTs or the field (control or open plants). The fan effect was estimated through an analysis of variance in which locations are confounded with blocks.

Larger PCTs 2019

One PCT was placed at each location. The 6 m × 6 m PCT (DWB10)

Table 1. Randomized plan of treatments with fan and no fan provisions for small tents at three sites in Oklahoma, USA.

Ardmore	Gene Autry	Burneyville
Tent DWB10, Fan	Open, Fan	Tent DWB24, no fan
Tent DWB24, Fan	Tent DWB10, Fan	Control, no fan
Control, Fan	Control, Fan	DWB10, no fan
Open, Fan	Tent DWB24, Fan	Open, no fan

Figure 2. An Onset HOBO data logger inside of a pollen control tent.

was placed at the Unit 3 Farm (34.11° N, 97.05° W; elevation 248 m) on a Windthorst fine sandy loam (fine, mixed, active, thermic Udic Paleustalf) and the 3 m × 12 m tent (DWB24) was erected at the Headquarters Farm (34.08° N, 97.12° W; elevation 252 m) on a Heiden clay (fine, montmorillonitic, thermic Udic Chromusterts). Both of these sites were located in Ardmore. Each tent contained 40 tall fescue plants (4 genotypes cloned 10 times each = 40). A control isolation (open pollinated) was planted at each location containing 40 tall fescue plants (same 4 genotypes used in the PCTs cloned 10 times each = 40) at a minimum of 305 m away. As in the small PCTs, all plants were grown and vernalized to stimulate flowering in early summer. PCTs were set up on 5th June at both locations. Both the inside and outside plants were harvested on 5th July and tents taken down on 6th July 2019.

Microclimate assessment

A HOBO MX temperature and relative humidity data logger (Onset Computer Corporation, Inc.) was placed in each PCT by suspending them from the roof (Figure 2). These data loggers are battery powered and record temperature, relative humidity and the dew point. Since the loggers have Bluetooth connection, we were able to collect data without disturbing the environment in the PCT.

Weather data were also collected from weather stations (Mesonet.org) located on the Noble Research Institute farms for comparison for the duration of the trial period. The same parameters were collected, along with the average maximum wind speed and maximum wind gusts, at each site.

Data collection

Biological traits

(1) After pollination and seed set, data on a number of biological traits were collected: plant height (cm) = PH and growth habit (1 = decumbent, 2 = semi-erect, 9 = fully erect scale) = GH. A visual disease score (1 to 5; 0 = no disease, 5 = death due to disease) = DS was also given to each plant.
(2) The seed related data were collected on: seed yield per plant (g) = SY, 1000-seed weight (mg) = SW, the presence of ergot (%), a visual seed quality score (1 to 5; 1= excellent, 5 = extremely poor) = SQ and germination rates on 7, 14 and 21 on the basis of 100 seeds from a sub-set of plants from each tent.
(3) The harvested seeds from all of the plants individually inside the PCTs were collected and assessed for various traits (Table 2).

Germination rate (%)

For germination rate 100 seeds per clone were sown for each genotype in treatments. The germinated seeds were counted on 7, 14 and 21 days after sowing to record percent germination.

Pollen contamination

For 2018, the measurement of contamination from ryegrass pollen from outside the PCTs on to the tall fescue plants was assessed by

Table 2. Summary of experimental details of pollination control tent (PCT) trials conducted on tall fescue in 2018 and 2019.

Tent size	Description	2018	2019
Small	Locations	3= Ardmore, Burneyville, Gene Autry	3= Ardmore, Burneyville, Gene Autry
	Treatments	3= DWB10, DWB24, Control	4=DWB10, DWB24, Control, Open
	Genotypes	3 = Geno 1, 2, 3	3= Geno 1, 2, 3
	Biological traits	PH, GH, SY, SW, DS, Ergot, SQ	PH, GH, SY, SW, DS, Ergot, SQ
	Germination (%)	7, 14, 21 days count	7, 14, 21 days count
	Fan effect	-	Fan in Burneyville but in other two locations Traits and germination count recorded
Large	Locations	-	2= HQ Farm, Unit 3 Farm
	Treatments	-	3= DWB10, DWB24 and control
	Genotype	-	4 with 10 replicate plants in each
	Biological traits	-	As in 2018
	Germination (%)	-	As in 2018

PH= plant height (cm), GH= Growth habit (1-9 scale), SY= seed yield per plant (g), SW= 1000-seed weight (mg), DS = Disease Score (1 to 5 scale), Ergot (%), and SQ = Seed Quality Score.

Figure 3. Photograph of the type of wax-coated paper bags that were used to test for pollen contamination in the field compared to the DWB10 and DWB24 bags provided.

looking at the possibility of hybridization of the perennial ryegrass and tall fescue. The hybrids are generally sterile and though fertility can be restored by chromosome doubling, such plants are unstable and experience chromosome loss (Scott and White, 1988). We examined some chromosomal pairing in the hybrids of the ryegrass and tall fescue.

To measure any contamination from outside pollen at the Research Park farm at Ardmore in the 2019 small PCT trials, an existing tall fescue population was chosen to measure for any pollen contamination due to the fabric (different nursery than the control and open nurseries at this site). Sixty plants were bagged (20 with DWB10 bags, 20 with DWB24 bags and 20 with wax coated paper bags). The type of wax-coated paper crossing bags (Lawson Bag Co.) that were used is represented in Figure 3. Plants were bagged between June 1 and 5th. The bags were sealed with weather proof tape and wooden stakes were used to support the tiller and bag in the field during the test period. The remaining heads in the plot were allowed to cross-pollinate. Bags were removed during the July 5-8 time-period. At this time, the bagged panicle was harvested. To determine if any seed were produced, panicles were later conditioned on a rubbing board. A total of four of the wax-coated bags were lost due to the weather. None of the

Table 3. Pollen proofing evaluation using small bags for individual panicles, 2019.

Bag type	Bags with seeds detected	Bags with no seeds detected	Bags lost	Total
DWB 10	0	20	0	20
DWB 24	0	20	0	20
Paper	1	15	4	20

other bag types was lost.

Statistical analysis of biological traits recorded on individual plants and germination percent per clone was performed following analysis of variance technique described by Sokal and Rahlf (2011). Fisher's Least Significant Difference (LSD) was used for pair-wise comparison of treatment means and significantly different means were labelled with different letters.

RESULTS

The first year with the PCTs (2018) was more of a learning experience as to how to assemble the PCTs, how they withstand the weather and how to take corrective measures. For instance, at Gene Autry (Dupy farm) on the evening of 31st May, covers of both small PCTs were blown about 200 to 300 m from their original location during a thunderstorm, which produced a wind gust of 80.14 km/h as recorded by the farm weather station located approximately 750 m from the PCTs. However, the frames remained intact. The plants inside were pollinating at the time of the failure, but we placed the tents back on their frames and continued the experiment. No damage was observed to the fabric of PCT DWB24, but we had one small tear along the seam of the DWB10 PCT along the roof, which was repaired with duct tape. Thus, the need to improve anchoring was noted.

During 2019, there was no failure from wind at any location. However, the fabric covers, having been re-used, were starting to show wear. There was no animal damage in any year at any location observed to the fabric and no fan failures occurred in 2019. The experiments in 2019 are thus more reliable for conclusions and we would lay more emphasis on these results.

Pollen proofing

During 2018, the measure of any contamination from outside pollen in the PCTs was looked at through the possibility of hybridization of the ryegrass and tall fescue. We had grown tall fescue plants inside PCTs that were surrounded by ryegrass plants. Since we do not have SSR markers to measure the rate of contamination in the progeny due to outside pollen entry into the PCT, we tried to look at some chromosomal pairing due to hybridization of the ryegrass and fescue. There was no evidence of any contamination in the progeny. However, we believe that this tedious method was not very reliable. In retrospect, fabric material in the form of small pollination bags to measure any selfing by bagging individual open pollinated plants would probably have been more reliable. This technique was selected for examining pollen contamination in the following year. Therefore, in 2019 we bagged reproductive panicles in an open pollinated plot at the Ardmore location. No seeds were produced by panicles covered by DWB10 or DWB24 bags. Of the 20 wax-coated paper bags used for comparison, 16 remained intact in the field, with only one bagged panicle producing a viable seed (Table 3). Results of the pollen study across both years showed that both the DWB10 and DWB24 tents were safe from contamination of foreign pollen from other grasses.

Microclimate within PCTs vs. weather data

In general, year 2019 was better for performance of grasses than 2018. Overall, mean seed yield per plant was higher in 2019 than in 2018. It was 15.2 g in 2019 against 11.2 g in 2018 (36% increase) at Ardmore; 20.3 g in 2019 against 11.3 g in 2018 (79% increase) at Burneyville and 19.7 g in 2019 against 10.5 g in 2018 (87% increase) at Gene Autry.

Climate factors within the PCTs along with weather data collected from the adjacent weather stations for the open pollinated controls are listed in Tables 4 and 5 for smaller PCTs in 2018 and 2019, and for larger PCTs in 2019. In 2018, the average minimum temperature inside the PCTs was higher by 2 to 8 degrees than the control at various locations, but in 2019, the outside temperature was higher at Ardmore by about 2 degrees than inside the smaller PCTs (Table 4). The average maximum temperatures were higher in both large and small PCTs compared to the controls at all sites in both years by 6 to 23°. The overall average temperatures were either equal at Burneyville in 2019 or higher in all other cases than the controls by up to 9°. It was suspected a malfunctioning data logger might have recorded some erroneous values at Burneyville in 2019. We can conclude that, in general, the temperatures within smaller PCTs had a wider range from slightly lower to slightly higher temperatures compared to control (Table 4). However, the larger PCTs in 2019 showed higher minimum temperatures by 2 to 3° at both sites (Table 5).

Table 4. Climatic data collected within the small pollination control tents (PCTs) by Onset HOBO data loggers and by local weather stations (Mesonet.org) for the open pollinated controls at each test site from May 18 to June 25 during 2018 and from June 5 to July 5 during 2019. Fans were added in tents at Ardmore and Gene Autry sites in 2019.

Year	Measure	Ardmore			Burneyville			Gene Autry		
		DWB10	DWB24	Control	DWB10	DWB24	Control	DWB10	DWB24	Control
					Temperature (°C)					
2018	Min	15	14	21	14	14	22	13	13	15
	Max	48	48	33	52	52	34	50	50	37
	Av	30	30	26	31	31	28	30	30	27
	Range	33	34	12	38	38	12	37	37	22
2019	Min	15	15	13	23	14	21	15	14	18
	Max	52	49	34	40	53	34	40	53	30
	Av	33	32	25	27	33	27	27	33	24
	Range	37	34	21	17	39	13	25	39	12
					Relative Humidity (%)					
2018	Min	17	17	49	15	12	47	16	16	29
	Max	100	100	89	100	100	88	100	100	73
	Av	72	70	71	62	61	66	71	70	69
	Range	83	83	40	85	88	41	84	84	44
2019	Min	20	6	36	23	19	39	24	26	37
	Max	100	100	100	100	100	100	100	100	100
	Av	49	53	76	53	50	72	57	54	80
	Range	80	94	64	77	81	61	76	74	63
					Wind (km/h)					
2018	Max gust			64			84			80
	Av Max			35			41			37
	Direction			SW			SSW			SW
2019	Max gust			72			70			99
	Av Max			37			35			28
	Direction			SSE			SSW			SSE
					Rainfall (mm)					
2018				49			23			32
2019				161			33			152

The maximum temperatures in larger PCTs were higher by up to 15° and the average temperature by up to 6° (Table 5).

Average minimum relative humidity values were lower in the PCTs compared to the outside controls at all three locations in 2019, but were higher at the Burneyville site in 2018. Maximum relative humidity was higher in PCTs than controls in 2018, but was consistently equal in 2019. Overall, averages for relative humidity values were variable compared to the outside controls at all three locations in two years; in 2019, they were lower in the PCTs compared to the control at all sites, but in 2018 they were lower at the Burneyville site only. The other sites showed similar results. In summary, the pattern appears that the range of temperature and relative humidity is greater inside the PCTs than outside (higher highs, lower lows), with smaller structures seeing slightly greater temperature and humidity range when compared to the larger PCTs.

There is some evidence to suggest that the PCTs made from DWB24 may record higher maximum temperatures than PCTs made from DWB10 despite being more air permeable; this may result from the more open structure, increasing light penetration in the longer wavelengths. The DWB24 also seems to have a greater range of relative humidity measurements through the day than the DWB10.

The range of average maximum gust of wind was not

Table 5. Climatic data collected within the large pollination control tents (PCTs) by Onset HOBO data loggers and by local weather station (Mesonet.org) for the open pollinated controls at both farms sites at Ardmore from June 5 to July 5 during 2019.

Measure	Parameter	Ardmore HQ farm DWB10	Ardmore Unit 3 farm DWB24	Control
Temperature (°C)	Min	16	15	13
	Max	47	49	34
	Av	30	31	25
	Range	31	34	21
Relative humidity (%)	Min	24	26	36
	Max	100	100	99
	Av	74	75	76
	Range	76	74	63
Wind (km/h)	Max gust	-	-	72
	Av gust	-	-	37
	Direction	-	-	SSE
Rainfall (mm)	Total (mm)	-	-	161

very different during the two year study ranging from 35 to 41 km/h in 2018 compared with 28 to 37 km/h in 2019 (Table 4). However, the maximum gust in 2019 was higher (up to 99 km/h) compared with 2018 (84 km/h). The direction of wind was generally SW in 2018 but SE at Ardmore and Gene Autry in 2019 (Table 4).

The average rainfall was low for the study period at all locations in 2018 with a minimum of 23 mm at Burneyville (Table 4). While it was low (33 mm) at Burneyville again in 2019 (Table 4), it was relatively higher at Ardmore (161 mm) and Gene Autry (152 mm).

Small PCTs-quantitative traits

Analysis of variance

Analysis of variance for 2018 and 2019 showed consistently significant differences among treatments for PH, SY and SW and among genotypes for GH only (Tables 6 and 7). The location effect was more pronounced in 2019 being significant for all traits, but only for GH in 2018. A significant interaction in 2018 was observed between treatments and genotypes (Table 6 and Figure 4) for seed yield, which arose from the reduced yield of genotype 1 in the DWB24 tent than in PCT DWB10. The other two genotypes did not change rank for seed yield between the two PCTs (Figure 4).

Interactions in 2019 were more pronounced for GH and SW in respect of locations vs. genotypes and treatments vs. genotypes, which are summarized in Figures 5 and 6. PCTs DWB24 and DWB10 showed cross over interaction at the Burneyville and Gene Autry locations for GH with higher values recorded at Gene Autry than at Burneyville (Figure 5). The location × genotypes interaction for GH was more pronounced for Genotype 2 at Ardmore than the other locations (Figure 5). Genotype 3 interacted significantly with PCTs due to its higher performance in PCT DWB10 and lower performance in the open treatment (Figure 5). All interactions for SW were similar to GH (Figure 6).

However, contributions of interactions SS to the total SS were very small which ranged from 0.4 to 11.4% in 2018 and from 1.1 to 12.5% in 2019 (Tables 6 and 7). On the other hand, the treatment SS was a significant contributor to the total SS in both years for most of the traits except for GH.

Mean performance

Fitted mean values for traits with significant differences were compared using Fisher's *t*-test in pairwise ways. Location means showed the highest GH score at Ardmore in both years compared to other locations, which were similar (Tables 8 and 9).

A significant location effect was also observed for PH, SY and SW in 2019 (Table 9). The mean plant height was significantly lower, but significantly higher for SW at Ardmore compared to the other two locations, which were similar. Significant mean SY differences for locations were in the order Burneyville > Gene Autry > Ardmore (Table 9). The highest mean SY at Burneyville in 2019 was accompanied with higher PH and average GH and

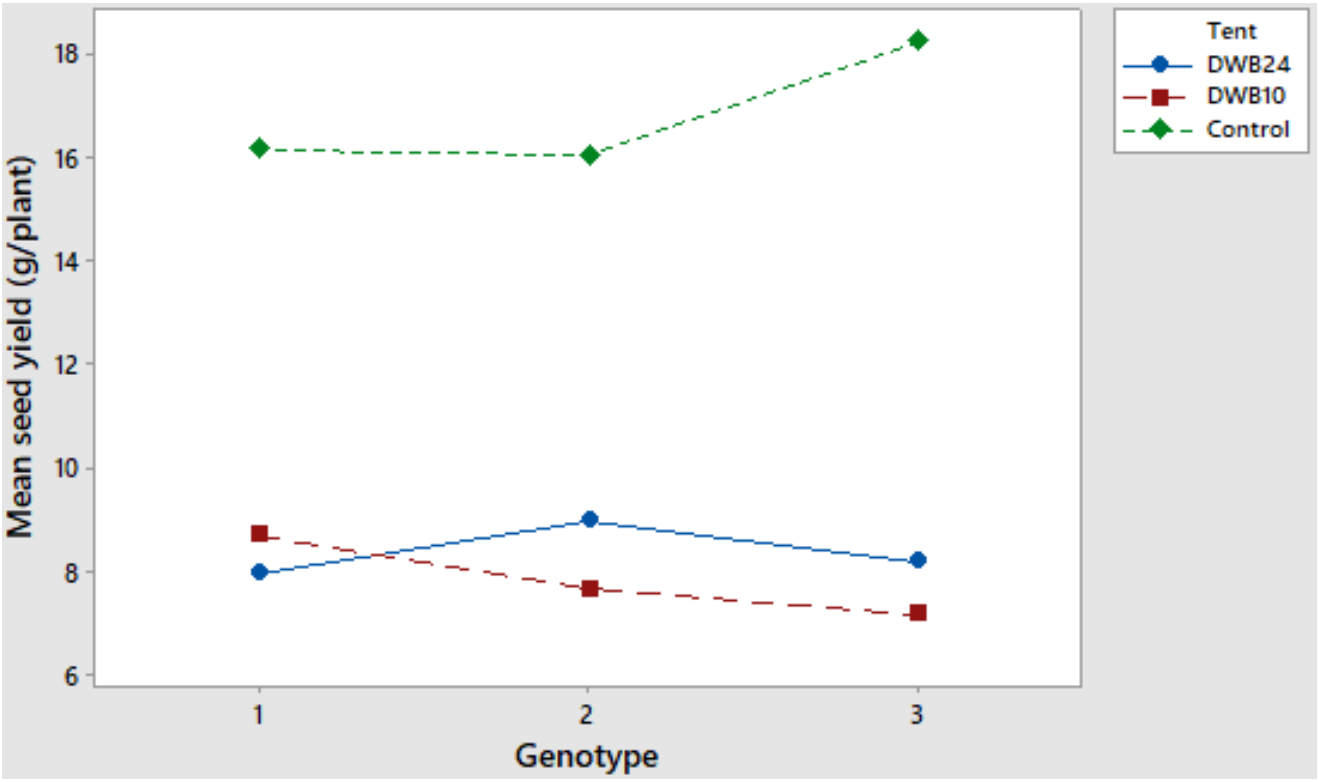

Figure 4. Interaction plot of genotypes vs. treatments (tents) for seed yield per plant (g) in small pollination control tent (PCT) trials in 2018.

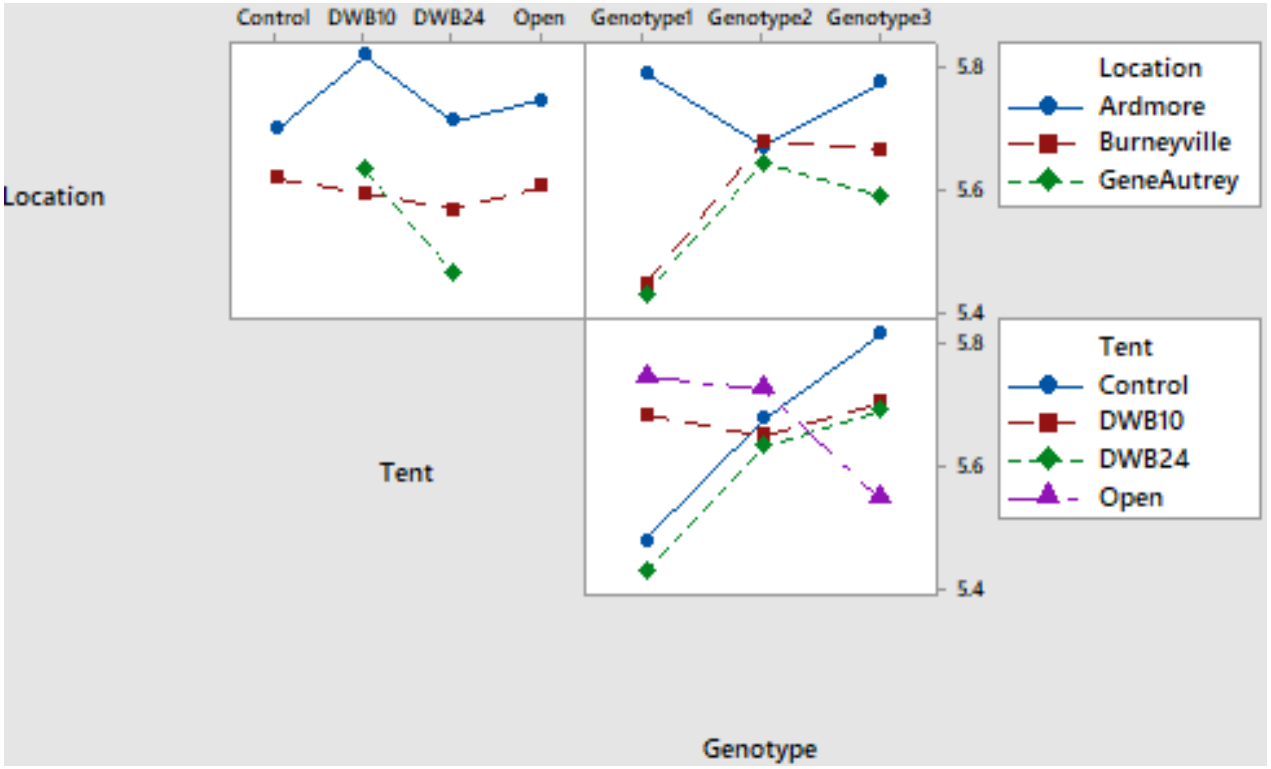

Figure 5. Plot for locations vs. PCT types and PCT type vs. genotypes for growth habit in 2019 trials.

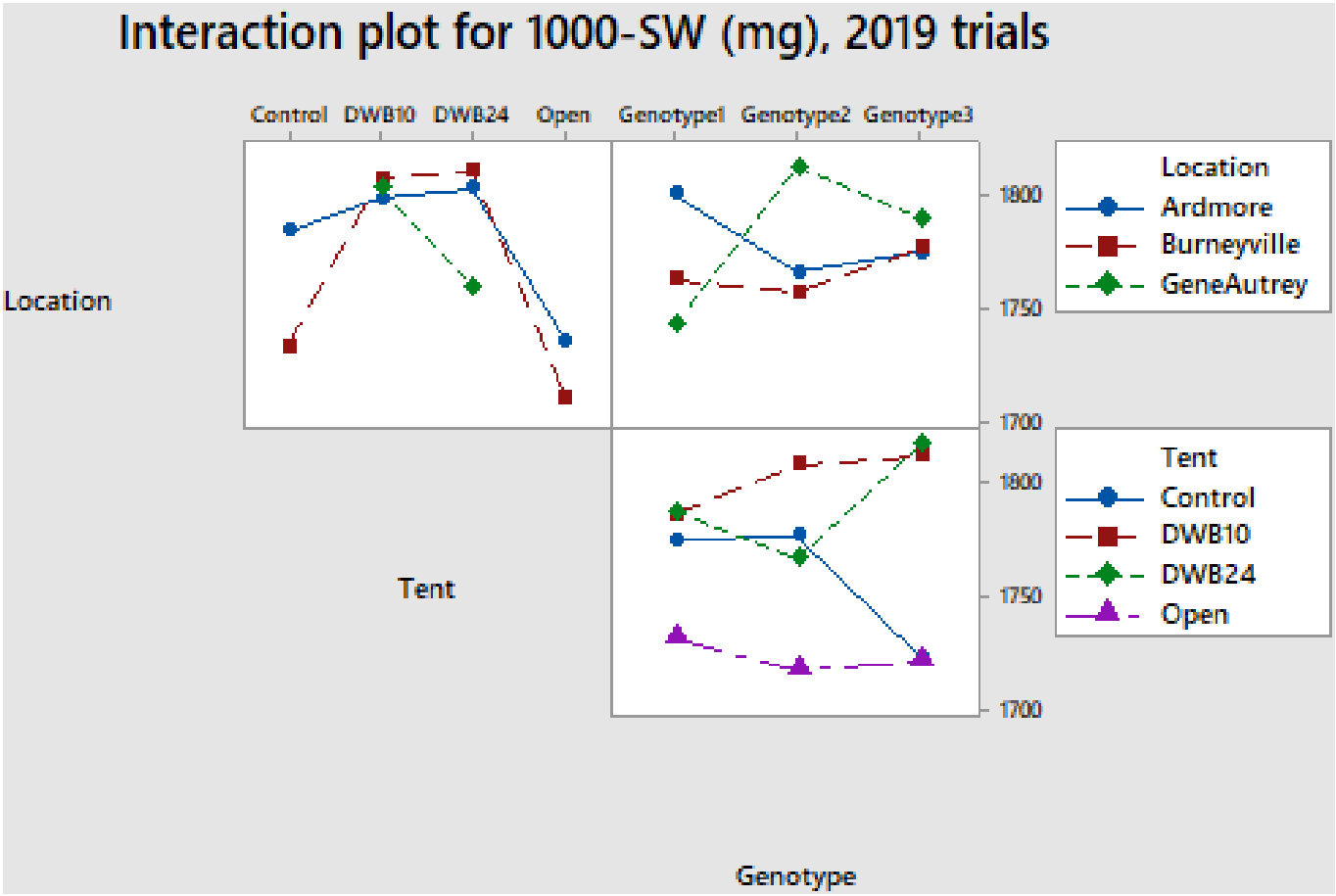

Figure 6. Plot for locations vs. PCT types and PCT type vs. genotypes for 1000-seed weight (mg) in 2019 trials.

Table 6. Mean squares (MS) from analysis of variance and their corresponding sum of squares (SS) as per cent of the total SS (in parentheses) for various traits in small pollination control tent (PCT) trials in 2018.

Source	df	PH	GH	SY	SW
Location	2	11.47 (2.1)	0.45 (4.8)*	8.40 (0.6)	6.96 (1.0)
Treatment	2	88.11 (16.4)**	0.15 (1.6)	1139.34 (78.0)**	111.17 (15.4)**
Genotype	2	2.85 (0.5)	0.47 (5.0)*	1.28 (0.1)	32.95 (4.6)*
Loc × Treat	4	5.99 (2.3)	0.21 (4.5)	3.17 (0.4)	10.38 (2.9)
Loc × Geno	4	6.20 (2.3)	0.10 (2.1)	8.36 (1.2)	15.64 (4.3)
Treat × Geno	4	7.76 (2.9)	0.08 (1.7)	17.40 (2.4)**	11.77 (3.3)
Loc × Treat × Gen	8	5.03 (3.8)	0.27 (11.4)*	11.10 (3.0)	17.21 (9.5)*
Error	108	6.92 (69.7)	0.12 (68.9)	3.86 (14.3)	7.94 (59.2)
Total	134				

*P<0.05, **P<0.01. Traits with non-significant mean squares not shown were: Disease Score, Ergot (%), and Seed Quality Score. PH= plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg).

SW (Table 9).

Of more interest are the significant differences between treatments where the control had the highest performance for PH, SY and SW in 2018 and the DWB10 and DWB24 PCT treatments being numerically equal (Table 8). Interestingly, in 2019, control and open-control

Table 7. Mean squares (MS) from analysis of variance and their corresponding sum of squares (SS) as per cent of the total SS (in parentheses) for various traits in small pollination control tent (PCT) trials in 2019.

Source	df	PH	GH	SY	SW
Location	2	358.2 (26.9)**	0.47 (10.5)**	384.41 (21.6)**	8.78 (3.0)*
Treatment	3	112.9 (12.7)**	0.07 (2.4)	170.48 (14.4)**	47.65 (24.2)**
Genotype	2	4.8 (0.4)	0.18 (4.0)*	0.05 (0.0)	2.27 (0.8)
Loc×Geno	4	7.4 (1.1)	0.20 (8.8)**	19.99 (2.3)	11.65 (7.9)**
Treat×Geno	6	7.3 (1.7)	0.19 (12.5)**	15.05 (2.5)	8.30 (8.4)**
Error	132	10.4 (51.5)	0.04 (61.7)	15.14 (56.2)	2.63 (58.8)
Total	149				

*P<0.05, **P<0.01. Traits with non-significant mean squares not shown were: Disease Score (1 to 5; 0= no disease, 5=death due to disease), Ergot (%) and Seed Quality Score (1-5; 1= excellent, 5= extremely poor). PH= plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg).

Table 8. Fitted mean values for main effects with significant mean squares in the analysis of variance for small pollination control tent (PCT) trials in 2018.

Factor	Detail	PH	GH	SY	SW
Location	Ardmore	-	5.89^{A}	-	-
	Burneyville	-	5.70^{B}	-	-
	Gene Autry	-	5.74^{B}	-	-
	SE m (±)	-	0.05	-	-
	LSD 5%	-	0.16	-	-
Treatment	DWB24	117.26^{B}	-	8.36^{B}	1813.4^{B}
	DWB10	117.31^{B}	-	7.83^{B}	1826.3^{B}
	Control	119.71^{A}	-	16.80^{A}	1905.2^{A}
	SE m (±)	0.39	-	0.29	13.30
	LSD 5%	1.28	-	-	43.64
Genotype	Genotype 1	-	5.89^{A}	-	1827.9^{B}
	Genotype 2	-	5.75^{AB}	-	1879.0^{A}
	Genotype 3	-	5.69^{B}	-	1838.0^{B}
	SE m (±)	-	0.05	-	13.30
	LSD	-	0.16	-	43.64

PH= Plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg). Means that do not share a letter are significantly different.

showed significantly lower mean performance for PH, SY and SW than the two PCT treatments, DWB10 and DWB24, which were higher than controls but statistically the same (Table 9). Genotype 1 showed the highest performance in 2018 for GH but was the lowest in 2019 while genotypes 2 and 3 were average in both years. The SW mean of genotype 2 was significantly higher than other two genotypes in 2018 (Table 8).

Large PCTs-quantitative traits

Analyses of variance were performed separately for the two sites since they had different sizes and types of fabrics of large PCTs in 2019 (Table 10). Both PCTs had significantly higher SY and SW than their respective controls.

The trial at Unit 3 Farm showed significant mean squares for SY and SW with significantly higher mean values for the DWB10 PCT. The trial at HQ Farm also showed significant differences for SY, SW; the mean values for DWB24 PCT were significantly higher for SY and SW than the control at the same site. Comparison of DS, Ergot (%) and SQ here were significantly more favourable inside the DWB24 PCT than outside (lower scores) (Table 10).

Table 9. Fitted mean values ± standard errors for main effects with significant mean squares in the analysis of variance for small pollination control tent (PCT) trials in 2019.

Factor	Detail	PH	GH	SY	SW
Location	Ardmore	113.4±0.42^{B}	5.75±0.03^{A}	15.20±0.50^{C}	1780.3±6.6^{A}
	Burneyville	118.2±0.42^{A}	5.60±0.03^{B}	20.26±0.50^{A}	1765.4±6.6^{AB}
	Gene Autry	117.0±0.66^{A}	5.55±0.04^{B}	17.84±0.79^{B}	1749.5±10.5^{B}
Treatment	DWB24	118.1±0.48^{A}	-	19.13±0.58^{A}	1791.0±7.6^{A}
	DWB10	117.6±0.48^{A}	-	20.04±0.58^{A}	1802.8±7.6^{A}
	Control	115.1±0.64^{B}	-	14.69±0.77^{C}	1750.9±10.1^{B}
	Open	114.1±0.64^{B}	-	17.20±0.77^{B}	1715.7±10.1^{C}
Genotype	Geno 1	-	5.55±0.03^{B}	-	-
	Geno 2	-	5.67±0.03^{A}	-	-
	Geno 3	-	5.67±0.03^{A}	-	-

PH= Plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg). Means that do not share a letter are significantly different.

Table 10. Mean squares from analysis of variance (above) and fitted mean values for various traits in large pollination control tent (PCT) trials at Unit 3 Farm and HQ Farm at Ardmore Noble Research Institute campus during 2019.

Farm	Source	df	SY	DS	SW	Ergot	SQ
Unit 3 Farm 6×6 tent (DWB10)	Treatment	1	119.81*	0.20	98701**	2.81	0.61
	Error	78	23.44	0.73	7571	2.11	0.29
HQ Farm 3×12 tent (DWB24)	Treatment	1	262.09**	0.61*	109520**	2.81+	3.20**
	Error	78	27.94	0.15	5789	0.89	0.32
Mean value							
Unit 3 Farm 6×6 tent (DWB10)	Tent	-	32.69	-	1853.8	-	-
	Control	-	30.25	-	1783.5	-	-
	SE m ±	-	0.77	-	13.8	-	-
HQ Farm 3×12 tent (DWB24)	Tent	-	31.96	0.03	1836.8	0.00	1.03
	Control	-	28.34	0.20	1762.8	0.38	1.43
	SE m ±	-	0.84	0.06	12.0	0.15	0.09

*P<0.05, **P<0.01, +P<0.08. SY= seed yield per plant (g), Disease Score (1 to 5; 0= no disease, 5=death due to disease), SW= 1000-seed weight (mg), Ergot (%) and Seed Quality Score (1-5; 1= excellent, 5= extremely poor). Mean squares for PH= plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect) were non-significant and are not reported.

Germination percent

Small PCTs-germination percent

The analysis of variance showed significant location effect on germination at 7 and 14 days in both years but also at 21 days in 2018 only (Table 11). The treatment effect was only significant at 7 days in 2018 (Table 11). Mean germination percent was significantly higher at Ardmore in 2018 for each time point. In 2019, the germination percent at 7 and 14 days was higher at Gene Autry (Table 11). Mean germination of treatments were significantly different only in 2018 at 7 days. The mean germination of seed produced under the DWB10 PCT fabric was significantly higher than seed harvested under DWB24 fabric and control which were both similar at 7 days in 2018 (Table 11).

Despite the effect of locations on seed development and subsequently on rate of germination, the final germination percent on the 21st day was the highest at

Table 11. Mean germination (%) for main effects at 7, 14 and 21 days after sowing in small pollination control tent (PCT) trials in 2018 and 2019.

Factor	Loc/Treat	Means for small tents 2018			Means for small tents 2019		
		7 day	14 day	21 day	7 day	14 day	21 day
Location	Ardmore	12.89^{A}	74.67^{A}	94.22^{A}	14.22^{A}	72.67^{B}	93.17^{A}
	Burneyville	10.67^{B}	66.00^{C}	89.33^{B}	12.30^{B}	71.17^{B}	91.67^{A}
	Gene Autry	11.56^{AB}	70.44^{B}	92.67^{A}	14.83^{A}	75.92^{A}	93.75^{A}
	SE m (±)	0.57	1.09	0.78	0.51	1.28	1.39
	LSD 5%	1.69	3.24	2.32	1.48	3.73	4.07
	Significance	*	**	**	**	**	NS
Treatment	DWB24	10.67^{B}	-	-	-	-	-
	DWB10	12.89^{A}	-	-	-	-	-
	Control	11.56^{B}	-	-	-	-	-
	SE m (±)	0.57	-	-	-	-	-
	LSD 5%	1.69	-	-	-	-	-
	Significance	*	-	-	-	-	-

*P<0.05; **P<0.01; NS= Not significant. The ANOVA (not given) had locations (2 df), treatments (2df) and error (18 df) since locations × treatment interactions were not significant in any case. Means that do not share a letter are significantly different. Means for non-significant treatments are not given.

all locations in both years (Table 11). The lowest overall germination of 89% was recorded for Burneyville in 2018. The significant difference between locations and for genotypes tends to disappear as the time from sowing seeds increased. The slower start of germination in some cases may be due to the effect of climate at different locations for the stored metabolites to be activated differentially.

Large tents-germination percent

There was no significant variation between treatments for germination percent at 7, 14 and 21 days following sowing. There was a linear increase in the percent of germinated seeds from 7 to 21 days and the germination reached more than 96% for seed from both farms and PCT types. At 7, 14 and 21 days DWB10 PCTs showed 12, 70 and 97% germination respectively, against 12, 70 and 96% for the control. Seeds from the PCT DWB24 showed 13, 66 and 97% germination at 7, 14, and 21 days vs. 14, 72 and 97% for the respective control. Since there was no significant difference in germination of seed from the larger PCTs or from the outside control, it can be concluded that the PCT microenvironment from either fabric in no way differed in its effect on the rate of seed germination or viability of seeds.

Fan effect-small tents

Fans were introduced in tents at Ardmore and Gene Autry, but not at Burneyville during 2019 trials on small tents. Fan vs. no fan effects were significant for PH, GH, SY and for germination percent at 7 and 14 days (Table 12). Seeds produced with fans in the PCTs and control always gave higher mean percent germination at all days of the count. Provision of fans tended to produce plants with lower PH, higher GH score and lower SY without affecting the seed size. Higher SY may not mean higher germination since healthy and viable seeds may be fewer than the actuals. Fans could have created a microclimate that produced seeds, which looked similar in weight to those under no fan, but had better metabolite reserves resulting in better germination that might translate in better establishment and stand in the field. Further, reduction in SY by fan airflow might be caused by pollen mobility to be adversely affected reducing settlement on stigmas. Thus, there is no apparent advantage of adding fans in the PCTs. Fans × treatment interactions were significant for PH and SY (Table 12 and Figure 7). The major source of interaction was the interaction of two types of tents with fans in them. The PH of DWB10 was reduced in the presence of a fan but SY increased in comparison with the DWB24 PCT. Perhaps conditions in the heavier and waxier fabric of DWB10 improved seed set and SY compared to the more aerated DWB24 PCT material (Figure 7).

DISCUSSION

The major objective of this study was to assess the comparative performance of grass genotypes in novel nonwoven synthetic fabric PCTs vs. isolated, open pollinated control conditions at different locations in

Table 12. Mean squares from analysis of variance (above) and mean values (below) for fan effect on quantitative traits and germination (%) at 7, 14 and 21 days after sowing in small pollination control tent (PCT) trials in 2019.

Source	Df	PH	GH	SY	Df	7 day	14 day	21 day
				Anova				
Treatment	3	168.91**	0.07	226.28**	3	2.32	4.02	5.41
Fan vs. no fan	1	621.72**	0.31*	721.29**	1	29.76*	45.76*	23.05
Treat × Fan	3	154.35**	0.03	77.11**	3	1.43	5.50	3.37
Error	142	8.44	0.06	14.61	22	4.76	8.03	8.06
Total	149	-	-	-	29	-	-	-
				Mean values				
No Fan	-	118.20±0.38	5.60±0.03	20.26±0.49		12.33±0.63	71.17±0.82	91.67±0.82
With Fan	-	113.94±0.33	5.69±0.03	15.68±0.43		14.42±0.55	73.75±0.71	93.50±0.71
% increase/decrease over no fan	-	-3.60%	1.70%	-22.63%		16.90%	3.63%	2.00%

*P<0.05, **P<0.01. PH= plant height (cm), GH= Growth habit (1-9 scale), SY= seed yield per plant (g).

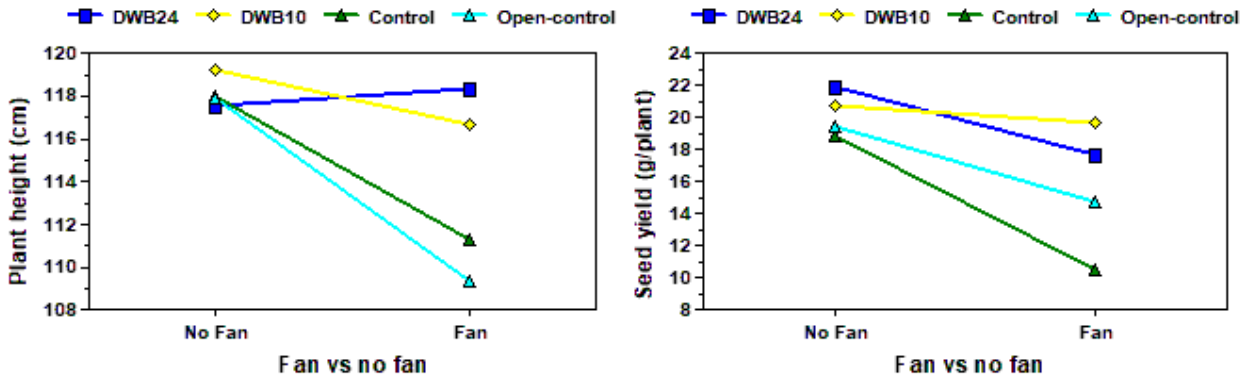

Figure 7. Interaction plots for fan effect vs treatments for plant height (cm) and seed yield (g/plant) in small pollination control tents (PCT) in 2019 trials.

Oklahoma. Locational differences were more pronounced in 2019 with significant differences for all quantitative traits when differences only existed for SW and GH in 2018. The treatment differences were consistently significant for most of the traits across years, which revealed possibilities of more productive options over open pollinated controls. There also existed significant interactions of treatments with locations and genotypes for SY and SW in two years, but the contribution of interaction sum of squares (SS) to the total SS was very small reaching a maximum of 13% for GH in 2019 (Tables 6 and 7). These contributions were very small in comparison with the larger contribution of the main effects to the total SS. Therefore minimal significance was attributed to these interactions and conclusions were based largely on main effects (Tables 6 and 7).

Of the two years, 36 to 87% more seed per plant was produced in 2019 across sites compared to 2018. The two PCTs showed a 2 to 5% decrease for PH, SY and SW compared with the control treatment in 2018 (Figure 8). However, in 2019 the performance of tall fescue was superior to control for PH, SY, SW in small PCTs, and SY and SW in large PCTs. SY from DWB10, DWB24 small PCTs were 37 and 30% higher, respectively, over the control (Figure 8). Similarly, SY from large DWB24 PCTs were 13% higher and the DWB10 PCTs averaged 8% higher over the control (Figure 8). Clearly, SY returns from both PCTs were higher than open controls (Tables 8 and 9); thus both PCT materials were equally useful in this particular climate and crop combination. However, the choice of PCT fabric for other crops and other climates may be different.

Viable pollen is important for species dispersal, fitness, and survival of the next plant generation (Impe et al., 2020). It is also essential for directed plant breeding and, consequently, crop improvement. The extent of seed set following pollination, fertilization and healthy seed development is conditioned by the ambient microclimate within the PCTs. Wang et al. (2004) assessed *in vitro* pollen viability from transgenic and non-transgenic tall

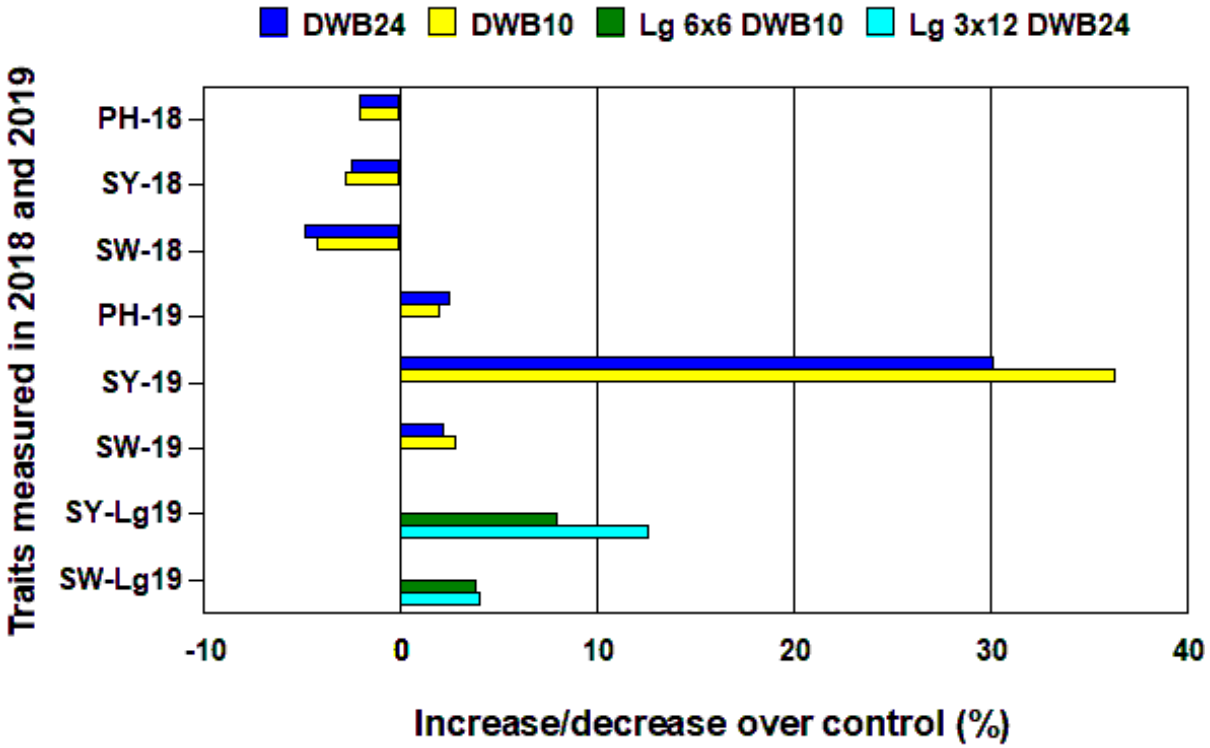

Figure 8. Percent increase or decrease of mean performance of various quantitative traits over control for small tents DWB10 and DWB24 in 2018 (with -18) and 2019 (with -19) and large tent (Lg) for 2019. PH= plant height, SY= seed yield, SW= 1000-seed weight.

fescue and found that treatment with relatively high temperatures (36 and 40°C) reduced pollen viability while relative humidity did not significantly influence pollen viability. They found that the viability of pollen from transgenic progenies was similar to that from seed-derived control plants. Plant disease can also decrease seed production, especially in tall fescue (Barker et al., 2003). In the Pacific Northwest of the USA, the most significant diseases affecting seed production of tall fescue are fungal diseases, including stem rust, caused by *Puccinia graminis* subsp. *graminicola* Pers., and blind seed, caused by *Gloeotinia temulenta* (Prill & Declacr.) (Alderman et al., 2009). However, the appearance of plant disease is highly influenced by environmental factors (Velásquez et al., 2018). Even when a host is susceptible, the plant may not be infected by a virulent pathogen if the environmental conditions are not optimal for disease. Therefore, the occurrence of diseases within plants and the developing seed in tent microenvironments is highly influenced by the inside temperature and relative humidity-the two major contributing factors. The appropriate humidity ensures leaves remain moist and the temperature ensures warmth for germinating spores of disease pathogens. In general, there was no difference in temperature and relative humidity between the two PCT fabrics across all locations and years. While the lower temperatures in the PCTs fell below the control by a few degrees the maximum and average temperatures were higher than outside. Similarly, the minimum humidity was generally lower in PCTs in both years and across all locations, but the maximum humidity was the same or higher in PCTs than outside. The average humidity was equal or lower in the PCTs vs. the outside groups. Moderate temperature and relative humidity in PCTs tended to favour higher SY and SW with disease free seeds of better quality (Table 10).

The effect of introducing fans within PCTs and controls to determine if they improved pollination, fertilization and subsequent seed output were also examined. We hypothesized that static air within the PCTs might reduce the free airflow of pollen grains leading to poor seed set. However, the introduction of fans unexpectedly decreased seed yield by 23% (Figure 9). While no explanation could be evidenced, it is possible that the draft created by the fans could have interfered with the settling of pollen on receptive stigmas during pollination and fertilization However, the reduction of SY with fans established that there is no need for increased airflow within the PCTs; perhaps the porous nature of the fabrics allowed enough aeration and airflow within the PCTs eliminating the need for increasing it by other means.

A measure of healthy seed development is germination capability (McDonald and Copeland, 1997) which

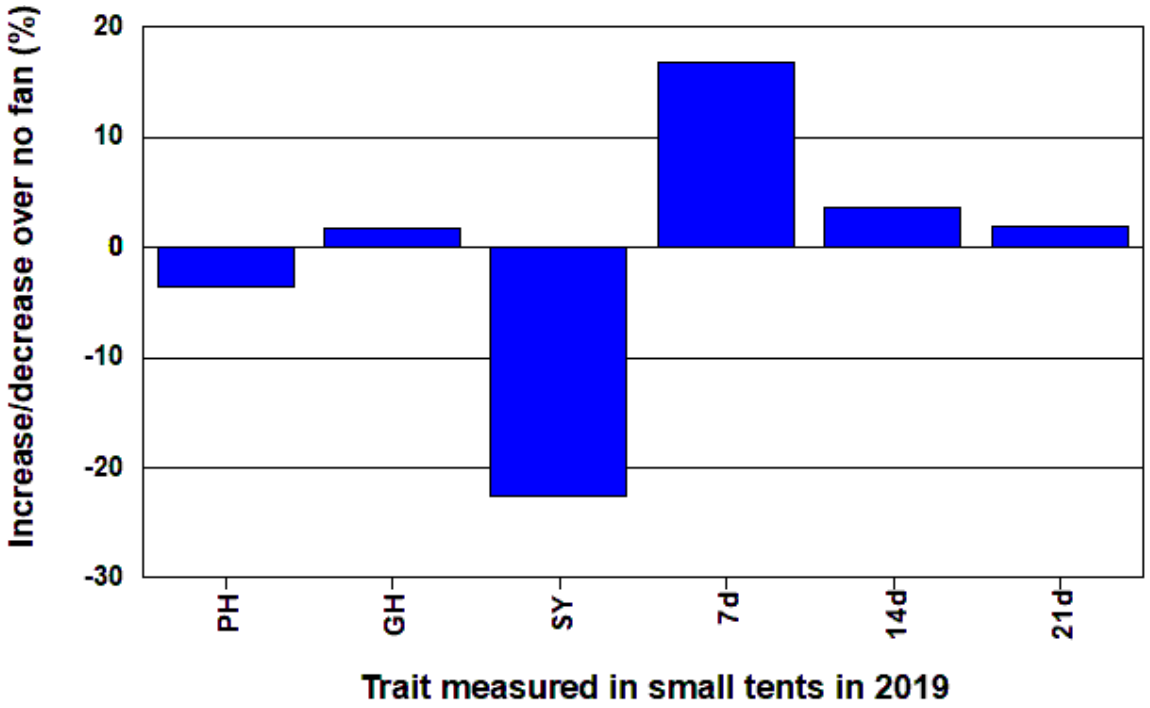

Figure 9. Fan effect as (percentage) increase or decrease of mean performance of various quantitative traits and germination (%) after 7, 14 and 21 days over no fan in small pollination control tents (PCTs) 2019 trials. PH= plant height, GH = growth habit, SY= seed yield.

indicates not only seed viability but also the extent of the store of differential metabolites responsible for faster or slower germination. There was evidence of significant differences for germination rate at all stages of count for different locations with Burneyville seed displaying average germination rates and the other two locations changing their ranks at some stages. This can be expected as seed produced at different locations can differ in quality and extent based on stored metabolites. However, one would not expect differences among seed produced in PCTs and outside controls if, conditions within PCTs are ambient. In general, germination of seed produced in the two PCTs and outside controls were comparable at all stages except at 7 days when seed produced within PCTs with the DWB24 fabric had higher germination. Overall, there was little difference among treatments and locations for the final seed germination at 21 days after sowing. This demonstrates that seed produced in PCTs exhibit similar germination to the seed produced under natural conditions and that the use of a PCT for seed multiplication could be a gainful possibility.

An important feature of hybridization or seed multiplication in PCTs is the maintenance of genetic identity of stocks from contamination of foreign or unwanted pollen. We did not have any evidence, though preliminary, for any contamination from outside pollen in the PCTs. This is a very useful indication to build the confidence of plant breeders and seed producers for the use of nonwoven fabric PCT's in grass breeding.

Economic implications

While a proper economic analysis was not a direct objective of the present research, we can examine the effect of various factors determining the economic impact of using PCTs in comparison with other means of isolation or self-pollination. This is a very preliminary analysis that could be used as a basis for future studies and follows a simplistic approach shown in Schaffert et al. (2016) and Gaddameedi et al. (2017) in sorghum. Our approach is based on explorative circumstantial evidence from the analyses provided by the available data that could be extrapolated for comparative assessments (Table 13).

While performing any economic analysis for grass breeding it should be remembered that a grass breeder is interested in: (i) attempting single or multiple interspecific crosses, (ii) seed increase of interspecific crosses for synthetic varieties, (iii) seed increase of advanced entries for multi-locational trials, (iv) maintenance of early generation of seed such as nucleus or breeder seed, and (v) maintenance of genetic stocks for use in breeding. While making interspecific crosses, objectives are usually identifying good combining components for synthetics or identifying specific cross combinations for releasing hybrid varieties for their increased heterosis. Interspecific single crosses between two species are made by hand using pollination control bags to get small quantity of seeds. However, for multiple crosses (e.g., several

Table 13. Factors for comparing pollination control tents (PCTs) for economic analysis.

Treatment	Seed yield	Diseases	Effect of natural factors	Bird damage	Labour, resources	Risk of loss of genetic stock	Reusability	Relative cost†
PCT	= or > control; >30% vs control for small tent sand 8-13% > for large tents	Variable	Wind, rain, storm effects small	Nil	Low, 30-40 mts for 3 people to assemble, and to remove	Nil	Yes	$$
Isolation plots	=control	Diseases occur, ergot	High impact	Can be high	High; 3 h of 1 person per week for full season	Low with costly watch and ward. Part or whole loss from uncontrolled animals.	NA	$$$
Isolation chambers	< control	Variable	Nil but expensive climate control	Nil	Permanent type, high cost of temperature, humidity, lighting etc.	Nil	Yes	$$$
Bagging	<, plant × plant crosses only	Variable	Wind, rain may tear or blow away	Variable	Only for bagging or re-bagging	Nil	Paper not; synthetic yes	$
No bagging	=control	As in isolation	High effect	Can be high	Nil	As in isolation	NA	Nil

†The dollar ($) sign indicates relative costing for each method. The method with one $ has minimum cost, $$ has double and $$$ has three times more price.

female parents crossed with one good combining male parent) or for seed increase, space isolation plots, isolation chambers or PCTs will be appropriate. In all of these scenarios, breeders place a high level of confidence in the genetic integrity, quality and viability of the seed produced.

Traditionally, plant breeders used pollination control bags made of paper, but recently synthetic fabrics with greater strength against bad weather, bird damage and wind resistance along with air permeability, lower moisture absorption and prevention of unwanted pollen have been developed (PBS Intl., 2020a,b). Pollination control bags made from nonwoven synthetic fabrics have been successfully trialed and proven to deliver better outputs and increased plant breeding efficacy than controls by Gitz et al. (2015), Schaffert et al. (2016, 2018, 2019) and Gaddameedi et al. (2017) in sorghum; Clifton-Brown et al. (2018) in sugar beet, wheat, *Arabidopsis* and *Miscanthus*; Hayes and Virk (2016) in *Miscanthus*; Vogel et al. (2014) and Adhikari et al. (2015) in grasses; and Bonneau et al. (2017) in oil palm. Encouraged with the superior performance and re-usability of nonwoven synthetic fabrics for pollination control bags we uniquely extended the use of such fabrics to PCTs in the present study with the objective of improving the efficiency of grass breeding and seed production.

Hayes and Virk (2016) compared the efficiency of isolation chambers (small pollen-proof compartments with controlled airflow and water supply) with pollination control tents in both external and glasshouse environments in *Miscanthus*. The comparative efficiency of tents and isolation chambers was measured by recording the total number of seeds and average number of seeds per head, which were both consistently higher for tents whether in external or internal glasshouse conditions. Thus the synthetic nonwoven polyester fabric of the tents, as used in the present study, provided an ambient climate for higher seed set. The temperature and humidity inside the crossing tent followed the same pattern as shown by the ambient conditions in the Venlo glasshouse. The temperature and humidity in the glasshouse isolation chamber was lower than both the crossing tent and the ambient conditions of the Venlo glasshouse. The difference in humidity and temperature within the different crossing environments was likely the reason there was reduced seed set, on average, between the isolation chambers when compared with the results from the crossing tents.

The seed yield, over a 15-year average, for a tall fescue plant in the breeding program at the Noble Research Institute ranges from 20.00 to 26.50 g. This means seed yields of 20 g/plant or higher would justify the use of PCTs or isolation chambers for seed increase on a regular basis. While 2018 was not a good year for seed yields, being much lower than expected, the yields in 2019 in smaller PCTs were closer to 20 g per plant and higher than in the outside control.

This showed that the use of PCTs could be an economic possibility for seed increases in grasses in at least Oklahoma climate conditions.

In 2018, the first small PCT took about 1.5 h (3 people) to assemble. This included digging and anchoring of the skirting and placing a soaker hose for irrigation of the plants under the PCT. In 2019, it averaged about 30 to 40 min per small PCT for three people to complete the task. However, more labour is required with the control isolation plots from a maintenance standpoint. Maintenance around the isolation controls usually requires planting a pollen screen of cereal rye (*Secale cereale* L.) as well as hoeing and/or spraying to reduce weeds or insect pests. In addition, there is the added issue of maintaining the land around the isolation plot. Experience in Oklahoma shows that one full time person spends about 3 h per week working on keeping the outside control nursery clean of weeds or insect pests. About 0.5 h per week (1 employee) were spent on maintenance of each of the large PCTs. However, it could be possible to raise revenue through the sale of the grain produced by the pollen screen (cereal rye) or potentially other types of crops, to offset the cost of maintaining open type nurseries.

With the open pollinated controls, the only way to maintain genetic purity is with distance or isolation from the same species. For open pollinated species, such as tall fescue, a minimum of 305 m of distance between seed fields is required for the production of breeder or foundation certified seed as recommended by the Oregon Seed Certification Service (Oklahoma Crop Improvement Association standards are the same). If a breeding program established 20 or more open pollinated seed increases each year, the distance requirements would be demanding, requiring a spread of isolation nurseries over lots of different farms at different locations creating administrative and logistical challenges. If land space is a factor, then the number of isolations planted could be an issue, which may cost a generation of advancement. PCTs with reliable seed production would allow the planting of many isolation plots in a much smaller area. This would reduce time for traveling to and from many different locations and maintaining the space around these locations. For this purpose, researchers may prefer the larger PCTs compared to the smaller ones. Since the small PCTs are portable and easy to move they could also be used at leased offsite locations, such as private agricultural producers and universities. The PCTs would be much easier to maintain at these types of locations vs. larger open pollinated plots since travel to these sites may be many kilometres away. PCTs may reduce costs since less time is spent at the location for nursery maintenance. In addition, the production of high-grade seed or breeder (nucleus) seed of a licensed cultivar normally costs a seed company around \$35 to 50 kg^{-1} to produce. In this scenario, pre-breeder seed would be a good target for the small PCTs, while the large PCTs would be ideal for breeder or nucleus seed production.

Since our experiments, a number of modifications for improvement of PCTs have been made. Previously the seams of the cover in the corner of the roof tended to show some wear and tear. This has been improved with the new robust frame structures and methods of fixing the cover fabric. It was also felt that some type of 'U' type anchor for holding the frame on ground could have been useful. This improved design is more robust and holds on the ground much more strongly than in previous versions. Options for windows are provided in the new design that allows viewing the interior of the PCT without disturbing it.

Future considerations

Although tents have been used for indoor and outdoor plant multiplications, the use of specifically developed PCTs as pollination control aids and seed increases are recent. Therefore, there is a market for the development of robust structures that can withstand high winds and bad weather, but are lightweight for transport, easy to assemble and include windows for examination and easy entry. Improvement regarding irrigation and agronomic operations within the PCTs without disturbance were needed following our experiments. Advances have been made since these trials and the PCT design has been improved to increase the benefit: cost ratio and for wider applicability to many crops. Flexibility in sizing the PCT covered area is also important for accustoming the protected area as per breeders' requirement in any season. Advances in this area reflect development of PCTs of specific capacity that can be joined together as a modular structure to a number of independent parts to cover as large an area as required.

The second important aspect is the use of the right fabric as a cover. The fabric needs to be easily fitted, but hard enough to withstand wear and tear on the corners where it touches the frame, be pollen proof, but have sufficient aeration for temperature and humidity control. Apart from the DWB10 and DWB24 synthetic nonwoven fabrics used in the study, there are a number of other fabrics that are available that have been tested in other crops such as sugar beet (Paul Townson Pers. Comm.) and mustard (S.S. Banga Pers. Comm.) with encouraging results. However, while the present study has established the superiority of synthetic PCTs, further studies to confirm wider utility in other crops and breeding scenarios will be needed in terms of estimating the economic implications in seed production.

Conclusions

Pollination control tents (PCTs) made from two nonwoven synthetic fabrics, DWB10 and DWB24, were tested against open controls across two years and three locations in Oklahoma for their seed production efficacies

and control of pollen contamination. The two types of PCTs showed similar and higher seed yield by up to 36% compared with open control treatment. The higher average temperature and a lower to average humidity within the PCTs compared to the control across locations and years could have led to the more optimal and healthier seed set in the PCTs. The introduction of fans in the PCTs to increase pollen flow was not beneficial as it reduced seed yield by about 23% demonstrating that natural conditions in the PCTs were conducive for higher seed yield. Knowledge gained from this study is being used to improve the PCT design structure and to test newly developed fabrics in different crops. The proposed economic analysis and the generalized possibilities regarding the application of PCT technologies in plant breeding and in particular grass breeding, seems encouraging for increasing seed outputs, the hybridization process and seed multiplications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank Rob Coy and Stuart Carter of PBS International, Salter Road, Scarborough YO11 3UP, UK for their help in standardising and providing the pollination tents.

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Vol. 12(3), pp. 219-227, July-September 2020
DOI: 10.5897/JPBCS2020.0898
Article Number: 7BF46A964561
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

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Journal of Plant Breeding and Crop Science

Full Length Research Paper

Performance evaluation and selection of new maize hybrids under sole and inter crop production systems

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Received 9 May, 2020; Accepted 3 August, 2020

Screening of maize genotypes under different cropping systems (sole and inter cropping) is very important to understand the genotypes response for different abiotic and a biotic stress. Nine maize genotypes including the standard check (BH-543) were planted and evaluated at research and farmers' fields in the 2011 and 2012 cropping seasons. Farmers were invited to evaluate the genotypes based on their criteria of selection. Hawassa-Dume common bean variety was used for intercropping purpose in 2012. The genotypes substantially varied for yield and other traits both under sole and intercropping systems. When combined across seasons, the high yielding genotypes, genotype-1 and genotype-5 showed 38 and 37% yield advantage over the standard check. Besides, genotypes markedly varied for their compatibility for intercropping system with land equivalent ratio (LER) <1 for most of genotypes. However, genotype-4 and genotype-8 had LER >1 highlighting the need to evaluate genotypes for intercropping system at early stage of breeding.

Key words: Hawassa-dume, inter-cropping, sole-cropping, *Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.) is the second most widely cultivated crop grown by smallholder farmers under rainfed condition in Ethiopia. Maize yield in Ethiopia vary considerably across seasons and locations making smallholders livelihoods vulnerable to climate variability. Maize and common bean are two of the leading crops in their respective category of cereals and pulses in southern Ethiopia. Accordingly, maize and common bean occupy 36 and 44% of the area devoted to cereals and pulses, respectively (CSA, 2017).

Intercropping systems play an important role in subsistence and food production in developing countries (Tsubo and Walker, 2002). It is most widely practiced in countries where arable land is scarce where it contributes to biodiversity and food security (Mushagalusa et al., 2008). Land scarcity is one of the constraints facing small farmers in Ethiopia. In the southern Ethiopia, 40% of farmers have an average land holding of 0.1 to 0.5 ha with a further 30% having 0.51 to 1 ha (CSA, 2017). This led farmers to use multiple cropping mainly intercropping to increase yield per unit area and reduce the risk from crop failure due to climate change.

Maize-common bean intercropping is an integral part of the cropping system in small-holder farmers expecting better yield and weed suppression (Getahun and Tenaw, 1990), and provides balanced diet compared to the

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predominant cereal monoculture and gives high total productivity compared to sole crops of bean and maize (Walelign, 2014; Workayehu, 2014). However, all varieties released so far in the country were evaluated under monocropping system and has not been tested for intercropping system at early stage of breeding. Selection of genotypes both under sole and intercropping systems is of paramount importance to enhance yield and varietal adoption in the region. Therefore, the objective of this study was to identify best performed genotypes under sole and intercropping systems.

MATERIALS AND METHODS

The experiment was conducted under rain-fed condition at Hawassa research station (07°03′71″ N, 38°30′88″ E, 1689 masl elevation) and on-farms (farm1; 07° 79′43″ N, 37° 04′ 31″ E, 1696 masl elevation and farm2; 07° 78′ 28″N, 37° 04′ 31″E , 1692 masl) in 2011 and 2012 main rainy seasons in Ethiopia. This area is characterized by bimodal rainfall between March and September with mean annual maximum and minimum temperatures of 27.3 and 12.6°C, respectively. Nine hybrid maize genotypes were planted in 2011 and 2012 cropping seasons. The hybrids were planted both under sole and inter cropping systems at research station in 2012. These genotypes were also planted only under sole-cropping on two farmers' field in 2011 and 2012 without replication at each farmer's field but for analysis farmers were used as replication. Similarly, in 2011, these genotypes were planted at research field without replication. For analysis of the data collected in 2011, farmers and research station were used as replication because the trial was not replicated both at farmers' and research field. The recently released hybrid variety (BH-543) was included as check in 2011 and 2012 cropping seasons. The genotypes were planted using randomized complete block design with three replications at research field in 2012. Each genotype was planted on two rows 7.65 m^2 area at research station and three rows 11.48 m^2 area at farmers' field. Maize genotypes were planted in 75 and 30 cm spacing between consecutive maize rows and plant, respectively. Common bean genotype named Hawassa-Dume was planted between two rows of maize in one to one ratio for intercropping. At research field, sole common bean was also planted for land equivalent ratio determination. Common bean was planted at the spacing of 40 cm between rows and 10 cm between seeds within a row. Grain yield and other important agronomic traits of component crops were recorded to evaluate the genotypes grown under the sole and inter-cropping systems. Plants from the whole plot were hand harvested at physiological maturity. Ears were shelled, grain weight and grain moisture content measured, and yield was adjusted for 12.5% grain moisture content. However, for common bean yield was adjusted to 10% grain moisture content. In both seasons, farmers were participated to set selection criteria and evaluate maize genotypes. Yield deviation due to inter cropping from the sole maize yield was calculated using the formula

$$\text{Deviation (\%)} = ((\text{inter crop maize yield} / \text{sole maize yield}) \times 100) - 100 \quad (1)$$

Land equivalent ratio calculated was computed as in Adu-Gyamfi et al. (1997).

$$LER = ((Y_m/Y_{sm}) + (Y_b/Y_{sb})) \quad (2)$$

where Y_m and Y_b were grain yields of intercropped maize and bean; Y_{sm} and Y_{sb} were grain yields of sole cropped maize and bean.

Grain yield, number of ears (NE), plant height (PH), ear height (EH), gray leaf spot (GLS), turcicum leaf blight (TLB) and common leaf rust (CLR) were analyzed as randomized complete block design in SAS statistical package (SAS, 2002) version 9.0. Performance and stability of genotypes were visualized graphically through an average environment coordination (AEC) view of GGE biplot based on genotype-focused SVP (that is, "SVP=1") (Yan and Rajcan, 2002). Graphs were developed using R software (Table 1).

RESULTS AND DISCUSSION

Analysis of variance

Highly significant grain yield (GY) variation was observed among genotypes for sole cropping system in 2011 ($p < 0.01$) (Table 2). Similarly, Mossisa et al. (2019) reported significant difference between 12 early to intermediate maturing and the other with 13 intermediate to late maturing hybrids tested at farmers' for participatory assessment of new stress tolerant maize hybrids in Eastern Africa but in contrast to the finding under this study, Daniel et al. (2014) reported non-significant difference between six released varieties tested at farmers field in Chilga District of North Western Ethiopia. No significant difference was observed among genotypes for yield for both sole and intercropping systems in 2012 cropping (Table 3). This could be due to high drought stress in 2012 cropping season resulting into low genotype variation. This is because variation among genotypes in the optimum condition is high than under stress environments leading to lower chance of genotypes to express their genetic potential under stressful conditions (Mohammadai et al., 2012). In 2011, significantly high variation ($p< 0.01$) was observed among genotypes for plant height (PH), ear height (EH), gray leaf spot (GLS), turcicum leaf blight (TLB), common leaf rust (CLR) and number of ears (NE). Genotype-6 was more tolerant to across the three major foliar diseases (GLS, CLR and TLB) compared with the other genotypes (Table 4). Genotype-5 was also showed relative tolerance to these major foliar diseases. Similarly, in 2012 cropping season, significant variation was observed among genotypes tested under sole system for EH and GLS (Table 5). Genotype variation for PH, EH, GLS and CLR under sole cropping system has been previously reported (Berhanu, 2009). However, in this study, genotypes tested under both cropping systems responded consistently excepting small variation. For instance, high GLS score was recorded for genotype 2 and 3 under sole cropping compared with under intercropping system (Tables 5 and 6). However, our study highlights for PH, EH and GLS, Kariuki et al. (2016) reported significant difference between single crosses treatments tested in Kalro experimental stations in Kiambu and Embu counties in 2012. In 2012 under sole crop, significant variation among genotypes was observed for EH and GLS. Genotype variation for GLS, TLB and CLR has been previously reported for this area

Table 1. Pedigree of hybrid maize genotypes used for on-station and on-farm experiments in the 2011 and 2012 cropping seasons.

Pedigree	Code	Type	Seed color
CML395int/CML202//30H83-5-1-3-2-1-1	Genotype-1	CN	White
CML395/CML202//Gibe1-91-1-1-1-1	Genotype-2	CN	White
SC 22/124-b (109)//Gibe1-91-1-1-1-1	Genotype-3	CN	White
SC/22CML395//CML197	Genotype-4	CN	White
CML 197/ BH660 (F2)-10-2-1-2-1//CML395 int	Genotype-5	CN	White
CML 197/BH-660(F2))-10-2-1-2-1//CML312	Genotype-6	CN	White
30H83-7-3-4-1-1-1-1//Gutto LMS5	Genotype-7	CN	White
DE-78-Z-126-3-2-2-1(g) CML312//Gibe1-91-1-1-1-1	Genotype-8	CN	White
BH-543	Genotype-9 (Check)	CN	White

Where CN = Conventional normal maize.

Table 2. ANOVA for maize yield tested under sole and intercropping systems in the 2011 and 2012 cropping seasons.

ANOVA for genotypes tested under sole cropping in 2011			
Source of variation	**Degree of freedom**	**Mean square**	**Computed F**
Rep	2	13.15***	13.78
Genotype	8	3.64**	3.81
Error	16	0.95	
ANOVA for genotypes tested under sole cropping in 2012			
Rep	2	0.95ns	0.26
Genotype	8	2.85ns	0.77
Error	16	3.69	
ANOVA for genotypes tested under inter-cropping in 2012			
Rep	2	3.80ns	2.31
Genotype	8	1.09ns	0.66
Error	16	1.65	
ANOVA combined for genotypes tested across seasons and cropping systems			
Rep	2	8.25*	3.79
Genotype (G)	8	3.66ns	1.68
Cropping Systems (CS)	2	2.15ns	0.91
CS*G	15	1.25ns	0.58
Error	50	2.17	

ns, *, ** indicate non-significant and significant at P < 0.05 and 0.01, respectively.

(Berhanu, 2009). Similarly, Daniel et al. (2014) and Goshime (2019) also reported significant difference between treatments for PH and EH. In the combined analysis, the difference was significant for PH, EH, CLR and NE whereas the variance was non-significant for GLS, TLB and GY (Table 7). For yield, in contrast to the current finding for grain yield, O'Leary and Smith (1999) reported highly significant variation between three cropping systems (monoculture, maize-bean inter cropping and maize-clover inter cropping). For PH of maize, Zaeem et al. (2019) reported significant variance between cropping system with the overall higher value obtained for inter cropping with soybean in their study. The highest PH and EH was showed by genotype-6 and by genotype-5, respectively (Table 7).

Mean performance of the genotypes

Combined over seasons and cropping systems, mean maize grain yield performance of genotypes showed that the highest grain yield advantage was obtained from

Table 3. Mean grain yield (GY) (t ha^{-1}) and percent yield advantage of genotypes over the check (BH-543) for intercropping and sole cropping for participatory on-farm and on- station trials in the 2011 and 2012 cropping seasons.

Genotype	Mean GY					% GY advantage over the check			
	IC 2012	SC 2012	SC 2011	Combined	Bean	IC 2012	SC 2012	SC2011	Combined
Genotype-1	8.23^{a}	9.05^{a}	10.10^{a}	9.13^{a}	0.30^{a}	23	33	60	38
Genotype-2	8.47^{b}	8.56^{a}	8.10^{bc}	8.38^{ab}	0.14^{c}	27	26	29	27
Genotype-3	7.63^{a}	8.27^{a}	8.40^{bc}	8.10^{ab}	0.14^{c}	14	21	33	23
Genotype-4	8.24^{a}	7.10^{a}	8.40^{bc}	7.91^{ab}	0.19^{bc}	24	4	33	20
Genotype-5	8.07^{a}	10.05^{a}	9.10^{ab}	9.07^{a}	0.32^{a}	21	47	44	37
Genotype-6	7.67^{a}	8.07^{a}	8.20^{bc}	7.98^{ab}	0.26^{ab}	15	18	30	21
Genotype-7	7.67^{a}	8.25^{a}	6.90^{cd}	7.61^{ab}	0.27^{ab}	15	21	10	15
Genotype-8	6.97^{a}	6.75^{a}	8.20^{bc}	7.31^{b}	0.14^{c}	4	-1	30	11
BH-543	6.67^{a}	6.82	6.30^{d}	6.60^{ab}	0.32^{a}	-	-	-	-
Mean	7.74	8.26	8.21	8.01	0.23	-	-	-	-
CV (%)	16.58	10.98	11.99	18.31	25.99	-	-	-	-
LSD	2.22	1.57	0.78	1.57	0.1	-	-	-	-
SE	17.38	5.86	9.9	1.92	0.02	-	-	-	-

Columns with the same letter are not significantly different at P<0.05. IC = intercrop; SC = sole crop.

Table 4. Mean grain yield (t ha^{-1}), plant height (cm), ear height (cm), gray leaf spot (GLS), turcicum leaf blight (TLB), common leaf rust (CLR) and number of ears harvested (NE) of maize genotypes tested under sole cropping in 2011 cropping season.

Genotype	PH	EH	GLS	TLB	CLR	NE	GY
Genotype-1	240^{bc}	136^{bcd}	1.7^{bc}	2.0^{b}	2.0^{c}	89.3^{a}	10.1^{a}
Genotype-2	244^{ab}	148^{ab}	1.8^{bc}	2.0^{b}	2.5^{c}	69.3^{b}	8.1^{bc}
Genotype-3	246^{ab}	143^{abc}	1.7^{bc}	1.7^{b}	2.0^{b}	71.7^{b}	8.4^{bc}
Genotype-4	251^{ab}	153^{ab}	2.0^{ab}	2.0^{b}	2.0^{c}	71.0^{b}	8.4^{bc}
Genotype-5	262^{a}	159^{a}	1.5^{c}	1.7^{cd}	2.0^{c}	64.7^{b}	9.1^{ab}
Genotype-6	261^{a}	156^{ab}	1.7^{ab}	1.5^{d}	1.5^{d}	68.0^{b}	8.2^{bc}
Genotype-7	223^{bc}	111e	2.0^{ab}	1.8^{bc}	2.7^{ab}	68.3^{b}	6.9^{cd}
Genotype-8	235^{bc}	122^{cd}e	2.3^{a}	2.3^{a}	2.8^{a}	69.3^{b}	8.2^{bc}
BH-543	212^{d}	115^{d}e	-	-	-	63.7^{b}	6.3^{d}
Mean	241	138	1.83	1.88	2.19	70.6	8.21
Genotype	***	***	**	**	**	**	**
CV (%)	4.99	9.03	10.93	9.20	6.36	7.40	11.99
R^2	0.77	0.79	0.80	0.80	0.94	0.77	0.78
LSD	21	21.58	0.35	0.30	0.24	9.1	1.69

Columns with the same letter are not significantly different at P < 0.05.

genotype-1 followed by genotype-5 with 38 and 37% yield over the check, respectively. However, in 2011 under sole cropping, all new genotypes had higher grain yield advantage over the check (Table 3). In 2011, the highest yield (10.1 t/ha) and the lowest (6.3 t/ha) were observed for genotype-1 and genotype-9, respectively. Similarly, in 2012, under inter cropping, the highest grain yield advantage was obtained from genotype-2 with 27% over the check. The second and the third yield advantage was obtained from genoypte-4 and genotype-1, respectively. Under sole cropping in 2012, except for genotype 8, all genotypes showed yield advantage over the check with the highest grain yield advantage observed for genotype-5. The maize yield of genotype-1, genotype-2, and genotype-5 were consistent under both cropping systems in 2012 (Tables 4 to 6). Generally, in 2012 under both sole and intercropping and combined analysis genotypes had higher grain yield advantage over the check (BH-543) except for genotype-8 (Table 3).The overall mean performance was higher from sole cropping compared with the yield harvested from inter cropping with yield penalty of 0.45 t ha^{-1} due to inter cropping

Table 5. Mean grain yield (t ha^{-1}), plant height (cm), ear height (cm), gray leaf spot (GLS), turcicum leaf blight (TLB), common leaf rust (CLR) and number of ears harvested (NE) of maize genotypes tested under sole crop at Hawassa Research Station in the 2012 cropping season.

Genotype	PH	EH	GLS	TLB	CLR	NE	GY
Genotype-1	177^{ab}	89^{bc}	1.8^{abc}	2.7^{a}	1.8^{c}	11.0^{a}	9.05^{a}
Genotype-2	194^{a}	100^{ab}	2.2^{a}	2.7^{a}	2.5^{ab}	13.6^{a}	8.56^{a}
Genotype-3	188^{ab}	92^{ab}	2.2^{a}	2.7^{a}	2.3^{abc}	10.3^{a}	8.27^{a}
Genotype-4	181^{ab}	91^{abc}	1.8^{abc}	2.5^{a}	2.3^{abc}	6.1^{a}	7.10^{a}
Genotype-5	190^{ab}	105^{a}	1.7^{bc}	2.5^{a}	1.8^{c}	8.7^{a}	10.05^{a}
Genotype-6	191^{ab}	99^{ab}	1.5^{c}	2.5^{a}	2.0^{bc}	10.7^{a}	8.07^{a}
Genotype-7	177^{ab}	76^{c}	2.0^{ab}	2.8^{a}	2.8^{a}	8.3^{a}	8.25^{a}
Genotype-8	163^{b}	97^{ab}	1.7^{bc}	2.3^{a}	2.5^{ab}	9.7^{a}	6.75^{a}
BH-543	196^{a}	92^{ab}	1.8^{abc}	2.7^{a}	2.0^{bc}	10.7^{a}	6.82^{a}
Mean	184	93	1.85	2.59	2.24	9.9	8.26
Genotype	ns	*	*	ns	ns	ns	ns
CV (%)	9.53	9.43	13.37	12.79	15.71	44.81	1.92
R^2	0.39	0.58	0.66	0.3	0.62	0.42	0.29
LSD	30	15	0.43	0.57	0.61	7.6	3.33

Columns with the same letter are not significantly different at P < 0.05.

Table 6. Mean grain yield (t ha^{-1}), plant height (cm), ear height (cm), gray leaf spot (GLS), turcicum leaf blight (TLB), common leaf rust (CLR) and number of ears harvested (NE) of maize genotypes tested under intercropping at Hawassa Research Station in the 2012 cropping season.

Genotypes	PH	EH	GLS	TLB	CLR	NE	GY
Genotype-1	193^{a}	98^{a}	2.2^{a}	2.5^{a}	1.5^{a}	13.0^{a}	8.23^{a}
Genotype-2	197^{a}	102^{a}	1.8^{abc}	2.5^{a}	2.2^{a}	13.0^{a}	8.47^{a}
Genotype-3	190^{a}	98^{a}	1.7^{bc}	2.3^{a}	2.0^{ab}	12.7^{a}	7.63^{a}
Genotype-4	189^{a}	105^{a}	1.7^{bc}	2.7^{a}	2.0^{ab}	15.3^{a}	8.24^{a}
Genotype-5	184^{ab}	97^{a}	1.5^{c}	2.3^{a}	1.7^{bc}	9.7^{a}	8.06^{a}
Genotype-6	193^{a}	102^{a}	1.8^{abc}	2.3^{a}	2.0^{ab}	12.7^{a}	7.67^{a}
Genotype-7	173^{ab}	77^{a}	1.7^{bc}	2.7^{a}	2.2^{a}	11.3^{a}	7.67^{a}
Genotype-8	192^{a}	95^{a}	2.0^{ab}	2.7^{a}	2.0^{ab}	11.3^{a}	6.97^{a}
BH-543	155^{b}	91^{a}	1.8^{abc}	2.7^{a}	1.8^{abc}	9.0^{a}	6.67^{a}
Mean	185	96	1.80	2.52	1.93	12.00	7.74
Genotype	ns	ns	ns	ns	ns	ns	ns
CV (%)	9.38	17.04	14.17	12.74	14.88	36.04	16.58
R^2	0.48	0.29	0.51	0.40	0.54	0.34	0.38
LSD	30	28	0.44	0.56	0.50	7.49	2.22

Columns with the same letter are not significantly different at P < 0.05.

without considering the bean harvest in 2012 (Table 2). Similar to this, O'Leary and Smith (1999) obtained higher maize grain yield from sole cropping than maize inter cropped with bean and clover. The result showed the existence yield penalty due to inter cropping when we see the overall effect but individual there were some genotypes which had higher yield under inter cropping compared with performance under sole cropping and in line with this finding, Rusinamhodzi et al. (2020) reported as intercropping had maize yields reduction effect due to increased competition.

When genotypes were compared in terms of yield reduction/deviation due to intercropping, most the genotypes showed reduction/negative deviation except for genotype-4 and genotype-8 (Figure 1). The highest deviation to the negative side was observed for genotype-5 (-20%) and genotype-9 (-19%) while the least negative deviation was observed for genotype-2 (-1%). The highest deviation to the positive side was observed for genotype-4 (16%) followed by genotype-8 (3%). The

Table 7. Mean grain yield (t ha-1), plant height (cm), ear height (cm), gray leaf spot (GLS), turcicum leaf blight (TLB), common leaf rust (CLR) and number of ears harvested (NE) of maize genotypes combined data across seasons (2011 and 2012) and cropping systems (sole and inter-cropping).

Genotype	PH	EH	GLS	TLB	CLR	NE	GY
Genotype-1	203^{abc}	107^{ab}	1.8^{ab}	2.4^{ab}	1.8^{e}	37.7^{a}	9.13^{a}
Genotype-2	212^{ab}	115^{ab}	1.8^{ab}	2.3^{b}	2.2^{bc}	33.2^{b}	8.38^{ab}
Genotype-3	207^{abc}	112^{ab}	1.8^{ab}	2.3^{ab}	2.3^{abc}	30.7^{b}	8.10^{ab}
Genotype-4	207^{abc}	116^{ab}	1.8^{ab}	2.4^{ab}	2.1^{cd}	31.7^{b}	7.91^{ab}
Genotype-5	212^{ab}	120^{a}	1.56^{c}	2.2^{b}	1.8^{de}	29.1^{b}	9.07^{a}
Genotype-6	215^{a}	119^{ab}	1.6^{bc}	2.1^{b}	1.8^{de}	29.2^{b}	7.98^{ab}
Genotype-7	191^{c}	88^{c}	1.8^{ab}	2.4^{ab}	2.6^{a}	29.3^{b}	7.61^{ab}
Genotype-8	196^{bc}	104^{b}	2.0^{a}	2.4^{ab}	2.4^{ab}	29.2^{b}	7.31^{b}
BH-543	175^{d}	92^{c}	1.8^{ab}	2.7^{a}	1.9^{de}	9.2^{c}	6.60^{ab}
Mean	203	109	1.8	2.4	2.1	29.6	8.01
Genotype	*	**	ns	ns	**	**	ns
CS	**	**	ns	**	**	**	ns
CS*genotype	ns	ns	*	ns	*	**	ns
R^2	0.86	0.85	0.49	0.68	0.69	0.98	0.38
CV (%)	7.54	11.57	15.5	13.1	13.88	15.56	18.31

Columns with the same letter are not significantly different at P < 0.05.

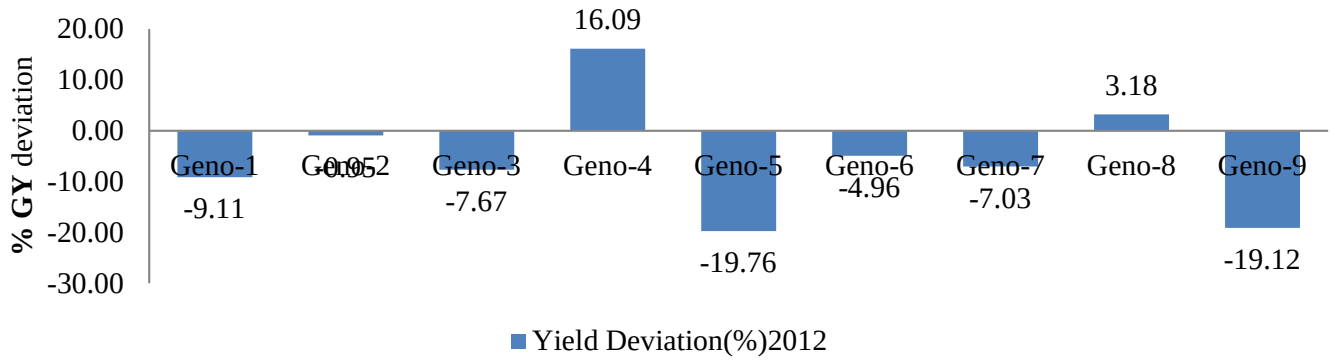

Figure 1. Percent grain yield deviation of genotypes for sole cropping system over the corresponding yield under intercropping system at Hawassa research field in the 2012 cropping season.

higher deviation to the negative side indicated that the genotypes were affected by common bean in intercropping whereas the genotypes which had yield deviation to the positive side indicated that the maize genotypes are suitable for inter cropping or not affected by intercropping (Figure 1). However, the common bean yields obtained from intercropped with genotype-4 and 8 were the least compared with common bean yields obtained from intercropping with other genotypes (Table 3). Higher common bean yield obtained from intercropped with genotype-1, genoype-5 and BH-543 could in part be due to good leaf structure/architecture of maize genotypes resulting to high radiation interception and hence higher common bean yields or common bean had a better competitive advantage over the genotypes (Table 3).

Land equivalent ratio

The overall LER was evaluated to derive land benefits associated with intercropping of maize genotypes and the bean variety Hawassa-Dume. The LER in intercrops ranged from 0.86 to 1.19. Only three genotypes, genotype-2, genotype-4, BH-543, and genotype-8 had LER of 1.02, 1.19, 1.04 and 1.06, respectively, which is greater than 1 (Table 8). The LER greater than 1 suggests that there is greater land area requirement for the monoculture system or greater relative yield for intercropping of maize genotypes with common bean

Table 8. Land equivalent ratio (LER) of maize common bean intercropping systems for maize genotypes tested at Hawassa research field in the 2012 cropping season.

Genotype	Maize		Common bean		LER
	Sole	Inter-crop	Sole	Inter-crop	
Genotype-1	9.05	8.23	5.63	0.30	0.96
Genotype-2	8.56	8.47	5.63	0.14	1.02
Genotype-3	8.27	7.63	5.63	0.14	0.95
Genotype-4	7.10	8.24	5.63	0.19	1.19
Genotype-5	10.05	8.07	5.63	0.32	0.86
Genotype-6	8.07	7.67	5.63	0.26	1.00
Genotype-7	8.25	7.67	5.63	0.27	0.98
Genotype-8	6.75	6.97	5.63	0.14	1.06
BH-543	6.82	6.67	5.63	0.32	1.04

Table 9. Genotypes selected by farmers and selection criteria during participatory maize genotypes selection.

Genotype	Desirable characters by which genotypes selected
Genotype -1	Earliness, Stay green, tolerant to diseases, Narrow leaf, Good grain filling, uniformity
Genotype -2	Cob size, tolerant to diseases, uniformity,
Genotype -3	Stay green, tolerant to diseases, Good grain filling
Genotype -8	Earliness, cob size

variety Hawassa-Dume. For instance, LER of 1.19 observed for genotype-4 indicates that there is 19% requirement for the monocropping system or 19% greater relative yield for the intercropping of genotype-4 and Hawassa-Dume. Previous studies on maize common bean intercropping in Ethiopia reported high LER of intercropping system (Walelign, 2014; Tolera et al., 2005; Assefa et al., 2016) and with maize-soybean in Indonesia (Yuwariah et al., 2018). The LERs of intercrops between maize and common bean can save lands up to 48 and 55%, which would have required as additional land for monoculture of each crop (maize or common bean) if not intercropped (Nassary et al., 2019). However, most genotypes in this study had <1 LER indicating that the land productivity will be greater when genotypes are planted in monocropping than intercropping even if the difference was not that much high (Table 9). This is consistent with non-significant difference for cropping system x genotype interaction indicating that maize genotypes responded similarly for cropping systems (Table 7). This study highlights that varieties selected based on monocropping performance may not necessarily do well under intercropping system.

Genotypes are grouped into two mega environments. SC2011 and IC2012 grouped together in one mega environment and SC2012 grouped in the other mega environment (Figure 2). Genotype-1 was the ideal genotype followed by genotype-5 (Figure 3). From ranking biplot graph, genotype-1 and genotype-5 showed better performance in yield and were highly responsive to cropping systems. Genotype-2, genotype-3 and genotype-6 were highly stable genotypes compared with other genotypes (Figure 3).

Conclusion

The results of this study showed significant variation among genotypes for yield and other traits. Genotypes used in this study were developed for monocropping system and hence most genotyes had lower LER indicating that they are not compatible to incropping system. However, genotype-4 and genotype-8 demonstrated higher compatability to the intercropping system providing an opportunity for famers to grow under both cropping systems. In regions with maize commonly grown as an intercrop, it is of paramount importance to evalate maize genotypes for their compatibilty to intercropping system at early stage of genotype evaluation. Some morphological traits such as canopy architecture and tolerance to high planting density could be considered for variatal selection. The results of this study highlights the need for particpatory varietal selection where farmers criteria could also be met for fast-track realease and better adoption of maize varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

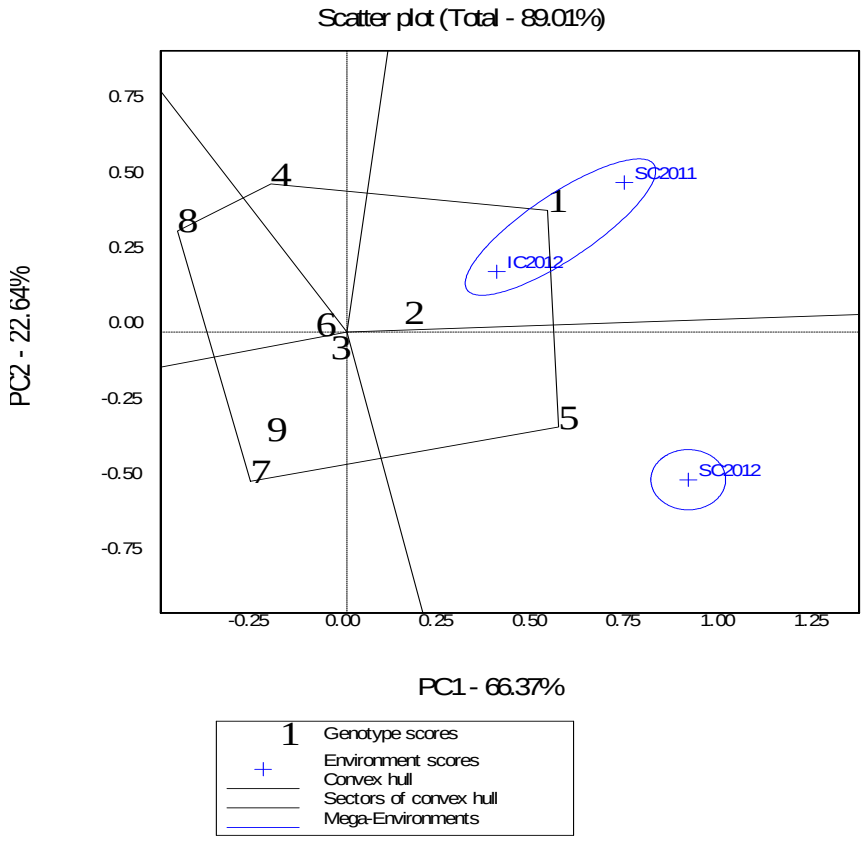

Figure 2. Genotypes mega environment classification.

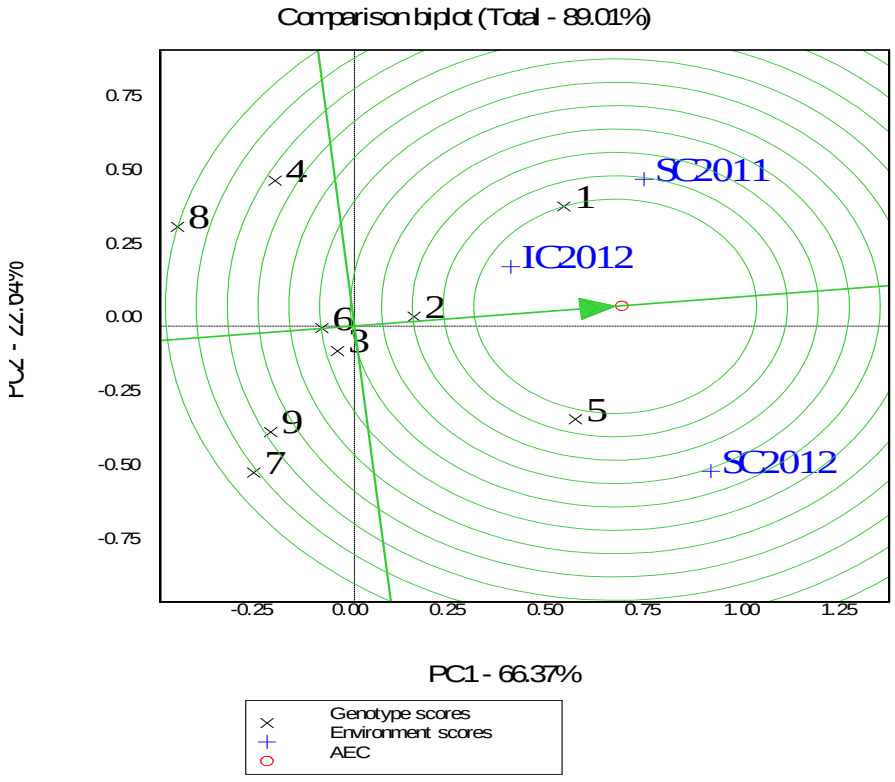

Figure 3. Genotypes identification for their performance and stability.

ACKNOWLEDGEMENTS

This research was funded by Australian Centre for International Agricultural Research (ACIAR) through Sustainable Intensification of Maize-Legume Cropping Systems for Food Security in Eastern and Southern Africa (SIMLESA) project. They thank Mr. Zerihun Beshir, Mrs. Maedin Tadesse and other Hawassa maize team for their assistance in data collection.

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Vol. 12(3), pp. 228-236, July-September 2020
DOI: 10.5897/JPBCS2020.0904
Article Number: 4B89B2264645
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Evaluating the pollen proofing of nonwoven synthetic fabric pollination control tents for sugar beet

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Received 22 May, 2020; Accepted 3 August, 2020

Three pollination control tents (PCTs) made from novel nonwoven synthetic fabrics with more open pore structure (DWB10, DWB23 and DWB24) were compared with the standard DWB01 fabric for pollen proofing in sugar beet (*Beta vulgaris* L.) at the research station of Lion Seed Ltd Essex, UK in 2019. PCTs of 63.5 x 63.5 cm footprint accommodated single potted plants. A completely randomised trial with five replications including an open pollinated control was conducted using cytoplasmic male sterile line 1TM37. Analysis was computed for (a) full data and (b) excluding three DWB23 defective tents. Differences among treatments were non-significant for all morphological traits except for number of secondary branches in (a) only. There was thus no micro-climatic difference among treatments for morphological traits of the 1TM37 CMS line. Among the seed related traits, 1000-seed weight and 10-day germination (%) were significant between treatments in (a) but only 1000-seed weight in (b). The mean 1000-seed weight was significantly higher for the open control than all other PCT treatment means which did not differ significantly from zero. Therefore, all four fabrics of PCTs were equally pollen proof in preventing pollen contamination. It is concluded for the first time that mini-tents of these novel nonwoven fabrics, engineered for both larger pores for air permeability and fibre architecture to prevent pollen transmission, adequately eliminated cross-pollination while maintaining ambient environmental conditions and are effective for sugar beet breeding. The PCT technology may be equally usefully deployed in other traditional, commercial and fibre crops for hybrid seed production.

Key words: Sugar beet, nonwoven synthetic fabric, pollination control tent, male sterility.

INTRODUCTION

Male sterility (MS) is the result of non-functional pollen in plants (Chen and Liu, 2014). Sugar beet hybrid seed production uses cytoplasmic-genetic male sterility (McGarth and Panella, 2018) by involving three parental lines: a cytoplasmic male-sterile (MS or A-line) family, an O-type maintainer family (also called B-line) and a pollinator with restorer gene (R-line). Commercial hybrid seed production is performed in open fields, where the pollinator and MS family (F1 of A-line and O-line) are grown next to each other. Pollination occurs in the following year once the parental lines have overwintered and vernalised for transition from vegetative to

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reproductive phase. Reproduction of the MS family (A-line) is achieved through pollination by an O-type maintainer that is equivalent in the nuclear genome; the seed so produced on the male sterile plants is intended to faithfully reproduce male sterile plants of the A-line (Brown et al., 2014). The male sterile families in sugar beet are phenotypically true-breeding and near-homozygous for practical purposes with all plants looking alike and any phenotypic observable variation among plants of the family is regarded as environmental or non-genetic although some of this variation may be residual genetic. We exploit this feature of male sterile family 1TM37 in the present investigation.

Sugar beet (*Beta vulgaris* L.) is pollinated by wind and possibly by insects (Bodnar, 2010). According to Hecker (1988) the mean diameter of pollen of diploid (2x) strains of sugar beet was 20.8 µm (19.3 to 22.5 µm) and that of auto-tetraploid (4x) to be 25.9 µm (23.4 to 27.4 µm). Clifton-Brown et al. (2018) reported average size of sugar beet pollen to be ~20-25 µm. The pollen may be carried up to 1200 m (Darmency et al., 2009). The Animal and Plant Health Agency (2016) in its Technical Standard Supplements laid out 1000 m isolation distance from any pollen source of genus Beta for producing basic or certified seed. When breeding sugar beet and other crops with small pollen, breeders typically rely on distances for seed production or open ended poly-tunnels angled away from the prevailing wind. Other methods such as pollination control bags, isolation chambers with controlled conditions and tents may be used for breeding operations. However, all these methods have their own limitations and advantages.

Commonly, plant breeders create artificial isolations with pollination control bags (PCBs) made of various materials. Commonly used PCBs made of paper, cellulose or polyethylene are cheap but are easily damaged by birds, wind and bad weather. Further, transparent film PCBs create higher temperature in them especially in the hot season and may adversely affect the pollination outcome (Gitz et al., 2015; Scheffert et al., 2016, 2018, 2019). More recently, specially developed nonwoven synthetic materials have been used for PCBs for their greater strength against bird damage and inclement weather conditions of heavy rains and wind, greater air permeability, lower moisture absorption, re-usability and pollen proofing (PBS International, 2020a, b, c). Such bags have been shown to have advantage over the controls for greater seed harvest by Gitz et al. (2015), Schaffert et al. (2016, 2018, 2019) and Gaddameedi et al. (2017) in sorgum; Clifton-Brown et al. (2018) in sugar beet, wheat, Arabidopsis and Miscanthus; Hayes and Virk (2016) in Miscanthus; Vogel et al. (2014) and Adhikari et al. (2015) in grasses; and Bonneau et al. (2017) in oil palm. Anecdotal evidence suggests that certain nonwoven materials when used as pollination control in very hot climates can result in the plant getting too warm affecting plant health and pollen viability. Therefore, finding more open nonwoven materials having greater air flow but retaining pollen proofing is important in increasing pollination performance. In this study, we uniquely and purposely included pollination control tents (PCTs) made from nonwoven synthetic fabrics designed to have greater pore sizes than the standard for air permeability, but a complex fibre arrangement to optimise pollen proofing ability in sugar beet, with the ultimate goal of using them in hybrid seed production and other breeding operations.

Comparing performance of stable cytoplasmic-genetic male sterile (MS) line in mini-isolation tents with an open control should provide tests for the following hypotheses:

1) The morphological plant traits of the MS line under mini-tents and in open control do not perform differently, H0. Significant variation indicates differential micro-environment within tents from the control and rejects H0.
2) The mean number of seeds set on MS plants in the tents should be zero; H0. In the event of seed set being significantly higher than zero there ought to be pollen contamination and null hypothesis stands rejected.

Usually, pollination bag materials have porosity smaller than the pollen size to avoid contamination (Hayes and Virk, 2016). However, in the present study, we use for the first time, specially designed single plant mini-tents made from nonwoven synthetic fabrics with greater strength and air permeability including pores larger than the sugar beet pollen to be controlled. As such this investigation lays a foundation for a new research area on pollination tents which has been sparingly investigated. It opens up new avenues for enhancing pollination performance in crop breeding. The major objectives were to: (i). Evaluate PCTs if they create within them a micro-climate different from the open control and (ii). Establish the pollen proofing ability of different fabrics with more open structures for use in sugar beet breeding.

MATERIALS AND METHODS

The germplasm used for the experiment was Lion Seeds family 1TM37. This is an established cytoplasmic-genetic male sterile line which has shown excellent performance in the past. There were five treatments of pollination control tents:

1. Open control with no cover. This treatment indicated the adequacy of pollen pressure in the field.
2. Currently used standard control at Lion Seeds as classic duraweb® or DWB01.
3. Three new nonwoven synthetic fabric tents: DWB10, DWB23 and DWB24.

The specific characteristics of new nonwoven fabrics specially obtained from PBS International, Scarborough, UK, in comparison to presently used DWB01 are given in Table 1. DWB01 is a standard nonwoven fabric used in sugar beet which has thin filtration layer. It is heat bonded making the fabric smooth and easy to working with. On the other hand, the new nonwoven fabrics are spun-bond and thicker with a more complex filtration layer (Table 1). This gives the fabrics greater strength. All new fabrics have

Table 1. Physical properties of nonwoven fabrics used in pollination control tents (PCTs) including the standard DWB01.

Property	Measure	DWB01	DWB10	DWB23	DWB24
Polymer		Polyester	Polyester	Polyester	Polyester
Manufacturing technique		Heatbond	Spunbond	Spunbond	Spunbond
Thickness	mm	0.20	0.33	0.40	0.40
Mass per unit area/ weight	g m^{-2}	101	100	110	110
Air permeability	$l/m^2/s$	110	550	1470	1218
Light transmittance	% (350-800 nm)	c. 35%	c. 35.5%	c. 39%	c. 39%
Max pore size	microns	31.7	152	219	205
Fibre cross section		Simple	Simple	Complex	Complex

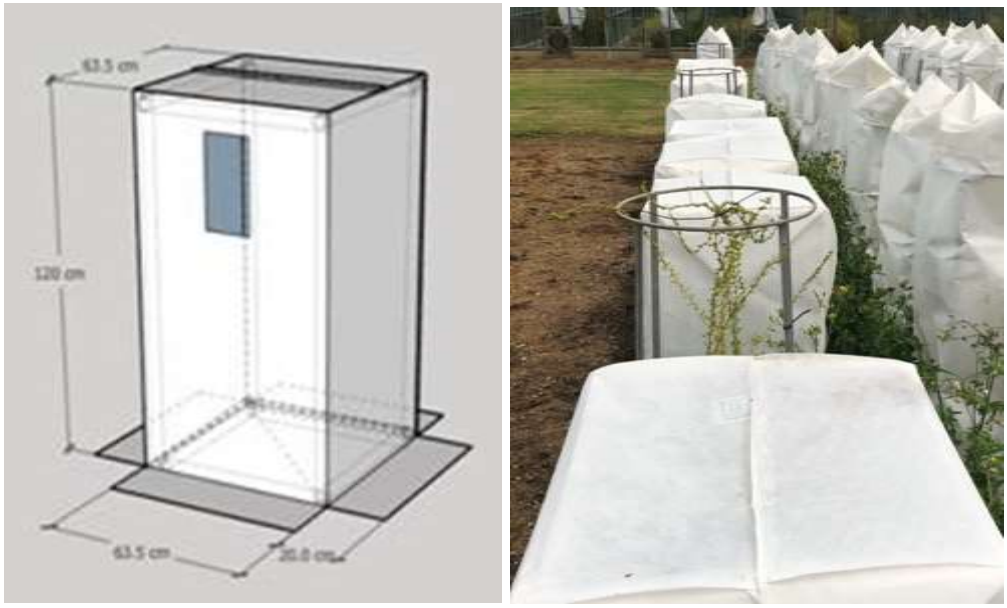

Figure 1. Design of mini-tent (left) and field arrangement of treatments and controls (right).

similar mass per unit area but greater pore size that makes them more air permeable for better temperature and humidity control. Light transmission in the range of 350 to 800 nm was 4% higher for DWB23 and DWB24 than the standard (Table 1).

Five replicate plants were used in each treatment to cover the possibilities of failures from damage by accident or a plant dying and to provide enough degrees of freedom for a valid statistical analysis. The design used was a completely randomised design with all plants of all five treatments being randomised together. Mini-tents with dimension of 63.5 x 63.5 cm, 120 cm height plus a 20 cm skirt at the base were designed for the experiment by PBS International (Figure 1). Each mini-tent accommodated a single potted plant (Figure 1). Tents were placed 50 cm apart in a single line of 22.1 m length that ensured equal pollen pressure over the whole experiment.

Single plants of male sterile line 1TM37 grown in pots were placed inside mini-PCT enclosures of various fabrics. Pollen pressure was generated by flowering sugar beet plants in adjacent poly-tunnels surrounding the experiment. To generate sufficient pollen pressure, the mini-tents were located in the down- wind direction from the pollen producing plants in the prevailing wind(s). Any seed observed on male sterile plants must result from the foreign pollen that might pass through the cover-fabric of the tent. The covers were adequately fixed to prevent pollen entry underneath the skirt and the frames adequately anchored to minimise the chance of wind damage. The frames were anchored to the ground on at least two sides and each side of the cover was anchored with one or more sandbags (Figures 1 and 2).

The plants were enclosed on 10th of June 2019 to ensure their isolation before flowering. The plants were harvested on 9th August 2019 and various data were collected.

Data collection

Length of primary stem was measured in centimetres and secondary branches counted. Cut weight of the whole plant was recorded in grams. Seeds were separated and weighed for each plant in grams and divided into those <2.8 mm ø (diameter) and >2.8 mm ø (Figure 3). The latter were taken as prospective seeds and weighed. These were divided into four replicates of 100 seeds each and weighed separately and then 1000-seed weight was

Figure 2. Skirt of each mini-tent dug-in to avoid wind-borne contamination from below.

Figure 3. Examples of seed return from the control and one of the pollination control bag treatment. Left: Seeds recovered from one of the unprotected controls were better formed. This lot returned just over 13 g in weight but achieved 70% germination. Right: DWB24 returned from one of the four 100-seed replications a weight of just over 13 g of seed sized material but only 1.5% germination.

derived. Each of these four replicates of 100 seeds per plant was sown for germination test. Final germination (%) over four replicates was computed after 10 days.

The amount of seed from an un-bagged plant can only be best assessed by considering the results from the controls because the amount varies from season to season. Sugar beet breeders usually expect at least 10 g with 75% germination – but much more is possible in good conditions. The germination certainly is an important aspect that needs to be taken into account. What is recorded as seed weight is really the mass of 'seed sized material'

that was recovered by the sieving procedure. In many cases, aborted flowers will dry down into small seed shapes and are recovered but fail to germinate as they are not real, viable seed.

Implied number of seeds (IS)

Any seed-like material with >2.8 mm ø (diameter) was taken as probable seed and weighed together for each plant in grams (X).

Four samples of 100 seeds were taken and weighed, and weight for 1000 seeds was extrapolated from weight of four hundred seeds in grams (Y). The implied number of seeds (IS) was computed using X and Y:

$$IS = \left(\frac{X}{Y}\right) * 1000$$

Implied total number of germinated seeds (ISG)

Number of seeds obtained from materials that looked like seed may be misleading. If it were a viable seed then it should germinate. Therefore, the number of implied seeds that could germinate by 10 days was computed to find out the actual number of seeds per plant as:

$$ISG = IS * \left(\frac{Germ\ \%}{100}\right)$$

There were three cases of damage to pollination tent DWB23 on cage number 52, 62 and 70. Seams of 5 cm (at position 52) to 30 cm length (62 and 70 positions) were observed opened suspecting that they may allow pollen through. There was overgrowth of plant at position 62 that hit the top of cage forcing the seam open. In order to assess and exclude the effect of open seems on three plants of DWB23 treatment the statistical analysis was performed twice; once for the whole data set (a) and then by excluding (b) three defective cases of DWB23.

Statistical analysis of individual plants data and germination percent was performed following the analysis of variance technique described by Sokal and Rahlf (2011) using MINITAB17 statistical package. Standard errors of mean (SE) and *lsd* at 5% were computed as *lsd* = √2.*SE.t* value at 20 df (2.086). Fisher's 'Least significant difference (*lsd*)' was used for pair-wise comparison of treatment means and significantly different means were labelled with different letters.

RESULTS AND DISCUSSION

In the context of our experiment seed on CMS 1TM37 under mini-tents can develop in two ways: (i) as a result of pollination by foreign pollen that passed through the tent fabrics and (ii) due to occasional failure of male sterility resulting in maternally produced selfed seeds. The occurrence of (ii) is very rare phenomenon and, in general, we do not expect seed set on CMS plants without pollination from extraneous pollen. The progeny of seeds from (i) will differ from the plants of mother family but that of (ii) will be phenotypically similar to the plants of the near-homozygous mother family within error limits. We anticipated confirming, if the seeds in tents resulted from outcrossing (i) or selfing (ii) through molecular marker studies by taking leaf samples of each of the trial plants before bagging for comparing with the progeny of seeds produced on CMS plants. However, this could not be accomplished and the simple criterion of the number of viable seed set under bagging was considered to be an indication of pollen contamination.

Analysis of data proceeded in two stages: (a) for the full data set and (b) for the data set excluding three defective cages for DWB23 and we shall refer to it in this way below.

Morphological plant traits

We do not expect significant variation for phenotypic traits among plants in the near- homozygous male sterile family 1TM37. The full data analysis (a) showed non-significant variation among treatments for cut plant weight and primary stem length but significant for number of secondary branches (Table 2). However, when analysis (b) was performed it turned out to be non-significant (Table 2). The examination of mean values for number of secondary branches shows that the significance among treatments in analysis (a) was due to the significantly lower performance of plants in the control than in PCT treatments, which were on par (Table 3). The number of secondary branches was on average 65% higher under cover than the control. It could be specific response of sugar beet to reduced light under cover. Wang and Feng (2004) reported typical leaf morphological responses to different light conditions in two species (*Eupatorium adenophorum* and *Gynura* sp.). At low light levels, plants enhanced light interception by means of increased biomass allocation to leaves and formation of large, thin leaves with high specific leaf area, leading to a high leaf area ratio. With a decrease in light intensity, plant of both species grew taller and produced more branches to intercept more light energy.

The mean number of secondary branches for DWB 23 in analysis (a) was 28.20 but for (b) it was 26.50. This reduction in number of branches reduced variation among treatments to a non-significant level in analysis (b). As seams of three mini-tents of DWB23 were open they encouraged plants to overgrow and push at the top. This perhaps is the physiological effect of shading. Plants growing in shade often tend to grow taller than they would grow outside under full sunlight. However, this is at the expense of energy and resources that could result in thinner main stem with fewer leaves or weaker roots and lower seed amount (Kniss and Schambow, 2016).

The mean values following analyses (a) and (b) showed (Table 3):

1. Mean number of secondary branches for the open control was significantly lower than the standard DWB01.
2. Mean number of secondary branches of the three new fabrics (DWB10, DWB23 and DWB24) were on par and non-significantly different from DWB01.

It can be concluded from the non-significant difference

Table 2. Mean squares from analysis of variance for different measured and derived traits. The probability (P) values are given in brackets.

Item	df	PW (g)	PSL (cm)	SB (No.)	TSW (g)	>2.8 mm seed weight (g)	1000 seed weight (g)	IS (No.)	10-day germ. (%)	ISG (No.)
					Full data set (a)					
Cover	4	6588(0.62)	890.2(0.51)	122.7 0.03*)	28.72(0.46)	27.27 0.41)	35.02(0.00**)	551123(0.52)	650.0 (0.04*)	105384(0.06*)
Error	20	9715	1048.0	35.2	30.15	26.09	2.28	666734	205.9	38228
Total	24									
					Excluding defective three mini-tents for DWB23 (b)					
Cover	4	6545(0.67)	835.7(0.61)	78.74(0.13)	28.45 0.47)	29.31(0.38)	33.99 (0.00**)	424245 (0.61)	650.5 (0.07)	105684(0.09)
Error	17	10940	1220.1	38.41	30.86	25.94	2.53	614458	240.8	44736
Total	21									

*P<0.05; **P<0.01. PW= plant weight; PSL =primary stem length; SB =secondary branches; TSW=total seed weight; IS = Implied number of seeds; ISG =implied number of seeds germinated.

Table 3. Fitted mean values for morphological and seed-related traits.

Cover type	PW (g)	PSL (cm)	SB (No.)	TSW (g)	>2.8 mm Seed wt (g)	1000 seed wt (g)	IS (N0.)	10-day germi (%)	ISG (no.)
					Full data set (a)				
Control	240.8	149.4	15.00^{B}	13.91	10.17	9.885^{A}	1000	26.55^{A}	339.7^{A}
DWB01	172.8	123.4	23.20^{A}	7.53	3.67	3.295^{B}	946	0.10^{B}	0.9^{B}
DWB10	213.2	138.2	23.80^{A}	11.11	7.51	4.420^{B}	1527	0.15^{B}	1.9^{B}
DWB23	217.2	119.8	28.80^{A}	12.14	6.87	3.900^{B}	1632	2.96^{B}	40.3^{B}
DWB24	271.0	146.4	23.20^{A}	12.45	7.78	4.800^{B}	1563	1.55^{B}	25.7^{B}
SE mean	44.1	14.5	2.65	2.46	2.28	0.676	365	6.42	87.4
LSD 5%	NS	NS	7.82	NS	NS	1.99	NS	18.94	257.83
					Excluding defective three mini-tents for DWB23 (b)				
Control	240.8	149.4	15.00^{B}	13.91	10.17	9.885^{A}	1000	26.55^{A}	339.7^{A}
DWB01	172.8	123.4	23.20^{A}	7.53	3.67	3.295^{B}	946	0.10^{B}	0.9^{B}
DWB10	213.2	138.2	23.80^{A}	11.11	7.51	4.420^{B}	1527	0.15^{B}	1.9^{B}
DWB23	220.0	112.5	26.50^{A}	12.32	5.08	3.570^{B}	1422	0.90^{B}	11.00^{B}
DWB24	271.0	146.4	23.20^{A}	12.45	7.78	4.800^{B}	1563	1.55^{B}	25.7^{B}
SE other	46.8	15.6	2.77	2.48	2.28	0.711	351	6.94	94.6
SE DWB23	74.0	24.7	4.38	3.93	3.60	1.120	554	11.00	150.0

NS= Non-significant; PW= plant weight; PSL =primary stem length; SB =secondary branches; TSW=total seed weight; IS = Implied number of seeds; ISG =implied number of seeds germinated. Means that do not share a letter are significantly different.

among mean values of PCT treatments for any morphological trait that the hypothesis of no micro-climatic difference among nonwoven synthetic fabrics and control is accepted. Thus, PCTs did not alter the plant environment to any significant effect on performance. However, Trammell et al. (2020) reported that the average temperature within tents was higher with lower average humidity than the open control but it produced a microclimate that gave 36% higher seed yield and disease free seeds. In the present study, because of the near-homozygous status of male sterile family all plants in it responded similarly to the changed environment under PCT covers.

Seed related traits

The sieving process for the seeds resulted in different type of seed sizes (Figure 3). It can be seen in Figure 3 that from one of the unprotected controls the grains were larger and better formed with the same counting board for size reference compared with those from DWB24. While about 13 g seed from control achieved 70% germination the same amount from DWB24 in four 100 seed replicates returned only 1.5% germination (Table 3). The mass that looked quite convincing as seed was mostly non-seed inert plant material that was not viable in DWB24.

Of special interest are seed-related traits since seed on plants in the open control resulted from cross pollination and that on plants in tents from pollen contamination or by parthenogenesis without contribution of pollen that could pass through the cover (Zhuzhzhalova et al., 2016). Of the six seed related traits the full analysis (a) showed significant variation between treatments for 1000-seed weight and 10-day germination (%) only (Table 2). However, in the reduced analysis (b) the significant variation was retained for 1000-seed weight only as the 10-day germination (%) became non-significant (Table 2). Therefore, we shall discuss the 1000-seed weight further.

Looking at mean values in Table 3 we find that 1000-seed weight for the control was significantly and 140% higher than mean of all PCT treatments together in (a). The high mean seed weight in control must arise from viable seeds resulting from cross pollination (Table 3). The comparison among treatment means showed that:

i. Open pollinated control's mean seed weight was significantly higher than the standard DWB01.
ii. New fabrics (DWB10, DWB23 and DWB24) all had mean seed weight on a par between themselves and with the standard, DWB01.

Therefore, the significant variation between treatments in both analyses of variance largely arose from the deviant seed weight of the control. However, the smaller mean seed weights from all four PCT treatments were significantly higher than zero value when compared with the SE and LSD. This means our null hypothesis of no differences among treatments does not hold at this stage. The important question, however, arises: did the total seed mass representing seed weight contain viable seeds? This could be verified through germination test.

The implication of heavier and real viable seeds in open control is further reflected in its out-rightly higher 10-day germination (27%) and implied seed number at 10-day germination (340) in both analyses. On the other hand, germination for the PCT treatments in analysis (a) ranged from 0.10 to 3% with implied average seed number at 10-day germination ranging from 0.9 to 40.3. The highest value of 40.3 implied seeds belonged to DWB23 which might be the result of contamination in the defective tents. The analysis (b) excluding the defective tents showed germination range of 0.10 to 1.6% and implied seed number at 10-day germination ranging from 0.9 to 26. When we tested the mean values of germination (%) and implied seed number at 10-day germination in both analyses (a) and (b) there was no mean value that was significantly higher than zero (Table 3). All germination and implied seed number means were as good as zero.

The between plant variances within treatments were highly and positively correlated with treatment mean values for 10-day germination ($r = 0.99$; $P<0.01$) and implied seed number at 10-day germination ($r = 0.99$; $P<0.01$) but non-significantly correlated for number of secondary branches ($r = -0.42$; $P>0.05$) and 1000-seed weight ($r = 0.66$; $P>0.05$). The within variances of treatments did not differ significantly on a Bartlett's Chi-square test (T) for number of secondary branches ($T= 0.03$ at 4 df; NS) and for 1000-seed weight ($T= 0.54$ at 4 df; NS). Apparently, there was no differential response of plants of MS line for these traits within tents and outside in the open.

Statistically there was zero mean seed set in all PCTs. It proved our hypothesis of no contamination by foreign pollen in all the pollination control tent fabrics and all fabrics of pollination tents were pollen proof. It may be recognised that maximum pore size of the PCT materials was greater than the average pollen size of ~20-25 µm in sugar beet but the structural arrangement of the fabric resulted in no contamination from outside pollen. Clifton-Brown et al. (2018) also observed no pollen contamination on plants covered with nonwoven fabric pollination control bags from externally placed red hypocotyl sugar beet variety. This is attributed to physical complexity of nonwoven spun-bound fabrics that have torturous path through the fibrous mesh ensuring that the entry of external pollen is restricted. Wang and Gong (2006) reported that the pore structure, pore size distribution, air permeability, and fabric area density of the 3D thermally bonded nonwoven filter samples consisted of multiple filtration layers of interconnected pores and tortuous pore paths through the fabric

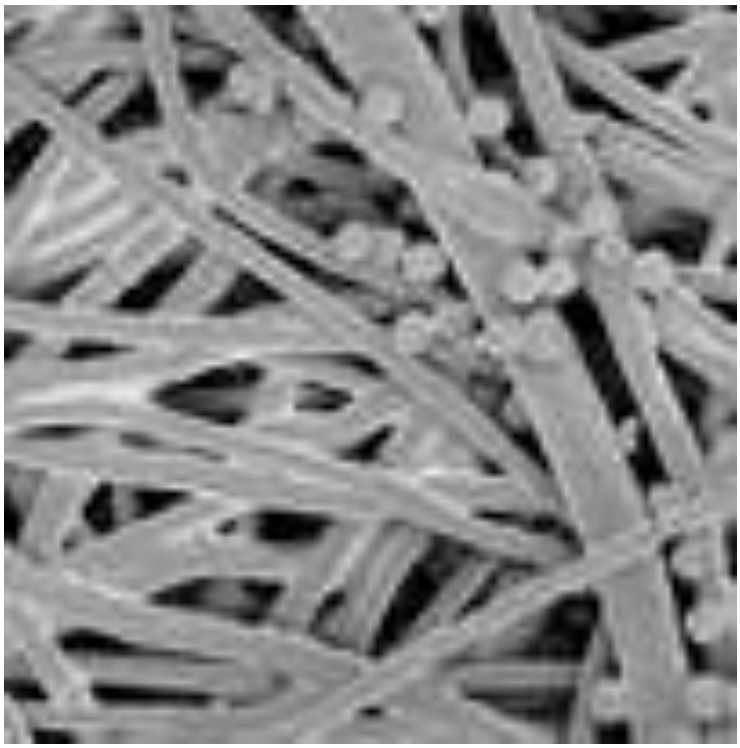

Figure 4. Scanning electronic microscope image showing arrangement of fibres of nonwoven spun-bond fabric layer that creates a torturous filtration path for pollen grains and hence reduce contamination despite larger pore size.

thickness. This torturous but purposefully effective filtration of pollen through larger pore size may not assure impermeable conditions yet it provides a trade-off in pollination performance (Figure 4). With the tested fabrics of PCTs we clearly find an acceptable filtering level of co-optimisation of pollen exclusion in the present experiment. However, unlike Clifton-Brown et al. (2018) who tested the nonwoven fabrics in glasshouse we have established their pollen proofing ability in field conditions although some of the previous studies suggested that maximum pore size be kept under the pollen size of the crop (Vogel et al., 2014).

The nonwoven fabric pollination mini-tent technology is relatively new for sugar beet. This study, for the first time, tested novel nonwoven fabrics in the form of pollination control tents in sugar beet breeding. None of the fabrics caused significant deviation in their micro-climate that could adversely affect biological performance of plants grown under their covers. However, reduced light under tents increased number of secondary branches (Wang and Feng, 2004). Further, all new fabrics with larger pores and hence more air-permeability were pollen proof. It therefore creates a possibility of optimisation of pore size and pollen filtering in different crops in future developments of fabrics. However, future studies in sugar beet should confirm the pollen contamination by studying the progeny of seed set on male sterile family to establish its maternal or cross-contamination origin. Further studies will confirm it by using a dominant morphological marker such as red hypocotyl pollen parent or molecular markers or simply studying the quantitative variation for different traits of the progeny of seeds in comparison with the mother parent.

The present study though carried out in one season at one location indicates that the tent technology can be usefully deployed for maintaining genetic integrity in various breeding operations such as attempting single crosses, generation advance of progenies, seed increase of selected progenies for multi-locational testing and providing multiple isolations simultaneously over limited space in a season. However, before full confidence is placed on the technology more research involving multi-environmental trials will be desirable. Further, exact categorisation of seed set on male sterile lines, however small in number, whether of maternal or contamination origin will establish pollen proofing ability of nonwoven fabrics beyond doubt. Therefore, experiments will continue to resolve these issues in the future. The simple sequence repeats (SSR) will be used to distinguish maternal and outcross seeds based on the amplified alleles of the progeny and the seed parent (Adhikari et al., 2015).

Recent advances in tent technology allow covering of larger areas with bigger tents and with modular extendable provisions (Trammell et al., 2020). Flexibility in size from mini tents to a modular design allows units of 1.5 x 3 m cubes (of 1.5 or 2 m tall) to be joined together to make larger structures as intended, for example 1.5 x 6 m, 3 x 3 m or bigger (PBS International, 2020c).

Flexibility in sizing the tent-covered area will go a long way in adjusting the protected area to breeders' requirement in any season for any crop. Further developments must be directed towards stronger but lighter frames that are robust in bad weather and easy to transport. More research on new nonwoven synthetic fabric covers needs to be conducted for universal extension of technology. The present study has opened up new avenues of research on PCT technology for different crop plants and situations with possibilities of use in hybrid seed production in traditional, commercial and fibre crops. An in-depth economic analysis of technology needs to be conducted for its wider use in seed production and breeding.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thankfully appreciate Ian Hansell and Naomi Reece-Ford from Lion Seeds Ltd, Essex CM9 6SN, UK for helping in the conduct of trial, and Rob Coy and Stuart Carter from PBS International, Scarborough YO11 3UP, UK for standardising and providing the single plant mini-pollination tents.

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Vol. 12(3), pp. 237-245, July-September 2020
DOI: 10.5897/JPBCS2019.0810
Article Number: 682274D64867
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Performance and adaptability of common bean genotypes at different agro-ecological environments in Kagera region

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Received 8 March, 2019; Accepted 19 August, 2020

Sixteen common beans (*Phaseolus vulgaris* L.) genotypes were used to study the genotype by environment interaction and grain yield stability. The randomized complete block design was used with three replicates. Data on yield were analyzed using additive main effects and multiplicative interaction (AMMI) model, genotype plus genotype by environment interaction (GGE) biplot model was used to display graphical representation of the yield data and the yield stability index (YSi). The analysis of variance of the AMMI model indicated that environments accounted for 56.9% of the total sum of square; genotypes effect explained 9.2% and the G x E interaction effect accounted 8.9% of the total sum of squares for the 16 genotypes tested across three environments and were all significant (P < 0.01). The average grain yield were 2.7, 1.38 and 1.20 t ha^{-1} for Karagwe, Bukoba and Muleba respectively. According the results, the GGE biplot revealed that, the genotypes SSIN 1240, SAB 659 and DAB 219, SMR 101, SMC 162 and DAB 602 showed greater stability with the average closer to the overall average of the tested genotypes. Therefore they are recommended to be used as varieties or parents for further improvement of available cultivars.

Key words: Adaptability, *Phaseolus vulgaris,* Kagera, genotypes, environment.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the major sources of dietary proteins, vitamins, and minerals to millions of resource-poor farmers, particularly in developing countries (Broughton et al., 2003). Beans are the main grain legume crop grown in Tanzania, where they are often intercropped with maize. Cultivation of beans can be seen in most areas of Tanzania (Hillocks et al., 2006).

In agricultural experimentation, a large number of genotypes are normally tested over a wide range of environments (locations, years, growing seasons, etc). Due to the variation of the climate, soil properties and the inherent potential of genotypes, crop yield may vary from one environment to another as a result of interaction

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between the environment and genotypes. The presence of a genotype x environment interaction automatically implies that the behavior of the genotypes depend upon the particular environment in which they are evaluated (Nchimbi-Ms and Tryphone, 2010). Therefore, it was important to study the genotype and environment interaction of the genotypes in order to identify high-yielding and stable cultivars and discriminating and representative test environments (Yan, 2001).

The genotype x environment interaction for certain bean characteristics, such as yield, may hinder cultivar recommendation for large geographical areas (De Araújo et al., 2003). The selection of genotypes to maximize yield when genotype rank changes occur across environments is complicated because of the complexity of genotype responses (da Silveira et al., 2013). A recently developed graphical data summary, called Genotypes main effects and Genotype x environment interaction effects (GGE) biplot, can aid in data exploration. GGE biplot is a Windows application that performs biplot analysis of two-way data that assume an entry × tester structure. A multi – environment trial data set, in which cultivars are entries and environments are testers, was used to demonstrate the functions of GGE biplot (Yan, 2001). These include but are not limited to: (i) ranking the cultivars based on their performance in any given environment, (ii) ranking the environments based on the relative performance of any given cultivar, (iii) comparing the performance of any pair of cultivars in different environments, (iv) identifying the best cultivar in each environment, (v) grouping the environments based on the best cultivars, (vi) evaluating the cultivars based on both average yield and stability, (vii) evaluating the environments based on both discriminating ability and representativeness, and (viii) visualizing all of these aspects for a subset of the data by removing some of the cultivars or environments. GGE biplot has been applied to visual analysis of genotype × environment data, genotype × trait data, genotype × marker data, and diallel cross data (Yan, 2001). GGE biplot identifies G x E interaction patterns of data and clearly shows which variety performs best in which environments and thus facilitates mega- environment identification (Gurmu, 2017; Shiri, 2013; Yan, 2001). Therefore, there is need for understanding the nature of G x E interaction, quantifying its magnitude and identifying stable and widely adapted common bean genotypes before release (Gurmu, 2017).

G x E due to different responses of genotypes in diverse environments, makes choosing the superior genotypes difficult in plant breeding programmes. Traditionally, plant breeders tend to select genotypes that show stable performance as defined by minimal G x E effects across a number of locations and/or years. The term stability is sometimes used to characterize a genotype which shows a relatively constant yield independent of changing environmental conditions. On the basis of this idea, genotypes with a minimal variance for yield across different environments are considered stable (Kundy and Mkamilo, 2014). The current study was conducted to evaluate the G x E interaction for the plant yield of common bean genotypes in Kagera Region, in order to identify stable high yielding and stable genotypes.

MATERIALS AND METHODS

Experimental sites and Materials used for the study

The study was conducted during 2017/2018 cropping season in three different agro ecological sites of Kagera Region which includes Bukoba, Karagwe and Muleba Districts (Table 1) where farmers grow common beans as food and commercial crop as well. A total of 16 common bean genotypes, 13 introduced genotypes from the International Center for Tropical Agriculture CIAT, two released varieties (Lyamungu 90 and JESCA as control) and one landrace (Ibwera as local check) were used during the experimentation across three environments. The list of these genotypes is presented in Table 2.

Experimental design and field layout

The experiment was laid out in Randomized Complete Block Design (RCBD) arranged in a split plot layout with three replications in each site (Table 1). Two factors were used; the first was location (the main factor), three Districts of Kagera Region (Bukoba, Karagwe and Muleba) with different agro climate was involved during the experiment. The second factor was genotypes (the sub factor): sixteen common bean genotypes were used in the experiment. The experimental unit size was 3 by 1.5 m, consisting of four rows; spacing was 50 cm between rows and 20 cm with row, two seeds per hill. Hand- hoe weeding and fertilizer application were done twice when beans had one trifoliate leaf and before flowering. Fertilizer used was NPK: 20:10:10 at recommended rate. All recommended agronomic practices for common bean productions were followed.

Statistical model

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + c_{ik} + e_{ijk} \quad (1)$$

Where
μ is a population mean.
α_i is the main effect of location (A).
β_j is a main effect of genotypes (B)
$(\alpha\beta)_{ij}$ is the interaction effect of A and B
c_{ik} is the plot error distribution, k = 1, 2.
$e_{ijk}$is the sub – plot error distribution, k = 1, 2.

Data collection

Days to 50% flowering (DF)

This was measured in days-after-planting and coinciding with the initiation of developmental stage R6 when 50% of the plants have one or more flowers (Schoonhoven and Pastor-Corrales, 1987).

Days to physiological maturity (DPM)

This was measured in days-after-planting and coinciding with the

Table 1. Characteristics of the experimental sites.

	Location		
	Bukoba	**Muleba**	**Karagwe**
Altitude (masl)	1349	1153	1160
Latitude	01°25'1"	01°37' 27.1"	01°18.027'
Longitude	031°46' 41"	031°37' 13.1"	031°21.494'
Soil Type	Sandy clay loam	Sand Clay Loam	Loamy Sand
pH (H2O)	5.26	5.42	5.87
N Total (%)	0.24	0.18	0.17
P (mg/kg) Bray 1	17.90	19.20	23.40
Organic Carbon (%)	2.39	2.34	2.41
Mg (meq/100 g soil)	0.12	0.14	0.36
Ca (meq/100 g soil)	0.66	0.78	2.04
EC (mS/cm)	0.33	0.28	0.30
CEC	3.10	3.80	5.20
Agro – ecological zone	High rainfall	Medium rainfall	Low rainfall

Table 2. Characteristics of the common bean genotypes used under experimentation.

Genotype	Seed size	PSC 1	SCP 2	SCB 3	GH
DAB 378	Large	R	2	3	Type I
DAB 219	Large	M	6	2	Type I
DAB 291	Large	M	6	3	Type I
SAB 659	Large	M	6	1	Type I
SCR 59	Medium	O	6		Type II
SSIN 1128	Medium	O	2	1	Type III
SSIN 1240	Medium	M	6	1	Type III
IBWERA	Medium	R	2	1	Type I
JESCA	Large	O	2	1	Type I
Lyamungu 90	Large	M	2	2	Type I
SMC 162	Medium	O	1	1	Type II
SMC 24	Medium	O	1	2	Type III
SMR 101	Large	O	1	1	Type I
DAB 602	Large	M	2	1	Type I
DAB 582	Large	R	2	1	Type I
DAB 362	Large	R	2	3	Type I

GH, Growth habit
1CIAT Seed color Pattern: O – No pattern, M – Mottled, R – Striped, J – speckle, P – pinto, B – bicolor,
2 CIAT Seed color Scale: 1 – white, 2 – Cream-beige, 3 – yellow, 4 – brown maroon, 5 – pink, 6 – Red
3CIAT Seed Brilliance Scale: 1 – Dull, 2 – Semi-Shine, 3 – Shiny.

initiation of developmental stage R9 when 50%of the plants have reached physiological maturity (Schoonhoven and Pastor-Corrales, 1987).

Number of pods/plant

Number of pods per plant were recorded from ten plant selected randomly in the net plot and the average of the plot was calculated.

Number of seeds/pod

The number of seed per pod was recorded from ten randomly selected pods in the net plot and the average of the plot was calculated.

Seeds size

Seed size is expressed as the weight in grams of 100 randomly chosen seeds and categorized as follows; Small: Less than 25 g, Medium: 25 g to 40 g, Large: More than 40 g (Schoonhoven and Pastor-Corrales, 1987.

Grain yield (kg/ha)

Harvesting was done for two middle rows of each plot and grain

Table 3. Summary of analysis of variance and partitioning of the G X E interaction using AMMI method.

Source of variation	df	Means squares of individual analysis of variance by location			Combined analysis of variance				
		L1	L2	L3	Source	df	SS	MS	%SS
Replication	2	0.55	0.28	0.64	Genotypes	15	10.49	0.7**	9.22
Genotype	15	3.29**	10*	7.38**	Location	2	64.80	32.40**	56.94
Error	30	2.91	9	4.3	Interactions (GxL)	30	10.18	0.34**	8.94
Mean(t ha-1)		1.38	2.7	1.20	IPCA	16	6.20	0.39*	5.45
CV%		9.5	3.4	11.8	IPCA	14	3.98	0.28*	3.5
s.e		0.13	0.09	0.14	Error	96	18.16	0.19	15.96

L1: Bukoba, L2: Karagwe L3: Muleba ** significant at 01%; * significant at 5% level; df – degree of freedom; SS – sum of square, MS – mean sum of square, %SS – percentage sum of square.

yield was adjusted by converting plot yield (at 14% moisture content) to seed yield per hectare (Kadhem and Baktash, 2016).

Statistical analysis

The additive main effect and multiplicative interaction Analysis

The data for grain yield were pooled to perform the analysis of variance across the environment. Since the pooled analysis of variance considers only the main effects, the additive main effect and multiplicative interaction model (AMMI) was computed using Genstat software. The AMMI analysis is a combination of analysis of variance (ANOVA) and principal component analysis (PCA) in which the sources of variability in genotype by environment interaction are partitioned by PCA (Ana et al., 2011).

The main idea of the AMMI models is: (i) first apply the additive of the variance model (ANOVA) to a two-way table and (ii) secondly apply the multiplicative PCA model to the residual from the additive model (Gauch, 1992). The AMMI model with multiplicative terms can be written as:

$$Y_{ij} = \mu + G_i + E_j + \Sigma_{k=1}\lambda_k\gamma_{ik}\alpha_{jk} + \rho_{ij} + \varepsilon_{ij} \quad (2)$$

Where: Yij is the yield of genotype i in environment j; μ Grand mean; G_i the genotype means deviations (the genotype means minus the grand mean); E_j the environment mean deviations; λ_k the singular value for the PCA axis k; γ_{ik} and γ_{ik} α_{jk} are the genotype and environment PCA scores for PCA axis k; K is the number of PCA axes (Kadhem and Baktash, 2016).

The AMMI model was used to identify genotypes(s) which are adapted in different environment. The AMMI's stability values (ASV) were computed using Equation 3.

$$ASV = \sqrt{\left(\left[\left(\left(SSIPCA1/SSIPCA2\right)(IPCA1SCORE)\right]^2\right) + \left((IPCASCORE2)^2\right)\right)} \quad (3)$$

Where SSIPCA1/SSIPCA2 is the weight given to the IPCA1 value by dividing the IPCA1 SS by the IPCA1 SS; and the IPCA1 and IPCA2 scores are the genotypic scores in the AMMI model (Rad et al., 2013).

Genotype and genotype by environment (GGE) – Biplot analysis

The GGE biplot methodology was used to analyze the multi - location genotype yield trial data to evaluate the grain yield stability and identify superior genotypes using the GenStat v.13 software. GGE biplot analysis was also used to generate graphs for: (i) comparing environments to the ideal environment; (ii) the "which-won-where" pattern; (iii) environment vectors. The angles between environment vectors were used to judge correlations (similarities/dissimilarities) between pairs of environments (Shiri, 2013).

RESULTS AND DISCUSSION

Analysis of variance

The single site analysis of variances (Table 3) revealed the high significance differences among the genotypes in each tested environment but the results shows variability of the genotype rank from one environment to another, this justifying the conduction of a more refined analysis so that to increase the efficiency of the selection and indication of cultivars. In this sense, AMMI analysis represents a potential tool that can be used to deepen the understanding of factors involved in the manifestation of the G × E interaction (da Silveira et al., 2013).

The analysis of variance of the AMMI model indicated that environments accounted for 56.9% of the total sum of square; genotypes effect explained 9.2% and the G x E interaction effect accounted 8.9% for the 16 genotypes tested across three environments (Table 3) and were all significant ($P < 0.01$). A large SS for environments indicated that the environments were diverse, with large differences among environmental means causing most of the variation (da Silveira et al., 2013) in genotype grain yield. This means there were large environmental effects on the genotypes performance across the environments than the interaction between the genotypes and the environment.

Mean performance of the genotypes in each and across environments

The mean grain yield of the genotypes as presented in Table 4. Karagwe site (L2) was the best environment for

Table 4. The mean genotype yield (t h-1) AMMI stability value of the 16 genotypes tested across three environments.

Genotype	L1	L2	L3	MEAN	IPCA1	IPCA2	ASV
SSIN 1240	1.4	2.8	1.1	1.7	0.12	0.02	0.19
SAB 659	1.4	2.8	1.5	1.9	-0.15	0.04	0.23
DAB 219	2.0	3.1	1.4	2.2	0.16	-0.14	0.29
LYAMUNGU 90	1.0	2.7	1.2	1.7	-0.11	0.26	0.31
IBWERA	1.2	2.1	0.9	1.4	-0.09	-0.29	0.32
JESCA	1.4	2.7	1.5	1.9	-0.21	-0.03	0.32
SMC 162	1.7	2.4	1.1	1.7	0.03	-0.45	0.45
DAB 602	1.3	2.7	1.6	1.9	-0.28	0.08	0.45
SMR101	1.2	3.3	1.4	1.9	0.07	0.47	0.48
DAB 378	1.0	1.5	0.3	0.9	0.01	-0.50	0.50
SCR59	1.4	2.9	0.9	1.7	0.32	0.07	0.51
DAB 362	1.3	3.4	1.1	1.9	0.30	0.43	0.64
SSIN 1128	1.6	3.1	0.9	1.9	0.42	0.09	0.66
DAB 582	1.2	2.8	1.9	2.0	-0.48	0.22	0.78
SMC24	1.8	2.9	0.8	1.8	0.49	-0.21	0.79
DAB 291	1.1	2.2	1.7	1.7	-0.63	-0.08	0.98
Mean	1.4	2.7	1.2	1.8			

L1, Bukoba; L2, Karagwe; L3, Muleba.

common bean production that gave the average grain yield of 2.7 t ha^{-1}, followed by Bukoba which gave 1.38 t ha^{-1} and Muleba was the least with an average production of 1.20 t ha^{-1} (Table 4). In Karagwe, plant responded vigorously and most of the genotypes performed more than 2 t ha^{-1} with high scores of the plant vigor of scale 1 and 2 to most of the tested genotypes, while in Muleba which is the least site in the performances of the genotypes was poor with some of the genotypes scores plant vigor of scale 3 (good) and scale 5 (intermediate) according to Schoonhoven and Pastor-Corrales, 1987.

AMMI's stability values (ASV)

The ASV is the distance from zero in a two dimensional scatter gram of IPCA1 (interaction principal component analysis axis 1) scores against IPCA2 scores. Since the IPCA1 score contributes more to GE sum of scores, it has to be weighted by the proportional difference between IPCA1 and IPCA2 scores to compensate for the relative contribution of IPCA1 and IPCA2 total GE sum of squares. From the calculation of Equation 1, genotypes SSIN 1240, SAB 659, DAB 219 and Lyamungu 90 had shown higher adaptive capacity compared to others genotypes due to their lower AMMI stability values as shown in Table 4 as described by Al-Naggar et al., 2018, that a genotype with least ASV and IPCA scores (either negative or positive) are considered as the most stable while the genotypes SSIN 1128, DAB 582, SMC24 and DAB 291 had shown lesser adaptive capacity.

Some of the genotypes may perform better in one environment but the same genotype performs less in the other environment. For instance, the genotype DAB 362 ranked number one in performance with average yield of 3.363 t ha^{-1} in Karagwe site but it did less in other two environments, like – wise DAB 219 ranked number one in Bukoba and in Karagwe ranked number four but in Muleba it did not appeared in top four performed genotypes (Tables 4 and 5). As stated by Kadhem and Baktash (2016) the best genotype needs to combine good grain yield and stable performance across a range of production environments. In this study only two genotypes DAB 219 and SSIN 1128 appeared to perform well in Karagwe and Bukoba sites. This happened despite the fact that the environments were diverse and caused for a great variation in grain yield which is quantitative trait, so the environmental factors are crucial determinant of yield expression (Kadhem and Baktash, 2016). However, the AMMI stability values revealed that SSIN 1240, SAB 659, DAB 219 were the most stable genotypes across three tested environments above checks which were Lyamungu 90, JESCA (released varieties) and Ibwera (landrace). Among them DAB 219 (arranged in increasing order of stability) had environment average yield of 2.169 t ha^{-1} higher than any tested genotypes (Table 4), while the first two more stable genotypes SSIN 1240, SAB 659 had environmental average yield of 1.737 and 1.892 t ha^{-1} respectively.

Genotype plus genotype by environment (GGE) biplot

In biplot the differences among genotypes in terms of direction and magnitude along the X-axis (yield) and Y

Table 5. First four AMMI genotypes selections per environment.

Environment	Mean	Score	1	2	3	4
KARAGWE	2.70	0.589	DAB 362	SMR101	SSIN 1128	DAB 219
BUKOBA	1.38	0.381	DAB 219	SMC24	SMC 162	SSIN 1128
MULEBA	1.20	-0.971	DAB 582	DAB 291	DAB 602	JESCA

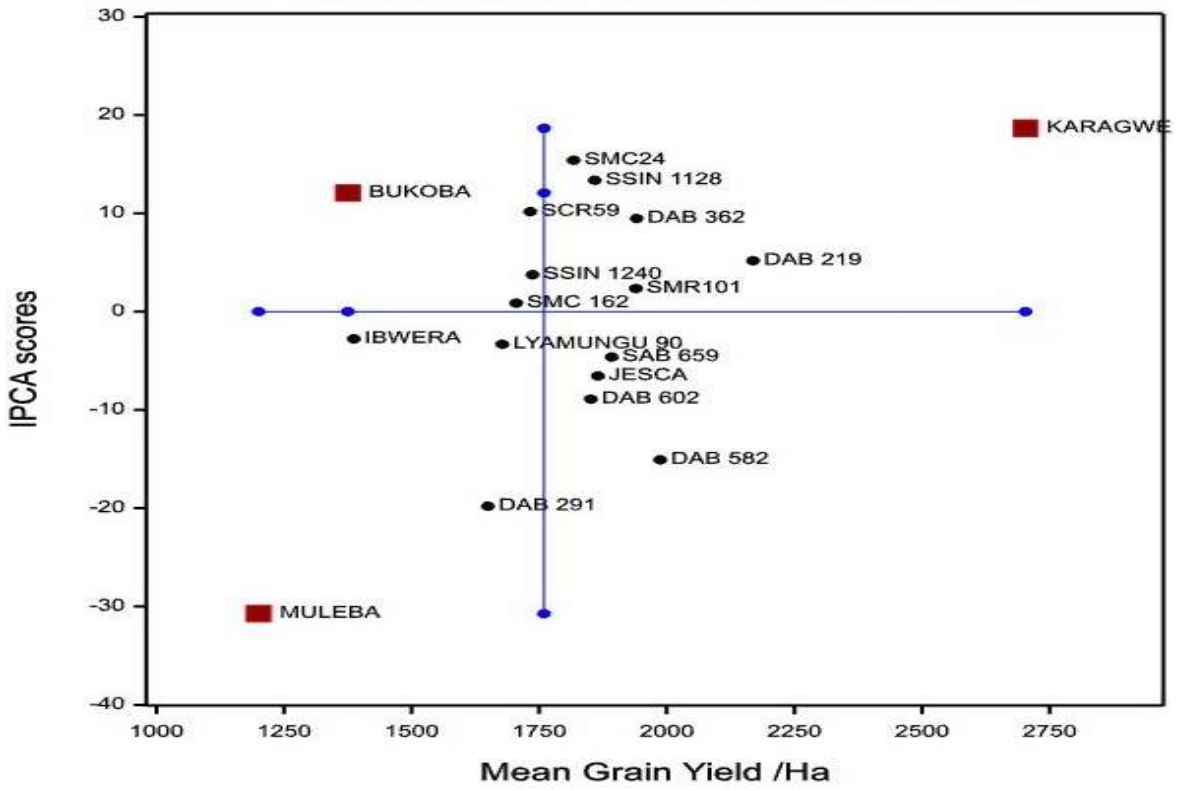

Figure 1. The biplot of 16 genotypes and environment IPCA score against means.

axis (IPCA 1 scores) are important (Kadhem and Baktash, 2016). In the biplot display, genotypes or environments that appear almost on a perpendicular line of the graph had similar mean yields and those that fall almost on a horizontal line had similar interaction (Alberts, 2004). Genotypes or environments on the right side of the midpoint of the perpendicular line have higher yields than those on the left side. The score and sign of IPCA1 reflect the magnitude of the contribution of both genotypes and environments to GEI, where values closer to the origin of the axis (IPCA1) provide a smaller contribution to the interaction than those that are further away (characteristic of stability), whereas higher score (absolute value) considered as unstable and specific adapted to certain environment (Psychometrika, 1968; da Silveira et al., 2013). The characterization of each promising lines (genotypes) to mean grain yield and contribution to GEI by mean of IPCA1 (Alberts, 2004) based on these attributes our study indicates that genotypes SMR 101, DAB 362, SSIN 1128, SMC 24 and DAB 219 were specifically adapted to Karagwe which was the high yielding environment as shown in Figure 2.

The genotypes SSIN 1240, SAB 659 and DAB 219, SMR 101, SMC 162, IBWERA, Lyamungu 90, JESCA and DAB 602 showed greater stability with the average closer to the overall average of the tested genotypes. However, genotypes SSIN 1240, SMC 162, IBWERA and Lyamungu90 were identified to be adapted to low yielding environment since they appeared on the left side of the mid-point representing grand mean in Figure 1. The GGE analysis was performed on the average grain yield of the 16 common beans genotypes tested in three different sites. The results showed that the GGE biplot explained 89.5% of the genotype main effects and the Genotype by Environment interaction. The primary (PC1) and Secondary (PC2) components explained 59.8 and 29.8%

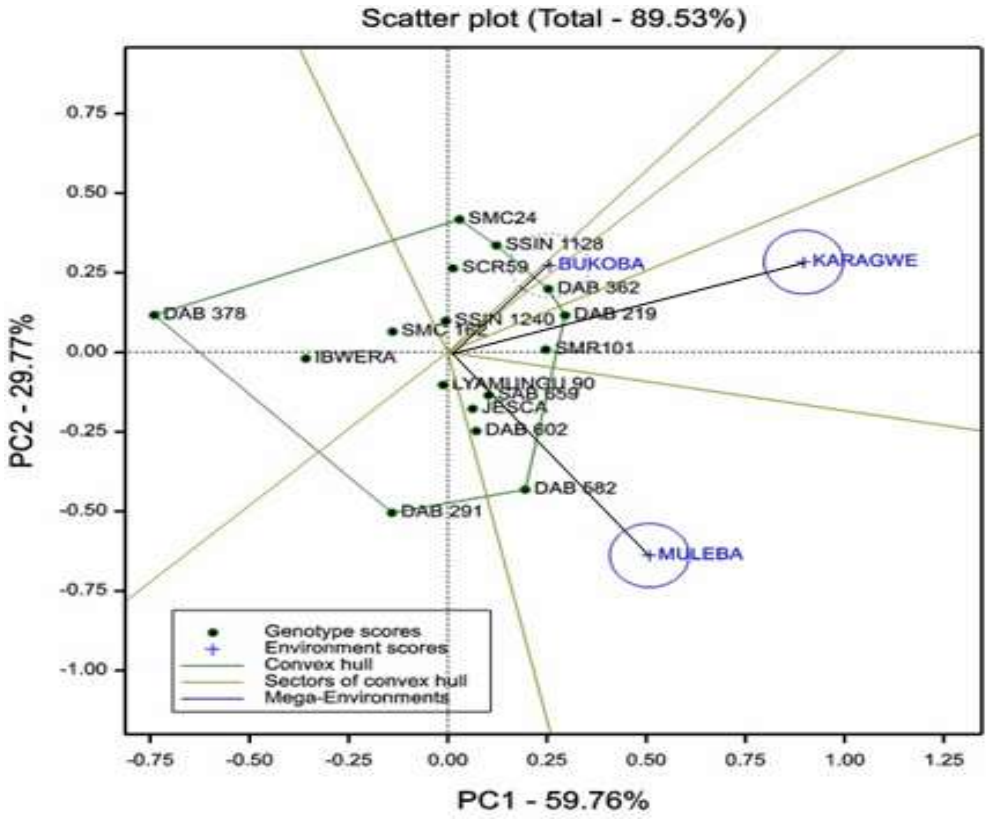

Figure 2. GGE biplot showing the two main axes of interaction (PCA1 vs. PCA2) in 16 genotypes across three locations.

of the genotypes main effects and G x E interaction respectively (Figure 2). The genotypic PC1 scores greater than zero classified the high yielding genotypes while PC1 scores less than zero identified low yielding genotypes, unlike genotypic PC1, genotypic PC2, scores near zero showed stable genotypes whereas large PC2 scores discriminated the unstable ones (Jalata, 2011).

The plot of PCA1 vs. PCA2 revealed that SSIN 1240, SAB 659, DAB 219 and Lyamungu 90 were the most stable genotypes due to the fact that, they were found closer or at a lesser distance from the center of the biplot when compared with other genotypes, while SSIN 1128, DAB 582, SMC24 and DAB 291 were considered as most unstable genotypes among all other tested genotypes across three environments as shown in Figure 2, similar result was also reported by Kadhem and Baktash (2016).

The GGE biplot was also used to show the association among the tested environment. Figure 2 show that Karagwe and Muleba exhibits longer vectors compared to Bukoba this contributed more to the environment sum of square as also indicated in the ANOVA table (Table 3). Genotypes and environments positioned close to each other in the biplot have positive associations, thus these enable the creation of agronomic zones with relative ease (Alberts, 2004). In the current study, the polygon view of GGE biplot for grain yield indicates the best genotype(s) for each environment(s). In Figure 3 the genotypes SMC 24, SSIN 1128, DAB 362, Dab 219, DAB 582, DAB 291 and DAB 378 were the best or poorest genotypes because they are located on the vertex of a polygon (Hagos and Abay, 2013).

The vector view of GGE-biplot (Figure 2) provides a succinct summary of the interrelationships among the environments; all environments were positive correlated because all angles among them were smaller than 90° (Rad et al., 2013). The correlation between Karagwe and Bukoba is stronger than that of Muleba and either of the other two locations. The results suggesting that indirect selection for grain yield can be practical across the tested environment, this means adaptable genotypes in Karagwe may also show a similar respond in Bukoba and less response in Muleba.

The GGE biplot was also used to draw the polygon for G × E interaction effect from which different interpretations can be derived. The polygon is formed by connecting the markers of the genotypes that were further away from the biplot origin such that all other genotypes were contained in the polygon as shown in Figure 2. The polygon view of a biplot is the best way to visualize the patterns of interaction between genotypes

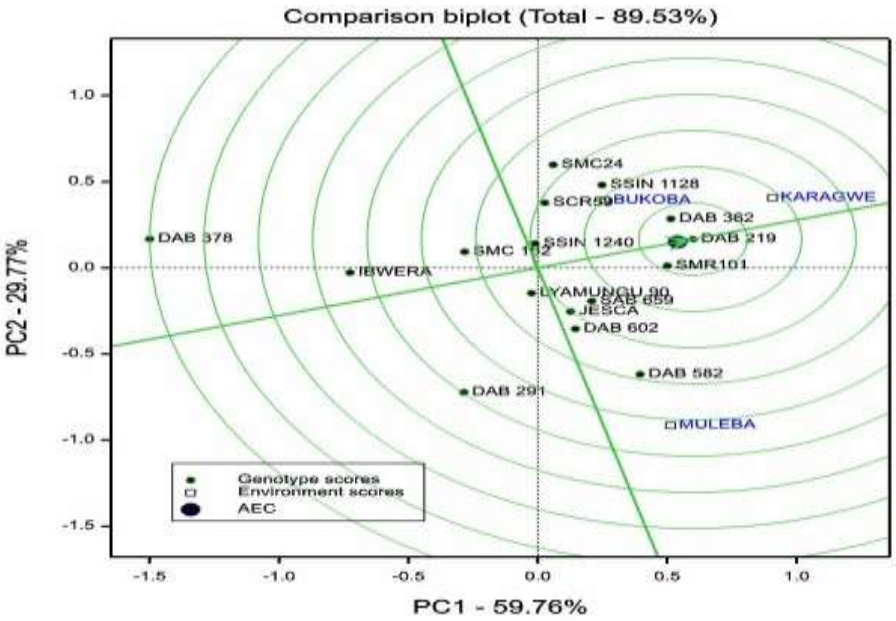

Figure 3. GGE – biplot based on environment – focused scaling for comparing the environments with the ideal environment.

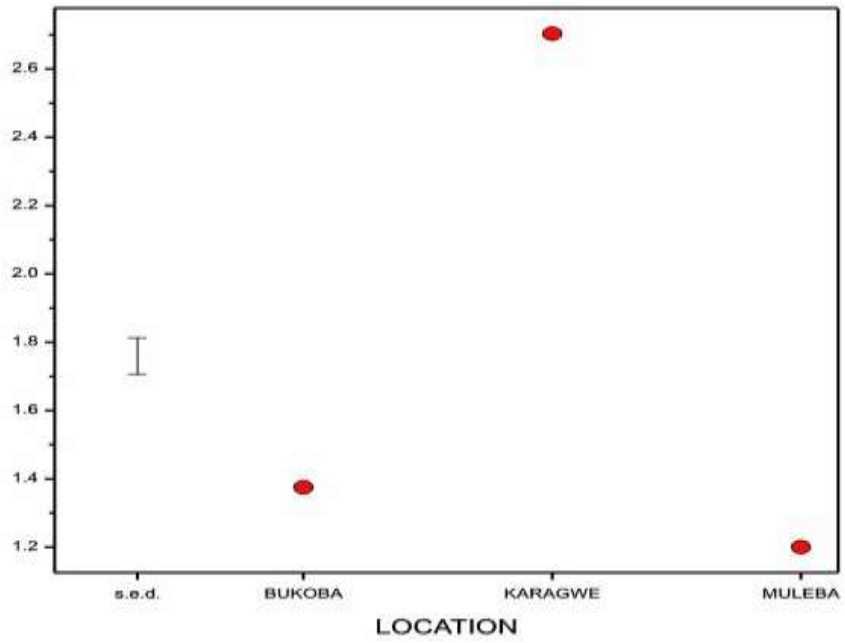

Figure 4. Genotypes grain mean yield per location.

and environments, and to effectively interpret a biplot (Shiri, 2013).

An environment is more desirable if it is located closer to the ideal environment. Thus, using the ideal environment as the centre, concentric circles were drawn to help visualize the distance between each environment and the ideal environment (Yan et al., 2000; Yan and Rajcan, 2002). Figure 3 shows that Karagwe was an ideal test environment in terms of being the most representative of the overall environment. The graphical representation of the means performances of the genotypes per location which indicates that, Karagwe is better performing environment (Figure 4). However, the vector of GGE-biplot shows interrelation among tested

environment in which all three environments were positive correlated and the GGE – biplot, for comparing environments with ideal environment, positioned Karagwe site at the center of the concentric circles (Figure 3). As stated by da Silveira et al. (2013) genotypes and environments positioned close to each other in the biplot have positive associations, thus these enable the creation of agronomic zones with relative ease. Both the genotype and the environment determine the phenotype of an individual. The effects of these two factors, however, are not always additive because of the interaction between them. The large G x E variation usually impairs the accuracy of yield estimation and reduces the relationship between genotypic and phenotypic values (Ssemakula and Dixon, 2007).

Conclusion

The results of this study indicates the significant genotypes environment interaction in grain yield across the tested environments, this means each genotype responded differently when exposed to different location due to variations in climate and edaphic factors. It was difficult to identify genotype which was superior for all tested environment. Therefore, based on GGE and AMMI multivariate analyses which performed evaluation of genotypes adaptability/stability across the tested sites, genotypes DAB 362, and SMR 101 could be recommended to be used in Karagwe. While genotypes SMC 24, SMC 162 and SSIN 1128 could be used in Bukoba, likewise genotypes DAB 582, DAB 602 and DAB 291 could be used in Muleba. SSIN 1128 and DAB 219 could be grown in Karagwe as well as in Bukoba.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 12(3), pp. 246-257, July-September 2020
DOI: 10.5897/JPBCS2020.0905
Article Number: C70E77564876
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Evaluation of the performance of advanced generation soybean [*Glycine max* (L.) Merr.] genotypes using GGE biplot

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Received 27 May, 2020; Accepted 3 August, 2020

Soybean is an important food and cash crop in Uganda. Despite the importance of soybean in Uganda's economy, its performance is highly affected by genotype × environment interaction making it difficult to select and recommend new superior soybean genotypes for diverse growing environments. The objectives of this study were to examine the nature of G × E interaction for soybean grain yield, to identify stable and high yielding soybean genotypes with desirable percentage protein and oil content for production in diverse environments and to determine ideal test location for future soybean breeding activities in Uganda. The experiment was conducted at six locations for two consecutive seasons of year 2018 (2018A and 2018B). Twenty-three newly advanced generation soybean lines and two commercial varieties were evaluated in a randomized complete block design replicated three times. Combined analysis of variance over locations and seasons was carried out for grain yield, protein and oil (%) content. The results for grain yield showed significant ($p<0.05$) differences for all the sources of variation except genotypes × season interaction. Percentage protein and oil content showed non-significant ($p>0.05$) for all the sources of variation except location. The genotype main effect plus G × E interaction biplot explained 65.74% of the total interaction sum of squares for grain yield and showed that the advanced generation soybean lines BSPS 48A-28; Mak 3N × 1N and NGDT 8.11×3N-2 were high yielding and stable and had other desirable agronomic traits. Nakabango was the most discriminating and representative test location.

Key words: Soybean, stability, GGE, ideal testing location, mega-environment.

INTRODUCTION

Soybean (*Glycine max* L.) is an important food and cash crop in Uganda (Ibanda et al., 2018; Gebremedhn et al.,

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2018). Due to its nutritional superiority, soybean flour is often blended with cereal flours such as maize to boost their nutritional value. In Uganda, Makerere University Soybean Improvement Centre is developing soybean varieties ideal for food and industrial purposes (Tukamuhabwa et al., 2016). Generally, majority of farmers like high-yielding, minimal stem lodging and non-shattering soybean varieties that are less susceptible to common diseases such as soybean rust and red-leaf blotch (Tukamuhabwa and Obua, 2015) and pests such as groundnut leaf miner (Ibanda et al., 2018) and bruchids (Msiska et al., 2018). Food processors also want soybean varieties with high protein and oil content. Farmers and food processors normally would want all these traits incorporated in one variety (Whaley and Eskandari, 2019). In most cases, the agronomic traits are highly heritable and can be easily selected with accuracy at early generation testing. However, the expression of quantitative traits such as seed yield, protein and oil content is highly influenced by genotype × environment interaction, hence complicates the identification and selection of superior genotypes (Gurmu et al., 2009; Hampango et al., 2017) and therefore multi-environment trials (MET) are recommended for evaluation of promising lines (Nyombayire et al., 2018).

Uganda's agro-ecological regions are highly diverse with variable climatic conditions accelerated by global climatic changes that influence mean annual rainfall (510-2160 mm), temperature (23-28°C) and varied soils influenced by soil depth, texture, acidity and organic matter (Agoyi et al., 2017; Tukamuhabwa et al., 2012). Due to the variability of abiotic and biotic factors from location to location, soybean performance remains exposed to the influence of huge genotype × environment interactions, leading to inconsistent genotypic responses (Bhartiya et al., 2017); therefore, the development of stable varieties will be the only sustainable way to cope with the ever-changing biotic stresses like the outbreak of groundnut leaf miner (Ibanda et al., 2018) and soybean rust (Maphosa et al., 2013; Gebremedhn et al., 2018) and abiotic stresses; like extreme temperature and rainfall changes (Tukamuhabwa et al., 2016). The presence of significant G × E interaction for grain yield, percentage protein and oil content in soybean has been reported by several researchers (Gurmu et al., 2009; Nascimento et al., 2010; Chaudhary and Wu, 2012; Atnaf et al., 2013; Hampango et al., 2017; Bhartiya et al., 2017) which could lead to the failure of genotypes to achieve the same relative performance in different environments (Noëlle et al., 2018; Thungo et al., 2019).

The differential performance of genotypes across several unrelated environments reduces responses to selection and subsequently progress in plant breeding programs (Crossa et al., 2002; Yan and Kang, 2002). Furthermore, the presence of significant crossover G × E interaction complicates the recommendation of new varieties from MET and the identification of ideal genotypes (Bernardo, 2002; Yan and Kang, 2002) which should be either specific or widely adapted across different agro-ecological zones. Therefore, characterizing the interaction between genotypes and environments is very important for the selection of genotypes with high adaptability to specific environments or with high stability across different environments (Yan et al., 2000; Yan et al., 2019). In this regard, Yan and Tinker (2006) presented some objectives of MET analysis that included mega-environments delineation to minimize negative G × E interaction, as well as identification of the most discriminating and representative testing locations within mega-environments and identification of superior genotypes. This is important in cultivar development in order to rationalize resources and confine genotype evaluation to ideal locations that are informative to facilitate a rapid response to selection (Tukamuhabwa et al., 2012).

Several statistical methods for analyzing G × E interaction have been reviewed (Westcott, 1986). However, not all ways of exploiting G × E interaction involve trying to reduce it (Bernardo, 2002). Some methods, like analysis of variance (ANOVA), are good at detecting G × E interaction but cannot reveal the pattern of the interactions (Gasura et al., 2015). Regression-based methods (Eberhart and Russell, 1966) use environmental scores, which have less to do with genotype plus G × E interaction and explains only a small part of genotype main effect plus genotype × environment interaction (GGE) (Yan et al., 2007). In the recent past, statistically effective multivariate techniques, such as biplots based on Singular Value Decomposition (SVD) and Principal Component Analysis (PCA) have been developed for G × E interaction analysis (Gauch, 2006; Yan and Tinker, 2006). Approaches such as the genotype main effect plus G × E interaction (GGE) biplot (Yan, 2001;Yan and Tinker, 2006) and the Additive Main effect and Multiplicative Interaction biplot (AMMI) (Gauch, 2006, 2013; Gauch et al., 2008) have been widely used to exploit significant G × E interaction in soybean MET data as they effectively capture the additive (linear) and multiplicative (bilinear) components of G × E interaction and provide meaningful display and interpretation of multi-environment data set in breeding programs.

The biplot model that is fitted to residuals after the exclusion of the environment-centered data is called a GGE biplot (Yang et al., 2009). The GGE biplot is a graphical display of G × E interaction data into a two-way table for simplicity visualization of the interrelationship and it can be subjected to several ways of singular value decomposition (SVD) (Yan and Tinker, 2006). Yan and Hunt (2001) suggested that, for cultivar evaluation and recommendation, genotype and G × E interaction are the only two sources of variation that are crucial and must be considered simultaneously for appropriate genotype and test environment evaluation. Using a site's regression model (SREG) Yan et al. (2000) combined genotype

Table 1. Experimental sites used in the study during season 2018A and 2018B.

Site	Coordinates	Altitude (m)	Mean annual temperature (°C)	Mean annual rainfall (mm)	Soil type
Nakabango	00° 31'N 33°12'E	1178	26	1400	Crysalline basic
Iki-Iki	01° 06'N 34° 00'E	1156	28	1200	Sandy
Kabanyolo	00° 28'N 32° 37'E	1300	22	1255	Sand-clay loam
Bulindi	01° 28'N 31° 28'E	1230	23	1700	Sandy loam
Ngetta	02° 17'N 32° 56'E	1085	29	1483	Sandy loam
Abi	03° 5'N 30° 56E	1140	24	1250	Sandy-clay loam

Source: NARO Ngetta-Zardi (2018).

main effect and genotype × environment interaction, denoted as G + G × E interaction or GGE and repartitioned this into crossover and non-crossover G × E interaction. For exploiting G × E interaction in MET data, the strengths of the GGE and AMMI biplots have been debated unequivocally (Gauch, 2006; Yan et al., 2007; Gauch et al., 2008; Yang et al., 2009). In MET data, the GGE biplot is crucial in assessing the genotype main effects plus the G × E interaction (Yan and Tinker, 2006). This multivariate analysis technique has been widely used for delineating soybean production mega-environments and soybean variety recommendations (Bhartiya et al., 2017; Hunde et al., 2019). The objectives of this study were to examine the nature of G × E interaction for soybean grain yield, to identify stable and high yielding soybean genotypes with desirable percentage protein and oil content for production in diverse environments and to determine ideal test location for future soybean breeding activities in Uganda.

MATERIALS AND METHODS

Materials and testing environments

The study was carried out at six locations namely; Kabanyolo, Iki-Iki, Nakabango, Ngetta, Abi and Bulindi that are located in different agro-ecological regions of Uganda (Table 1). These locations have different climatic conditions, and therefore may influence the expression of soybean grain yield, protein, oil content and agronomic traits differently. Furthermore, these locations represent major soybean growing areas of Uganda. Twenty-five soybean genotypes were used in this study. Among the genotypes used, 23 were advanced generation lines and two were commercial varieties used as checks (Table 2).

Experimental design

A randomized complete block design (RCBD) with three replications was used. Each entry was represented by three rows measuring 5 m long with an inter-row and in-row spacing of 60 cm and 5 cm respectively. The study was carried out for two consecutive seasons; first rains of 2018 (2018 A), and second rains of 2018 (2018 B). The trials were kept weed free by constant weeding.

Data collection

Data was collected on soybean rust, a major soybean disease in Uganda using a scale of 1-5 (Miles et al., 2006) where 1= no visible lesion, 2= few scattered lesions present, 3= moderate number of lesions on at least part of the leaf, 4= abundant number of lesions on at least part of leaf, and 5= prolific lesion development on most of the leaf. Days to 50% flowering and plant height were recorded as described by Obua (2013). The groundnut leaf miner (GLM) severity was scored using the standard scale of 1-5 as described by Ibanda et al. (2018). The number of pods per plant was recorded at harvest. Furthermore, at harvest the genotypes were threshed, and 100 seed weight and yield per plot were determined and later corrected to 12% moisture content before determining yield per hectare (Tukamuhabwa et al., 2012). Protein and oil content (%) were quantified using the data from first and second replications of selected four locations of Nakabango, Iki-Iki, Abi and Bulindi. The locations were selected based on their previous informative study of Tukamuhabwa et al. (2012). The analysis described by Owusu-Apenten (2002) was used to quantify the protein content, whereas, the oil content was determined using Near infrared spectroscopic analysis as described by Sato (2010).

Data analysis

Analysis of variance (ANOVA) was performed initially for each environment to determine the performance of the genotypes in different environments. Combined analysis of variance over locations and seasons was conducted using mixed model as suggested by Moore and Dixon (2015) (where genotypes and locations were fixed, whereas seasons, all the interactions involving seasons, replications and error were considered random) in Genstat software version 18 (Genstat, 2016). To determine the performance of different genotypes across seasons and locations, the following model for combined analysis of variance was used as described by Gasura et al. (2015);

$$Y_{ijkl} = \mu + r_1(pt)_{jk} + g_i + p_j + t_k + (gp)_{ij} + (gt)_{ik} + (pt)_{jk} + (gpt)_{ijk} + e_{ijkl}$$

Where, $Y_{ijkm(l)}$ is observed value of *i*th genotype in the *j*th location and the *k*th season in the *l*th replication, μ is the grand mean, $r_1(pt)_{jk}$ is the effect of the *l*th replication within locations and seasons, g_i, p_j and t_k are the main effects of the genotype, locations and seasons, $(gp)_{ij}$, $(gt)_{ik}$, $(pt)_{jk}$ are the first order interactions and $(gpt)_{ijk}$ is the second-order interaction, and finally e_{ijkl} is the pooled error term.

The proper F-test for a mixed model in which genotypes and locations were considered fixed effects and seasons treated as random effects was applied as suggested by McIntosh (1983) and recently by Moore and Dixon (2015). The assumption of sum to zero the effects of random interactions across each level of a fixed

Table 2. Names and codes of the soybean genotypes used in the study.

Code	Genotype name	Status
G1	Duiker × 3N-5	Advanced line
G2	GC × 2N-1	Advanced line
G3	BSPS 48A-27-1	Advanced line
G4	BSPSS 48A-28-1	Advanced line
G5	NGDT8.11×14.16B	Advanced line
G6	NII × GC 13.2	Advanced line
G7	BSPS 48A-25-1	Advanced line
G8	Nam II GC 17.3	Advanced line
G9	NII × GC 35.3-2	Advanced line
G10	NG 14.1 × UG5	Advanced line
G11	Nam 4M × 2N-2	Advanced line
G12	NII × 35.3-3	Advanced line
G13	G8586 × UG5	Advanced line
G14	NGDT 8.11× 3N-1	Advanced line
G15	BSPS 48A-28	Advanced line
G16	Bulindi 18.4B	Advanced line
G17	Maksoy 4N	Standard check
G18	BSPS 48A-24-1	Advanced line
G19	Bulindi 24.1A	Advanced line
G20	NII × GC 35.3-1	Advanced line
G21	NDGT 8.11×3N-2	Advanced line
G22	2N × GC	Advanced line
G23	Mak 3N × 1N	Advanced line
G24	NG 14.1 × NII-1	Advanced line
G25	Maksoy 3N	Standard check

factor for combined experiments was used as described by Moore and Dixon (2015). In brief, the mean squares for genotypes, genotypes × locations, genotypes × seasons and genotypes × locations × seasons were tested against the pooled error mean square, while locations, seasons and locations × seasons were tested against the mean square of replications within locations and seasons (Mclntosh, 1983). The variance components due to genotypes (δ^2g), genotypes × location (δ^2gl), genotypes × seasons (δ^2gs), genotypes × locations × seasons (δ^2gls) and random error (δ^2error) were obtained by solving the equations formed by equating the mean squares to their respective expected mean squares (Moore and Dixon, 2015). The variance components due to environments (location × seasons combinations) were estimated by summation of δ^2l, δ^2s and δ^2ls, whereas the variance component attributed to genotype × environment (δ^2ge) was estimated by adding up δ^2gl, δ^2gs and δ^2gls (Mclntosh, 1983). The broad sense coefficients of genetic determination (BSCGD) (broad sense heritability based on fixed genotypes) on a single plot basis, single environment basis and across environments basis were obtained by solving the following equations as; $\delta^2g/(\delta^2g + \delta^2gl + \delta^2gs + \delta^2gls + \delta^2error)$; $\delta^2g/ (\delta^2g + \delta^2gl + \delta^2gs + \delta^2gls + \delta^2error/ nr)$ and $\delta^2g/(\delta^2g + \delta^2gl/nl + \delta^2gs/ns + \delta^2gls/nls + \delta^2error/ nslr)$, respectively, where nr = number of replications, nl = number of locations, ns = number of seasons, nls = number of location × seasons combinations and nslr is the number of seasons × location × replications (Moore and Dixon, 2015).

Yield data was further subjected to GGE biplot (Yan and Tinker, 2006) analysis for identification of high yielding and stable soybean genotypes. The GGE biplot analysis was performed to determine the mega-environments and visualize the "which-won-where" pattern following the model for GGE biplot based on singular value decomposition (SVD) of *t* principal components as described by Yan and Tinker (2006).

GGE model: $Y_{ij} - \mu_i - \beta_j = \sum_{k=1}^{t} \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}$

Where, Y_{ij} is the performance of genotype *i* in environment *j*, μ is the grand mean, *j* b is the main effect of environment *j*, *k* is the number of principal components (PC); λ_k is singular value of the k^{th} PC; and α_{ik} and γ_{jk} are the scores of i^{th} genotype and j^{th} environment, respectively for PC_k; ε_{ij} is the residual associated with genotype *i* in environment *j*.

For mega-environment delineation of test locations, the which-won-where scatter plot was generated by a polygon drawn by connecting genotypes that are furthest away from the biplot such that the polygon contained all other genotypes (Yan, 2002). Then the polygon was further divided by perpendicular lines drawn to the polygon sides and running from the biplot origin (Yan and Tinker, 2006). The genotype focused comparison biplot for visualization and comparing genotypes based on mean yield and stability was determined by representing an average environment by an arrow. A straight line that dissecting the biplot origin to the average environment coordinate (average genotype axis) was drawn followed by a perpendicular line that passes through the biplot origin using the appropriate singular value partitioning (SVP) methods (Yan and Tinker, 2006). For the analysis of test locations, location comparison biplot was used for identification of ideal testing site (the most discriminating and representative locations) (Gasura et al., 2015). The environment vectors were drawn from the location comparison biplot origin to the markers of the environment (Yan and Tinker, 2006).

RESULTS

Combined ANOVA and broad sense heritability estimates

Combined analysis of variance for grain yield showed significant ($p<0.05$) differences for all components except genotypes × season interaction. The broad sense coefficient of genetic determination for grain yield (BSCGD) (equivalent to broad sense heritability of fixed genotypes) on single plot basis, single environment basis and across environment basis were 3, 6 and 40% respectively (Table 3). Percentage protein and oil content results showed non-significance ($p>0.05$) for genotype, seasons, genotype × location interaction, genotype × season interaction and genotype × location × season interaction except location which was significant ($p<0.05$) (Table 4).

Genotypes evaluation based on GGE biplots

The which-won-where biplot showed different winning genotypes in different environments (Figure 1). The biplot accounted for 65.74% of the genotype main effect and G × E interaction for grain yield of the genotypes. The biplot was dissected into eight sectors and four mega-

Table 3. Mean squares for grain yield of 25 soybean genotypes evaluated over locations and seasons .

Source of variation		GY (kg/ha)
	Df	MS
Season	1	161551161***
Location	5	60563205***
Season. Location	5	10263901**
Replication. Season. Location	24	2274739***
Genotype	24	320102***
Genotype × Location	120	195916**
Genotype × Season	24	152514^{ns}
Genotype ×Location × Season	120	193393*
Pooled Error	576	142233
LSD		770.1
CV (%)		23.4
δ^2g		4940.81
δ^2gl		8947.17
δ^2gs		571.17
δ^2gls		17053.33
δ^2error		142233
H2 on single plot basis		0.03
H2 individual environment basis		0.06
H2 on across environment basis		0.41

***=p<0.001; **=p<0.01; *=p<0.05; ns=not significant; GY= grain yield; G= genotype; H2= broad sense heritability; δ^2g= variance component due to genotype; δ^2gl= variance component due to genotype × location; δ^2gs= variance component due to genotype × season; δ^2gls= variance component due to genotype × location × season.

environments and showed six vertex genotypes. The biplot identified winning genotypes in each mega-environment as follows; BSPS 48A-28 (G15) for mega-environment I (Bulindi, Nakabango and Kabanyolo), BSPS 48A-28-1 (G4) for mega-environment II (Iki-Iki), Bulindi 18.4B (G16) for mega-environment III (Ngetta) and BSPS 48A-24-1 (G18) for mega-environment IV (Abi). Genotypes within the polygon were less responsive than the vertex genotypes.

The ranking plot (Figure 3) and genotype focused comparison biplot (Figure 2) ranked genotypes based on both mean grain yield and stability performance in order to identify the highest yielding and stable genotypes. Based on mean yield performance and stability, the biplots ranked G15>G16>G22>G17>G21, as ideal genotypes followed by a check variety Maksoy 3N and the rest of the advanced generation lines.

Test location evaluation based on GGE biplots

The environment vector plot showed that Abi, Nakabango and Bulindi had the longest vectors from the biplot origin. The angle between Abi and Bulindi was almost right angle and locations Ngetta and Iki-Iki had the shortest vectors from the biplot origin as well as a small angle between them. Abi, Nakabango and Bulindi were the most discriminating locations, while Ngetta and Iki-Iki were the least discriminating test locations (Figure 4).

The environment focused comparison showed the ideal test location was Nakabango which was located near the center of the concentric circles as the most representative testing location, while other test locations, Bulindi, Kabanyolo, Ngetta, Iki-Iki and Abi were not representative (Figure 5).

Genotypes mean performance for yield, protein, oil content and agronomic traits

The mean performance of 25 soybean genotypes evaluated for two seasons across six locations are summarized in Table 4. Genotype BSPS 48A-28 had the highest yield of 1767 kg/ha followed by Maksoy 3N and Mak 3N × 1N both with average grain yield of 1725 kg/ha; these genotypes had the longest days to 50% flowering as well as lowest groundnut leaf miner damage and rust scores (Table 4). The results of mean percentage protein content are shown in Table 4. The results showed that the overall mean for percentage protein content across

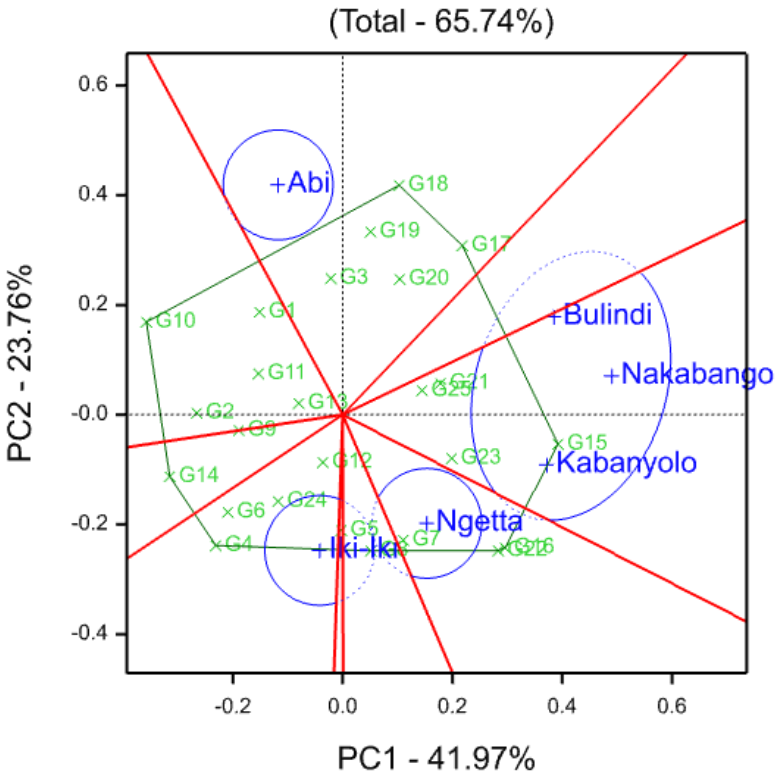

Figure 1. The which-won-where and mega-environment delineation biplot for yield of 25 soybean genotypes evaluated in six locations for two seasons (2018A and 2018B)

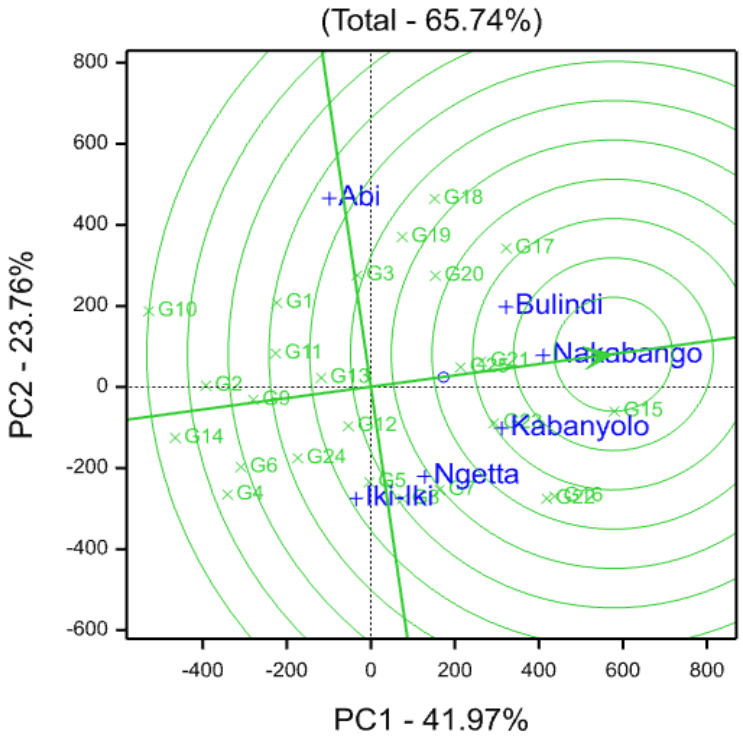

Figure 2. Genotype focused comparison biplot for yield showing the best genotypes based on mean performance and stability

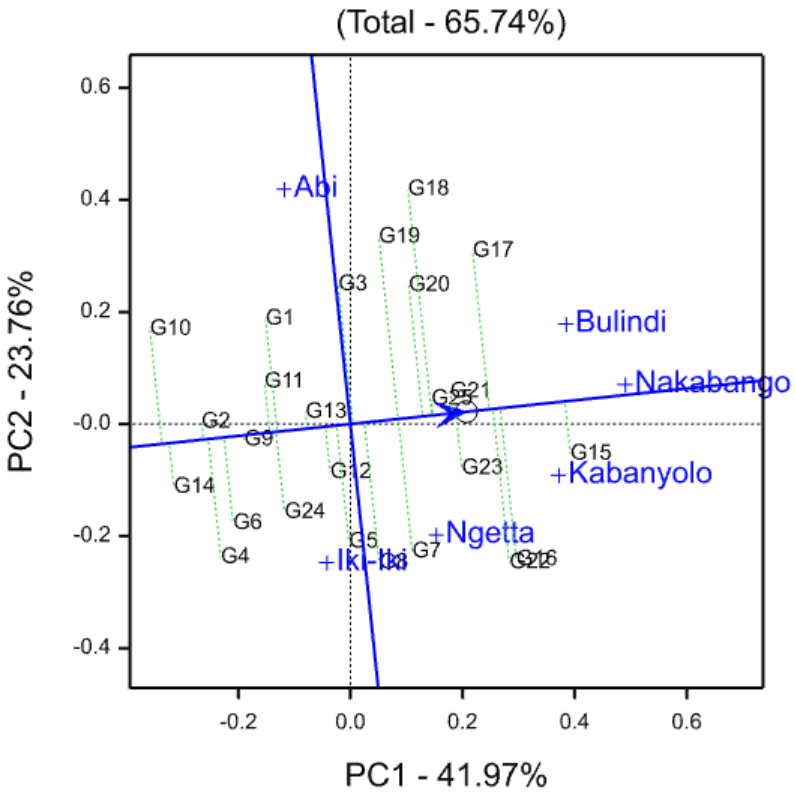

Figure 3. Ranking plot for yield showing the best genotypes based on mean performance and stability.

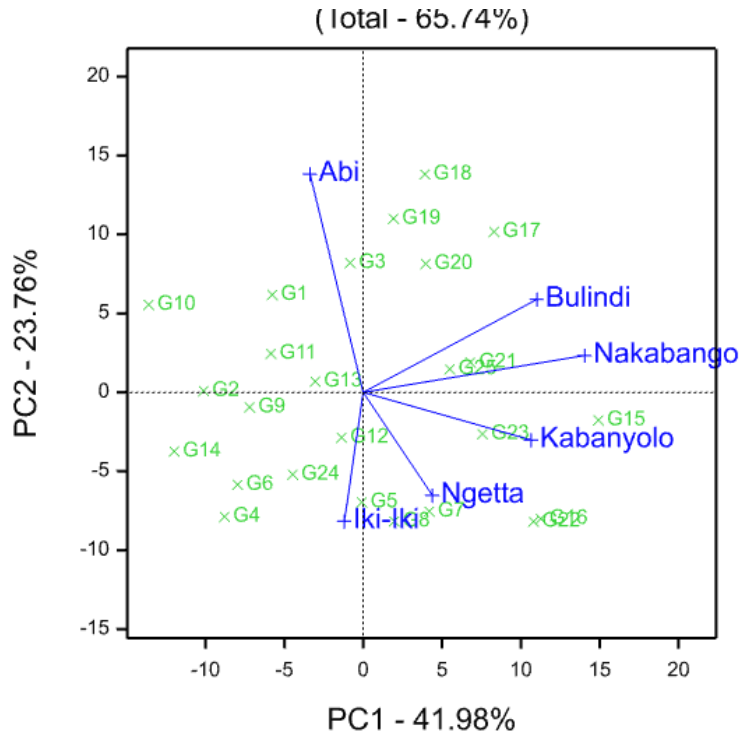

Figure 4. Environment vector plot showing discriminating ability of test locations based on yield

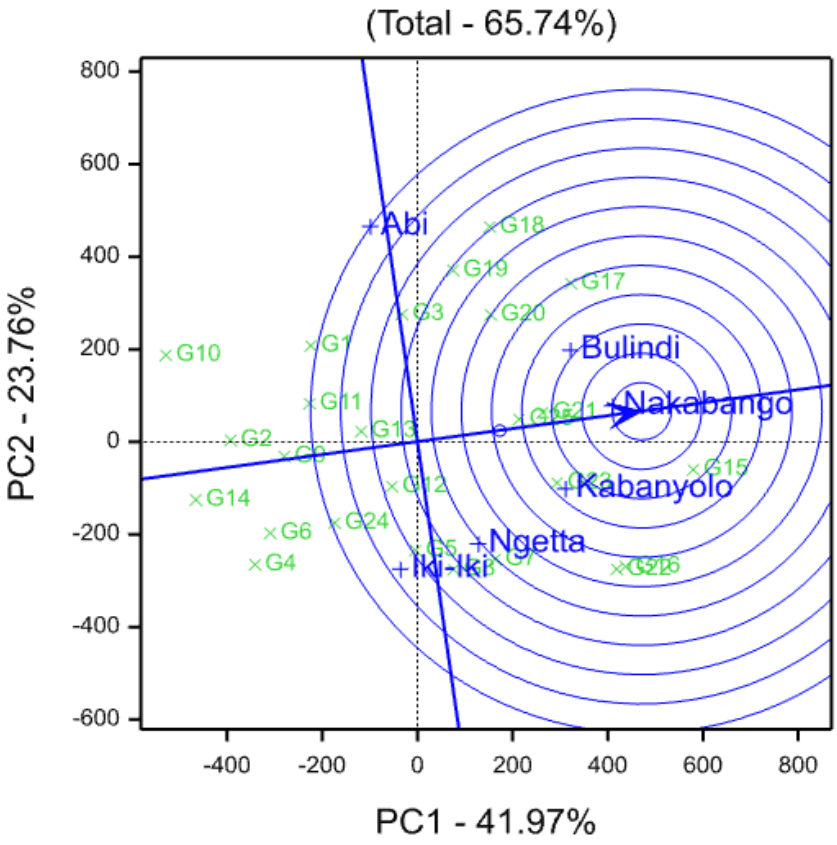

Figure 5. Environment focused comparison biplot showing the ideal testing location for soybean yield among the locations used in evaluations.

seasons and selected locations was 33.54%, with genotypes 2N × GC, G8586 × UG5 and Bulindi 24.1A had the highest percentage protein content of 34.67, 34.62 and 34.45, respectively. The results of mean percentage oil content analysis are presented in Table 4. The overall mean for oil content across seasons and selected locations was 16.01%, with genotypes Duiker × 3N-5, NDGT 8.11× 3N-1 and NGDT 8.11 × 14.16B, were ranked the best three with percentage oil content of 17.26, 16.62 and 16.55, respectively (Table 4).

For locations, Bulindi had the highest mean yield (2650 kg/ha) followed by Abi (1845 kg/ha), Nakabango (1698 kg/ha), Ngetta (1567 kg/ha) and Kabanyolo (1017 kg/ha) while Iki-Iki had the lowest mean yield of 889 kg/ha (Table 5). The overall mean yield performance for the genotypes across locations and seasons was 1611kg/ha.

DISCUSSION

Nature of the G × E interaction, variance components and heritability estimates

The presence of significant genotype main effect as well as G × E interaction for grain yield suggested differential responses of soybean genotypes across tested environments and implied the need to identify high-yielding and stable genotypes across the test environments. Similar results have been reported by several researchers (Gurmu et al., 2009; Atnaf et al., 2013; Kumar et al., 2014; Bhartiya et al., 2017). The large variance component attributed to locations alone justified the need to use genotype main effect plus G × E interaction (GGE) biplots, in which the GGE biplot captured much of the variation due to genotype plus G × E interaction as a fraction of the total sum of squares (G + E + GE) (Yan et al., 2007). The large variance component due to locations and seasons depicted that the locations used in the present study were very diverse across seasons. Indeed, Uganda has diverse agro-ecological zones with highly variable mean annual rainfall of 510-2160 mm, also varied with soil depth, texture, acidity and organic matter (Agoyi et al., 2017). The huge variability of these predictable factors (soil characteristics) and unpredictable factors (temperature and rainfall) (Table 1) from location to location leading into inconsistent genotypic performances (Obua, 2013) and therefore, widely adapted soybean genotypes with dynamic yield stability are recommended to strengthen soybean production country wide (Tukamuhabwa et al.,

Table 4. Grain yield, protein, oil content and agronomic performance of 25 soybean genotypes evaluated across two seasons in Uganda (2018A and 2018B).

Genotypes	Yield(kg/ha)	Protein (%)	Oil (%)	100SWT	DT50%F	GLM	NPODS	PH	RUST
BSPS 48A-28	1767	32.9	15.8	17.0	44	1.1	32	74.3	1.1
Mak 3N × 1N	1725	33.8	16.2	16.7	44	1.1	29	65.8	1.2
Maksoy 3N	1725	33.3	15.9	17.3	41	1.2	31	66.7	1.1
2N × GC	1710	34.7	16.0	15.2	43	1.2	33	67.4	1.6
NGDT 8.11× 3N-2	1702	33.7	16.3	15.8	44	1.3	24	61.3	1.3
BSPS 48A-27-1	1681	34.1	15.6	17.0	43	1.2	28	70.7	1.2
BSPS 48A-25-1	1678	33.4	15.8	16.8	43	1.3	31	75.8	1.3
BSPS 48A-24-1	1672	33.1	15.7	15.4	44	1.2	29	71.6	1.4
Maksoy 4N	1671	32.9	16.1	16.9	44	1.1	30	72.5	1.3
NGDT8.11×14.16B	1652	33.3	16.6	16.9	40	1.2	29	65.0	1.3
Bulindi 18.4B	1648	33.3	16.0	15.2	42	1.4	28	62.2	1.2
NII × GC 35.3-1	1633	33.1	16.1	15.0	44	1.2	30	74.8	1.6
Nam II GC 17.3	1624	33.9	16.4	15.8	44	1.2	27	48.3	1.3
Duiker × 3N-5	1609	34.3	17.3	17.4	43	1.1	33	85.0	1.6
G8586 × UG5	1606	34.6	16.3	15.3	43	1.5	29	52.9	1.6
NII × 35.3-3	1590	33.9	15.0	14.8	43	1.3	30	74.4	1.8
NII × GC 35.3-2	1585	33.5	15.8	15.0	43	1.2	30	73.3	1.5
Bulindi 24.1A	1572	34.5	15.5	16.0	43	1.2	31	81.0	1.8
Nam 4M × 2N-2	1543	32.8	15.6	15.8	42	1.2	30	67.0	1.7
BSPS 48A-28-1	1539	34.4	15.7	16.3	42	1.2	32	64.3	1.6
NG 14.1 × NII-1	1531	33.8	15.8	18.1	42	1.2	24	66.8	1.4
NG 14.1 × UG5	1491	33.8	15.3	16.2	45	1.2	31	80.5	1.4
GC × 2N-1	1469	33.2	16.4	15.5	42	1.2	27	71.6	1.5
NII × GC 13.2	1469	32.1	16.5	16.6	43	1.3	35	68.7	1.6
NDGT 8.11× 3N-1	1385	32.3	16.6	18.1	42	1.1	23	68.4	1.4
Mean	1611	33.5	16.0	16.3	42	1.2	29	69.2	1.5
LSD	174.6	5.4	4.4	2.5	2.5	0.4	14.6	14.3	0.7
CV (%)	23.4	8.3	13.4	9.0	3.1	19.7	27.7	11.1	29.8
F probability	<.001	NS	NS	<.001	<.001	<.001	<.001	<.001	<.001
Genotype × Location	0.009	NS	NS	<.001	NS	<.001	<.001	<.001	<.001
Genotype × Location × Season	0.012	NS	NS	NS	NS	NS	NS	NS	NS

100SWT=100 seed weight (gm); GLM=groundnut leafminner (scores); NPODS= number of pods; PH= plant height (cm); DT50%F= days to 50% flowering, Rust (scores); NS= non-significant.

2012).

The large G × E interaction and error variance components found in the present study could reduce selection progress by complicating the identification and recommendation of superior genotypes for a target environment (Nyombayire et al., 2018; Hunde et al., 2019). The results observed in this study, however, were of a lesser magnitude than that reported by Bhartiya et al. (2017) on 36 soybean genotypes evaluated in 3 environments in India, where G × E interaction almost doubled the genotypic main effects and five times larger than environmental effects. Large G × E interaction and residuals observed in multi-environment trials (MET) affect the repeatability of the experiment (Simion et al., 2018) could have contributed to the low broad sense coefficient of genetic determination (which is equivalent to broad sense heritability based on fixed genotypes) of 3% on a single plot basis and 41% on across environments which has improved as the number of locations and seasons increased. Similar results were reported by Gasura et al. (2015) in sorghum where broad sense heritability increased from 2.8% on single plot basis to 31.8% on across environments basis. Gasura et al. (2015) and Sousa et al. (2018) suggested that large G × E interaction and error variance components increase the cost of variety evaluation due to increase in numbers of replications, locations and seasons needed to improve broad sense coefficient of genetic determination, and hence the selection efficiency. Since crop growing locations have no precisely stated demarcations and most farmers tend to influence each other in the choice of variety that is grown (Gasura et al., 2015), the

Table 5. Grain yield performance in kg/ha of 25 soybean genotypes evaluated across 12 locations.

Genotype	Location							
	Abi	Bulindi	Iki-Iki	Kabanyolo	Nakabango	Ngetta	Mean yield	Rank
BSPS 48A-28	1683	3006	843	1165	2069	1836	1767	1
Mak 3N × 1N	1809	2773	1073	1317	1841	1538	1725	2
Maksoy 3N	2001	2642	817	1041	1937	1912	1725	3
2N × GC	1578	2739	988	1346	1932	1678	1710	4
NDGT 8.11 × 3N-2	1942	2592	850	1189	2021	1621	1702	5
BSPS 48A-27-1	2139	2844	1092	924	1717	1369	1681	6
BSPS 48A-25-1	1696	2686	1027	1181	1709	1766	1678	7
BSPS 48A-24-1	2194	2729	747	1038	2002	1321	1672	8
Maksoy 4N	1926	3036	630	983	1926	1526	1671	9
NGDT 8.11×14.16B	1805	2540	986	1143	1598	1838	1652	10
Bulindi 18.4B	1380	2938	926	1260	1867	1515	1648	11
NII × GC 35.3-1	1915	3030	674	1064	1623	1492	1633	12
Nam II GC 17.3	1694	2376	1014	1189	1861	1610	1624	13
Duiker × 3N-5	2112	2712	937	883	1491	1519	1609	14
G8586 × UG5	1928	2578	978	861	1716	1578	1606	15
NII × 35.3-3	1757	2652	963	973	1635	1563	1590	16
NII × GC 35.3-2	1978	2578	943	1064	1303	1643	1585	17
Bulindi 24.1A	2016	2631	446	1040	1851	1448	1572	18
Nam 4M × 2N-2	1935	2617	799	1111	1359	1438	1543	19
BSPS 48A-28-1	1790	2313	1034	849	1513	1735	1539	20
NG 14.1 × NII-1	1648	2561	1049	793	1670	1464	1531	21
NG 14.1 × UG5	2121	2362	869	733	1440	1419	1491	22
GC × 2N-1	1861	2392	802	865	1447	1447	1469	23
NII × GC 13.2	1616	2520	876	759	1462	1579	1469	24
NDGT 8.11× 3N-1	1603	2417	858	662	1453	1319	1385	25
Mean	1845	2650	889	1017	1698	1567	1611	
CV (%)	25.4	20.5	26.8	31.4	19.1	17.6		
LSD	538	621.5	272.6	365.8	371.1	316		

development of soybean varieties adapted to a broad range of environments is strongly recommended, rather than environment-specific varieties (Bhartiya et al., 2017).

Evaluation of soybean genotypes across environments

The significant difference for grain yield and yield related traits observed among genotypes across environments indicated the presence of genetic and environmental causes of variation. The significant G × E interaction observed in this study also showed the significance of environmental effects in the expression of soybean grain yield. These results are consistent with the findings of other researchers (Chaudhary and Wu, 2012; Atnaf et al., 2013; Krisnawati and Adie, 2018; Hunde et al., 2019). The absence of significant genotype, G × E interaction for protein and oil content observed in this study was inconsistent with previous studies (Gurmu et al., 2009; Nascimento et al., 2010; Chaudhary and Wu, 2012; Hampango et al., 2017) who reported the presence of significance genotype, G × E interaction for protein and oil content. The results obtained from this study showed that there was limited genetic variation among the tested genotypes for protein and oil content and therefore there is no need to advance this set of genotypes targeting commercial improvement of these two traits.

Based on scatter biplot for mega-environments delineation, only four mega-environments with their winning genotypes located at the vertices of the polygon were identified. Locations Kabanyolo, Bulindi and Nakabango were classified on mega-environment I, in which BSPS 48A-28 was the winning genotype. Mega-environment II had Iki-Iki with BSPS 48A-28-1 as the winning genotype, Ngetta was classified on mega-environment III where genotype Bulindi 18.4B was the most adapted. Mega-environment IV had Abi found in the West Nile region where BSPS 48A-24-1 was the winning genotype, indicating that Uganda had broad agro-

ecological regions with unique environmental characteristics with specific suited high yielding genotypes. Location Bulindi had the highest mean yield of 2650 kg/ha, while Iki-Iki had the lowest mean yield of 889 kg/ha. The reason is Bulindi received high rainfall (1700 mm/ annum) and the site has good soil types, with good nutritional status and water holding capacity (Table 1). The reason for low yielding in Iki-Iki might be the gradual changes in biotic and abiotic factors from season to season. On the other hand, Iki-Iki is characterized by poor sandy soils, with low water holding capacity (Tukamuhabwa et al., 2012). Also Iki-Iki is a hot spot for groundnut leaf miner, a new soybean pest which is devastating soybean in Uganda (Ibanda et al., 2018). Despite the relatively low yield potential for soybean in Iki-Iki, genotype BSPS 48A-28 managed to maintain its average performance implying that this genotype had good dynamic stability. This is a good attribute for any commercial variety given the unpredictable patterns of biotic and abiotic factors in most parts of the country (Obua, 2013). The existence of crossover G × E interaction in this study indicated that genotypes evaluation and recommendation typically based on any single location was unreliable because there is differential response of genotypes across locations (Mare et al., 2017). The presence of crossover interactions indicated genotype evaluation should be based on mean performance and stability (Yan and Kang, 2002).

The genotype focused comparison biplot indicated that the most stable and high-yielding genotype was BSPS 48A-28 probably due to having lowest groundnut leaf miner damage, rust scores, high number of pods and, is late maturing advanced line (Table 4). Based on mean yield and stability, the genotype maintained its above average performance in most of the environments. Genotype Mak 3N × 1N was comparable in yield performance to the commercial variety Maksoy 3N which was one of its parents. Meanwhile, a commercial variety Maksoy 4N performed well based on mean yield and stability, although it was ranked fourth (Figure 3) outperformed by three experimental genotypes and Maksoy 3N a commercial variety. Based on ranking plot for mean yield performance and stability (Figure 3), genotypes BSPS 48A-28; Mak 3N × 1N and NGDT 8.11×3N-2 are potential candidates for release since the variety release condition in Uganda advocate for broad instead of specific adaptation.

Evaluation of the test environments

The presence of G × E interaction for soybean yield justifies undertaking MET during cultivar selection and recommendations (Krisnawati and Adie, 2018). Based on test location biplot, the vector length of the biplot approximates the standard deviation within each location and a measure of the discriminating ability of the location (Yan and Tinker, 2006). Nakabango, Bulindi and Abi locations, which had the longest vectors from the biplot origin, were the most discriminating testing locations and, therefore these three testing locations could be used jointly as discriminating locations for testing early generation breeding materials (Yan et al., 2007; Yan and Tinker, 2006). Bulindi and Abi were discriminating genotypes but not representative and therefore, these two sites could be used together as “culling environments” for easily selecting against unstable genotypes during the breeding process (Yan and Kang, 2002). Nakabango was both discriminating and representative. Discriminating and representative test locations are useful for selecting superior genotypes while eliminating inferior ones (Atnaf et al., 2013).

CONCLUSION AND RECOMMENDATIONS

There was crossover G × E interaction for soybean grain yield which was twice larger than the effect of genotypes. Non-significant G × E interaction for percentage protein and oil content observed in the present study, hence no need to advance this same set of genotypes targeting commercial improvement of these two characters. We recommend BSPS 48A-28; Mak 3N × 1N and NGDT 8.11×3N-2 as widely adapted and higher yielding genotypes that could be advanced to the national performance trials before commercialization in Uganda. These three genotypes had lowest groundnut leaf miner damage, rust scores, high number of pods and, are late maturing advanced lines. They have almost all desirable attributes of a good soybean cultivar. Location Nakabango was both discriminating and representative, hence testing soybean genotypes at this location is ideal; it can save time and resources.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This research was implemented with funding from Intra-ACP Academic mobility for Crop Scientists for Africa Agriculture (CSAA) project. The authors also thank the Makerere University Soybean Improvement Centre and RUFORUM for partial financial support.

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