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Full Length Research Paper

GGE biplot phenotypic stability analysis of soybean [*Glycine max* (L.) Merril] genotypes

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The study on genotypes by environment interaction (GEI) and stability analysis was conducted to determine the G, E, and GEI variance magnitudes. The experiment was carried out at three locations in two consecutive years on 26 soybean genotypes using randomized complete block design (RCBD) design with three replications. The objectives were to (i) estimate the magnitudes of G, E, and GEI effects, (ii) stability analysis of 26 genotypes, and (iii) to identify the highest yielding genotypes for both specific and wide adaptability. The combined analysis of variance (ANOVA) of seed yield data was confirmed strongly significant ($p \leq 0.001$) for G, E, and GEI variances. At Kamash, the yield was increased by 47.6% as compared to Begi might be due to soil factors differences. The soybean plants therefore grew more produced, more yield where soil fertility is the highest as compared to poorest areas. The G, E, and GEI effects contributed 15.1, 51.6, and 30.2%, respectively. Such that the main variability is due to E and GEI variances being the largest proportions of the total treatment sum of square (TTSS). The genotypes main effect and genotypes by environment interaction (GGE) biplot is therefore the most appropriate recently used model's for stability analysis in efficiently utilizing and exploiting the existed GEI SS. The first two PC (PC1 and PC2) axes were used to create the two dimensional GGE biplots that explained 40.35 and 26.38% of GGE TSS, respectively. The biplots polygons vertex genotypes were categorized as the strongest and weakest as well as stable and unstable genotypes. The result of GGE biplot for G3 and G5 providing the best niche at A15, B15 and B16, G5, and G4 the highest at A16 and K16, while G4 and G12 are also best at K15. The highest and specifically performing polygon vertex genotypes contributed maximum MS for GEI SS. The highest scores for PC1, near zero absolute values for PC2, and the highest means were recorded from G5, G6, G19, G17, and G25 contributing nothing or little MS for GEI SS. These consistently performing genotypes showed high stability based on GGE biplots analysis growing vigorously in producing maximum means without changing their ranking across all sites for this economically interesting trait.

Key words: Genotypes main effect and genotypes by environment interaction (GGE) biplot, genotypes by environment interaction (GEI), seed yield, soybean genotypes, stability analysis.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is categorized under Fabaceae family, genus *Glycine*, and sub-genus *Soja* (Lackey, 1977). The *Soja* contains wild (*Glycine soja*) and cultivated (*G. max* (L.)) species where the *G. soja* species

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is the probable ancestors and gene sources for cultivated species (Hymowitz, 1970). Soybean is well adapting at 1300 to 1800 m altitudes receiving from 900 to 1300 mm rainfall, and 25 to 30°C temperature (Amare, 1987; Summerfied, 1975). The ideal soil types are light textured loams and medium black clay with pH of 6.5 to 7.0 (EIAR, 1982). Ethiopia is endowed with 18 main and 32 sub-agro-ecologies. This wide agro-ecological variability is the major challenges for field crops which resulted in high genotypes by environment interaction (GEI) effect. This GEI effect is a function of inconsistent responses of varieties due to genetic vs. location effects. The results of Rao et al. (2002) and Fekadu et al. (2009) confirmed strongly significant genotypes (G), locations (L), genotypes by locations interaction (GLI), and GEI effects for soybean genotypes. The ultimate goal of stability analysis is developing of consistently responding superior genotypes for broad adaptability (Kang, 1998). But, achieving of these objectives is generally difficult due to the probability of significant GEI effect (Gauch and Zobel, 1996). Accordingly, different parametric methods were developed for GEI partitioning (Kaya et al., 2006). The genotypes main effect and genotypes by environment interaction (GGE) model is more preferable for cross-over-type GEI describing via visual displaying of the which-won-where, and high mean *vs.* stability (Yan, 2001; Ding et al., 2007). The bilinear GGE model practically describes the first two PCs for effectively GEI partitioning via G *vs.* GEI effects variability exploring (Yan et al., 2000; Guach, 2006). This method is also graphically visualizing the G *vs.* GEI effects for means *vs.* stability estimation via mega environment (ME)'s identification (Yan and Tinker, 2006; Yan et al., 2007; Yan, 2014). The objectives of the study were (i) to determine the G, E, and GEI variances magnitudes, (ii) estimate the stability of 26 genotypes for seed yield, and (iii) to identify the highest yielding genotypes for both specific and broad sense adaptability.

MATERIALS AND METHODS

Descriptions of testing sites

The experiment was carried out at Assosa centre and also at Kamash and Begi sub-center experimental fields. The Assosa centre is one of the 17th centre for Ethiopian Institute of Agricultural Research (EIAR) positioned at Western Ethiopia at 10° 02.922 'N latitude and 34° 33.868 'E longitude at 1547 m elevation at a distance of 660 km from Addis Ababa city. The Begi is also situated at 9° 23.165 ′N latitude *vs.* 34° 24.380 ′E longitude at altitude of 1783 m and 125 km East of Assosa town. The Kamash is located at 1223 m and at a distance of 270 km at Northwest from Assosa town. The Assosa and Begi are characterized by a unimodal rainfall patterns receiving maximum mean during Jul, Aug, Sep, and Oct (AsARCMS, 2016). The mean annual rainfall during 2011 to 2016 was 1092.38 and 1289.10 mm for Assosa and Begi, respectively. The mean annual maximum and minimum temperatures, respectively was also reached at 28.6 and 15.4°C for Assosa and 26.0 and 13.0°C for Begi. The dominant soil types for Assosa are Dystric Nitosols and Fluvisols, while it is Eutric Nitisols followed by Orthic Acrisols and Eutric Fluvisols for Tongo-Begi areas (AsARCFSS, 2007). The soil textures are, respectively clay and sandy-clay for Assosa and Kamash with sand (22.5 and 51.0%), silt (22.5 and 12.0%), and clay (55.0 and 37.0%).

Breeding materials and experimental design

The study was carried out for two consecutive years (2015 to 2016) on 26 soybean genotypes in RCBD with three replications. The experimental materials used for the study were TGX-1740-2F, TGX-1935-10E, TGX-1987-10F, TGX-1987-62F, Gizo, Gishama, Awassa-95, Davis, Williyams, Nova, Crownford, Boshe, Jalele, Cocker-240, AGS-7-1, Clark-63k, Wello, Nyala, Gozela, TGX-1987-18F, Bellesa-95, TGX-1332644, Wegayen, Afgat, TGX-1987-38F, and TGX-1987-11F. The net area of each plot was 1.8 m^2 with one harvestable row. The inter and intra row planting distance was 60 and 5 cm, respectively. The yield data harvested from one central row of each net harvestable plot in g was converted into kg/ha by adjusting the grain weight at 12.5% moisture content.

Stability analysis

SAS PROC GLM of V-9.2 was used for both combined and separate analysis of variance (ANOVA) MS analysis to examine the existence of significant F-test for G, E, and GEI variances to discriminate the weakly performing as well as to identify superior genotypes (SAS, 2002). The error MS for individual environment was tested for homogeneity of error variance prior to pooling data for combined analysis. The homogeneity of error variance was determined by Bartlett's test. The objectives of pooled ANOVA for L *vs.* Y were to partition the total treatment sum of square (TTSS) into G, E, GEI, and pooled error variances as well as also to quantify magnitudes for main effect describing (SAS, 2002). The ANOVA explains only main effects, but it does not indicate the stability patterns *vs.* high mean squares (MS) contributed genotypes for GEI. The GGE model could implied the highest MS contributed as well as visually displaying the superior *vs.* stability for broad adaptability via GEI effectively partitioning. The contribution of G, E, and GEI effects were estimated by plotting of the means against PC1 scores (Zobel et al., 1988). These G *vs.* GEI effects displaying were effectively done by GGE model (Yan et al., 2000, 2007). The genotypes means of each environment were used for GGE biplots analysis, only GEI MS F-test was significant (Kang and Magari, 1995; R-V 3.4.3, 2017). The ANOVA for GGE was done by SAS (Burgueno et al., 2001). The GGE biplot GUI package of R-version 3.4.3 was used for GGE stability analysis following Yan et al. (2000) model. The polygon view for GGE biplots was also graphically plotted by connecting the highly projected vertex genotypes magnitudes on the first two PC axes for visual displaying of which-won-where patterns, environmental vectors, genotypes ranking for means *vs.* stability and environments comparison with ideal environment (R-V-3.4.3, 2017; Yan et al., 2000; Yan, 2001; Yan and Kang, 2003).

RESULTS AND DISCUSSION

Analysis of variances

The ANOVA showed that environments have significantly ($p \leq 0.001$) affected the seed yield of 26 tested genotypes (Table 1). The mean was highly varying with a range of 1545.79 at B16 to 3396.61 kg/ha at K15 for L *vs.* Y wise combined data analysis (Figure 1). The higher mean at

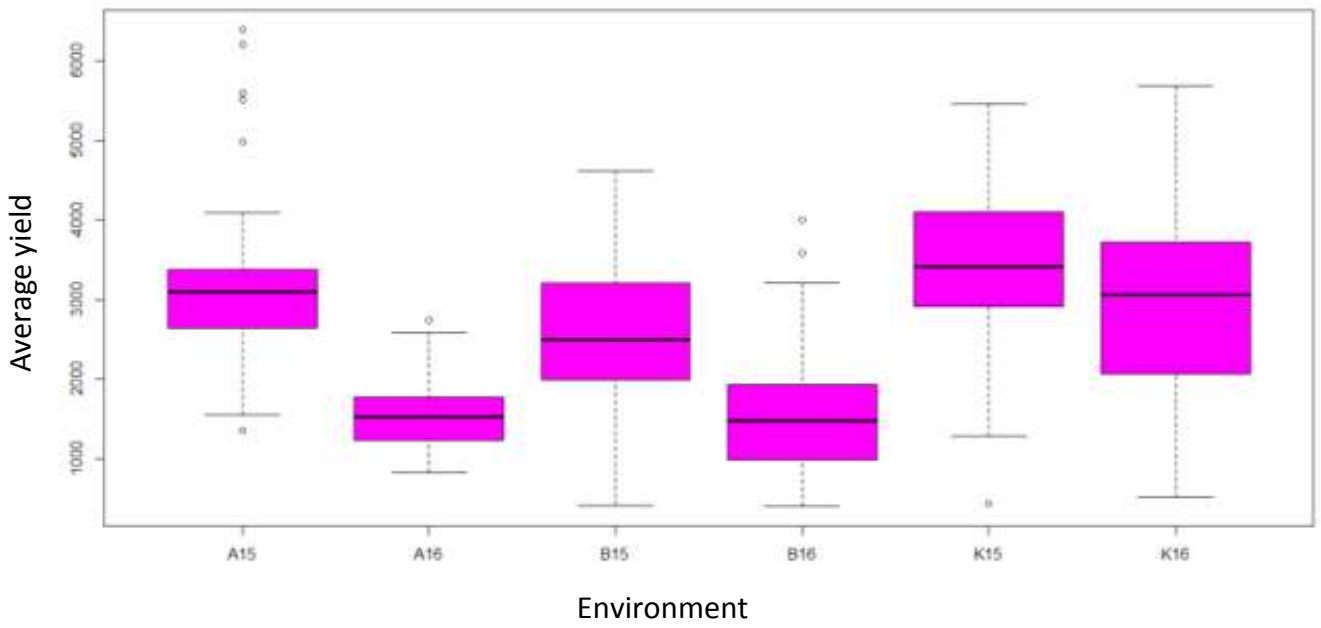

Figure 2. Average mean seed yield of six testing environments (Ls *vs*. Ys) across 26 genotypes in 2015 and 2016 at Assosa centre and Kamash, and Begi sub-center, Ethiopia.

Table 2. General soil chemical characteristics values for experimental sites at Assosa centre and Kamash and Begi sub-center, Ethiopia.

Soil property parameter	Assosa	Kamash	Begi
PH 1:1 H_2O	5.48	6.51	5.94
Nitrogen (%)	0.18	0.22	0.17
OC (%)	3.75	2.76	6.15
P (ppm) in Olsen method	4.30	18.80	14.80
CEC (meq/100 g)	21.78	13.00	28.26
Exchangeable K (cmol (+) kg^{-1})	0.17	1.73	1.50
Exchangeable Na (cmol(+) kg^{-1})	0.09	0.11	1.20

Kamash might be due to soil fertility variation indicating that Kamash is a more potential site for soybean production (Table 1). The mean was reduced by 47.6% at Begi due to location effect (Figure 1). The genotypes produced low yield at areas where soil fertility is a limiting factor as compared to those grown at a fertile soil. The soil at Kamash is more favorable for plant growth than Assosa and Begi with N (0.22 *vs.* 0.18 and 0.17%), P (18.8 *vs.* 4.3 and 14.8), K (1.73 *vs.* 0.17 and 1.5%), and pH (6.51 *vs.* 5.48 and 5.94), respectively (Table 3). The results of combined ANOVA also showed significant ($p \leq 0.001$) differences for genotypes (Table 4). This significant genotypes variance indicates adequate genetic variability. Strongly significant ($p \leq 0.001$) G, E, and GEI variances were reported by Fayeun et al. (2016). The G5 (3202.2), G19 (3170.9), and G17 (2933.2 kg/ha) were proved for the highest mean, ranked 1st, 2nd, and 3rd, respectively. The G5 and G19 were significantly superior except G17, G4, G13, G25, G3, and G6 (Table 5). Seven genotypes (G8, G9, G10, G11, G14, G15, and G16) were low yielding. The G3, G4, G12, G13, and G23 showed specific adaptability only at favorable sites (Figure 2). The GEI MS was also strongly significant ($p \leq 0.001$) and it might result from magnitude differences changing among tested genotypes (Table 6). This GEI effect was approved by the 1st and 2nd axes consisting both positive and negative values that resulted in cross-over-type interaction (Table 7). Strongly significant GEI effect was also reported by Fekadu et al. (2009). The breeders should be looking either for non-cross-over or absences of GEI effect in selecting of broadly adapting genotypes (Matus Cadiz et al., 2003).

The TTSS of ANOVA due to G+E+GEI was partitioned into G, L, Y, GLI, GYI, LYI, and GLYI variances attributed

Table 2. Mean squares of combined analysis of variance for 26 soybean genotypes studied in 2015 and 2016 at Assosa centre and Kamash and Begi sub-center, Ethiopia.

Source of variation	Degree of freedom	Mean square	%
G(DF=25)	25	2954344.2***	15.14
E(DF=5)	5	50340889.7***	51.58
Rep(DF=2)	2	263499.0^{NS}	0.11
L×Rep (DF=10)	10	1460634.5***	2.99
GEI (DF=125)	125	1178311.3***	30.18
Error (DF=300)	300	452808.9	-
CV (%)	-	26.58	-

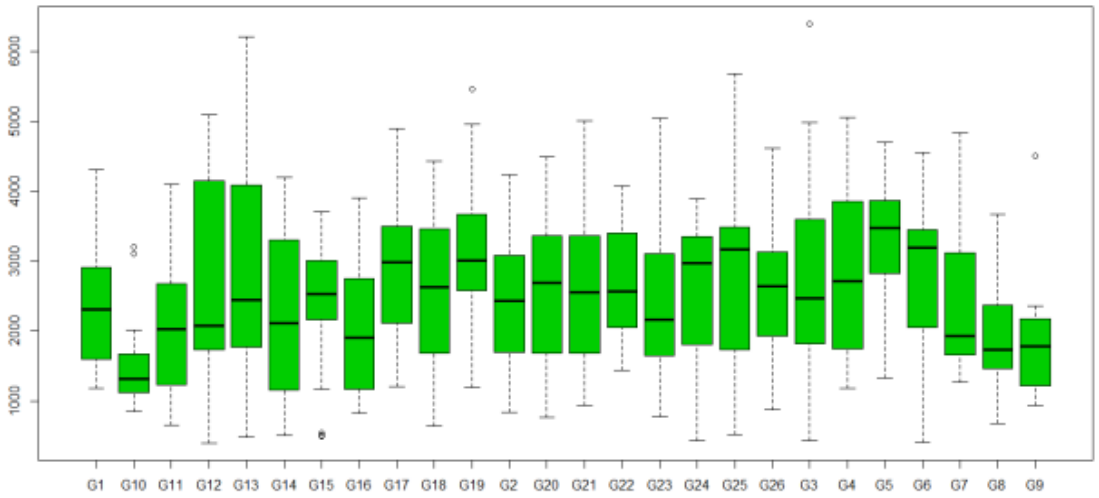

Figure 3. Mean performances of 26 soybean genotypes for combined data across three Ls *vs*. two Ys in 2015 and 2016 at Assosa centre, and Kamash and Begi sub-center, Ethiopia.

15.1, 20.9, 25.5, 15.9, 5.5, 5.2, and 8.8%, respectively (Table 8). The largest variance was explained by E (51.6%) consisted L, Y, and LYI effects. The GEI variance was also almost twice than the G that explained 30.2% of G+E+GEI variance. The interaction is not ignored for such large GEI than G (Yan and Kang, 2002). This large GEI effect was suggested by the differential responses of genotypes and possibility of ME existence with different winning genotypes (Yan and Kang, 2003; Fayeun et al., 2016). The larger GEI variance also indicated both predictable and unpredictable effects leading to specifically or broadly performing genotypes developing (Dehghani et al., 2006).

The ANOVA due to G+L+GLI also provided G, L, and GLI variances (Table 9). The G and GLI variances were 28.3 and 50.3%, respectively, out of the 94.4% for 2015. The G and L effects were also 27.1 and 50.1%, respectively, from total 96.9% variance for 2016. The GLI variance in comparison with G effect suggested the possibility of ME existence. This large variation due to G, L, and GLI effects suggested the suitability of SREG model for stability analysis (Gauch and Zobel, 1996). The GGE model is efficient for G *vs.* cross-over-type GEI effect interpretation (Karimizadeh et al., 2013). This model is also effective to identify highly stable *vs.* specifically adapting genotypes via GEI variance demonstrating *vs.* ME's delineating (Santos et al., 2016). Moreover, Kang et al. (2006) confirmed that the GGE biplot strength for stability *vs.* superiority was determined. The limitation of GGE biplot is the capturing of small portion of total variability (Yang et al., 2009).

The ANOVA of site regression model was significant ($p<0.001$) for G, E, and GEI variances (Table 10). The E (53.23%) and GEI (31.15%) effects took the largest proportion of the TTSS variance. The GGE MS variance was strongly significant for PC1, PC2, and PC3 with 81 df, cumulatively accounted 81.95% of the TSS. The 1st and 2nd PC axes with 40.35 and 26.38%, respectively and df of 29 and 27, respectively, effectively partitioned the existed GEI (Table 11). This 66.73% attributes was

Table 12. Percent contribution of sum of squares of each component variance to TTSS of 26 soybean [*Glycine max* (L.)] genotypes in 2015 and 2016 at Assosa centre and Kamash and Begi sub-center, Ethiopia.

Source of variation	Degree of freedom	Sum of squares	Mean squares	%
G	25	73858605	2954344.2***	15.1
L	2	101806746.9	50903373.4***	20.9
Y	1	124646535.8	124646536***	25.5
L×Y	2	25251166	12625583***	5.2
R (L×Y)	12	15133342.7	1261111.9**	3.1
G×L	50	77951262.2	1559025.2***	15.9
G×Y	25	26639534.3	1065581.4***	5.5
G×L×Y	50	42698115.1	853962.3***	8.8

Table 13. Mean square of ANOVA for G, L, and GLI Variances for year wise combined analysis of 26 soybean [*Glycine max* (L.) genotypes in 2015, and 2016 at Assosa centre and Kamash and Begi sub-center, Ethiopia.

Sources of variation	2015			2016		
	SS	MS	%C	SS	MS	%C
G (DF=25)	45281729.1	1811269.2***	28.3	55216410.3	2208656.4***	27.1
L (DF=2)	25212886.3	12606443.0***	15.8	101845027.0	50922513.3***	50.1
R (DF=2)	1969496.0	984748.0^{NS}	1.2	1176197.4	588098.7^{NS}	0.6
L×R (DF=4)	6936869.8	1734217.4*	4.3	5050779.5	1262694.9*	2.5
GLI (DF=50)	80439736.3	1608794.7***	50.3	40209641.0	804192.8***	19.8
G+L+GLI (DF=77)	150934351.6	16026506.9	94.4	197271078.3	53935362.5	96.9
Error (DF=150)	74884451.7	499229.7	-	60958206.0	406388.0	-

Table 14. VIPC1, and VIPC2 of GGE, and seed yield (kg/ha) of 26 soybean genotypes in 2015, and 2016 at Assosa centre and Kamash and Begi sub-center, Ethiopia.

Geno	Genotypes	PC1	PC2	Mean	Geno	Genotypes	PC1	PC2	Mean
G1	TGX-1740-2F	-0.63	-0.58	2390.5	G14	Cocker-240	-1.66	1.67	2291.8
G2	TGX-1935-10E	-1.03	1.77	2436.7	G15	AGS-7-1	-0.49	-0.91	2374.6
G3	TGX-1987-10F	0.69	3.56	2806.7	G16	Clark-63k	-2.73	0.47	2024.6
G4	TGX-1987-62F	2.00	-1.77	2903.4	G17	Wello	2.25	-0.02	2933.2
G5	Gizo	3.03	0.77	3202.2	G18	Nyala	0.67	-1.20	2662.6
G6	Gishama	1.25	-0.39	2796.2	G19	Gozela	3.93	-0.93	3170.9
G7	Awassa-95	-1.23	0.24	2363.3	G20	TGX-1987-18F	0.13	1.25	2573.9
G8	Davis	-2.99	-0.42	1914.1	G21	Bellesa-95	0.46	-0.53	2632.8
G9	Williyams	-3.59	-1.02	1817.2	G22	TGX-1332644	1.40	-1.36	2706.2
G10	Nova	-4.60	0.17	1553.6	G23	Wegayen	0.30	-2.44	2549.2
G11	Crownford	-2.25	-1.04	2072.7	G24	Afgat	0.65	2.20	2623.9
G12	Boshe	0.51	-3.17	2617.3	G25	TGX-1987-38F	2.36	0.13	2887.7
G13	Jalele	0.87	2.30	2903.3	G26	TGX-1987-11F	0.69	0.27	2591.9

predicted on the 1st and 2nd PC axes of the total G+GEI derived by G+GE centered to SVD for existed GEI variance visualizing. The results are in accordance with that of Edmore et al. (2015) who explained 36.8% (PC1) and 29.5% (PC2) of the GGE SS. This justifies the efficiency of GGE model in exploiting the G plus GEI variability. Similar reports were confirmed by Yan et al. (2000); Gauch (2013) captured 67% variation with the first two PCs. The positioning of G *vs.* GEI effects on PC1 *vs.* PC2 of GGE biplot is as shown in Figure 4. The GGE biplot is an effective method for which-won-where pattern and superiorly performing stable genotypes displaying via

Table 15. The results of ANOVA for GGE sum of squares of 26 soybean genotypes in 2015 and 2016 at Assosa centre and Kamash and Begi sub-center, Ethiopia.

SV	DF	SSGGE	MSGGE	%E
G	25	73858671.8	2954346.9***	15.62
E	5	251704507.4	50340901.5***	53.23
GEI	125	147288871.0	1178310.9***	31.15
IPCA1	29	89227410.0	3076807.2***	40.35
IPCA2	27	58336574.0	2160613.9***	26.38
IPCA3	25	33653544.0	1346141.8***	15.22
IPCA4	23	22802505.0	991413.3**	10.31

DF, Numbers in parentheses, degrees freedom; CV, coefficient of variation, NS, non-significant, *, **, and *** indicate the significance levels at 0.05, 0.01, and 0.001, respectively, and mean=2530.79 kg/ha, R^2=0.78, and CV=26.58% for all listed tables.

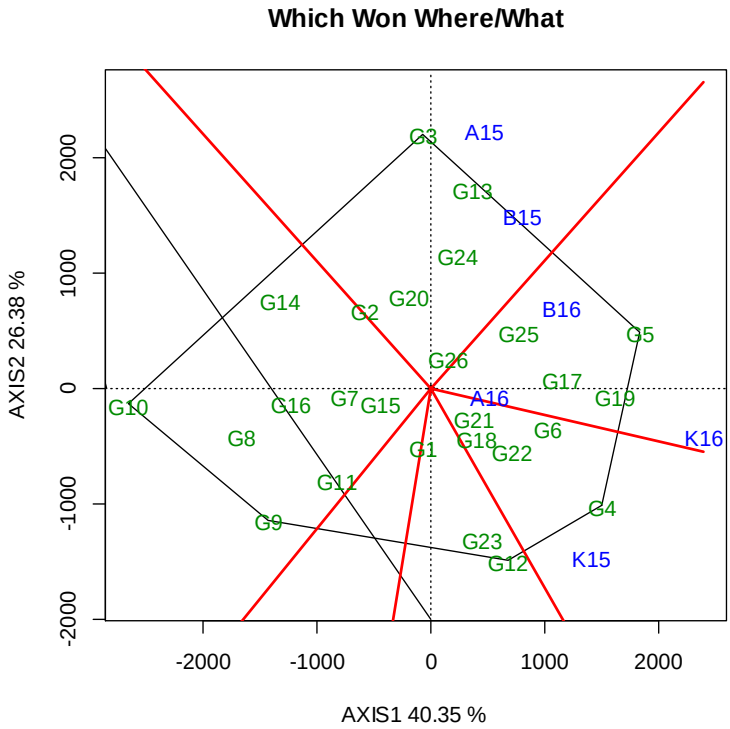

Figure 3. Vector views of GGE biplot when PC2 is plotted against PC1 for environments relations, winning genotypes, and MEs for yield of A15 *vs.* A16=Assosa year one *vs*. two, K15 *vs.* 16=Kamash year one *vs*. two; B15 *vs.* 16=Begi year one *vs*. two in 2015 and 2016.

GEI *vs.* ME's visualizing (Yan et al., 2007; Atnaf et al., 2013; Massaine et al., 2018). The results of GGE biplot showed that G3, G5, G4, G12, G9, and G10 were the highest and poorest located at the vertexes of polygon responding either positively or negatively for seed yield (Figure 5). G3 and G5 were the best winning at A15, B15 and B16, G5 and G4 are the highest, so niche at A16 and K16, while G4 and G12 are also well performing at K15 (Figure 6). The G3 and G12 were specifically adapted at favorable sites; contributed maximum MS to GEI SS due to high values for 2st PC (Figure 7). The polygon vertices are markers for highly projected genotypes indicating specific adaptability (Melkamu et al., 2015; Pavel et al., 2015). G8, G9, G10, G14, and G16 were the poorest that lied in opposite side of the vectors of all environments; not performed at all testing sites. The polygon vertexes G9 and G10 are the poorest genotypes that lied in opposite side of all vectors. Similar results were reported by Ashraful et al. (2017). Moreover, Figure 8 also provides a summary of E *vs.* G relationship. These

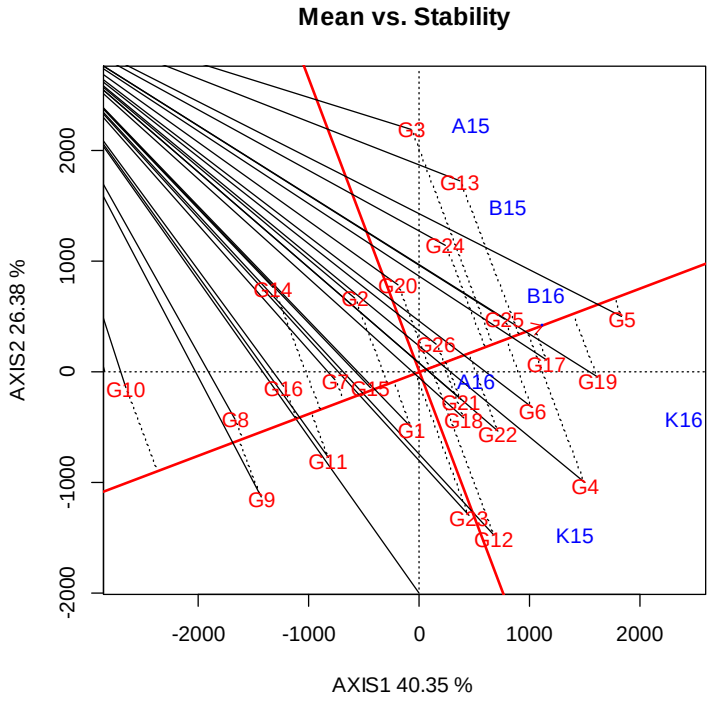

Figure 9. GGE biplot for mean *vs*. stability of 26 soybean [*Glycine max* (L.)] genotypes, and GEI in 2015 and 2016 at Assosa centre and Kamash, and Begi sub-center, Ethiopia.

variables were represented by vectors and markers, respectively. The correlation between vectors was determined by drawing a lines passed across origin aligned perpendicular to each polygon's sides. The line segments divided the polygon into sites and genotypes sectors. The environments were categorized into three major growing sectors based on the angles related with correlation coefficient (Figure 10). The first group was A15 and B15 with G3 and G5 as the most favorable genotypes. The second sector also includes B16, A16 and K16 where B16 is with G3 and G5, while A16 and K16 are with G5 and G4. The third one is K15 with G4 and G12 as favorable genotypes. The correlation for adjacent vectors was determined by cosine of the angles (Yan and Tinker, 2006). A15 and K15 were higher than 90° projected highly to positive and negative coordinates, respectively, witnessed negative association (Figures 3 and 7). A16, B16, and K16 showed less than 90 which implied strong correlation. This less than 90° indicates high correlation (Yan and Holland, 2010). The positive and negative relations were observed for Ls *vs.* ys combination analysis (Santos et al., 2016).

The horizontal axis drawn to pass via biplot origin and average genotypes was average tester coordinate (ATC) line used for visual displaying of both means *vs.* stability (Figure 11). The oval sign of an arrow is showing the positive end of ATC line. The average yielding capacity was estimated by mean projection onto ATC x-axis (Pavel et al., 2015; Ashraful et al., 2017). The double arrowed ATC lines passed via biplot midpoint divided the genotypes into the poorest (below average) *vs.* the highest (above average), and stable *vs.* unstable based on means and stability (Figure 12). The G5 is the highest, while G10 is the lowest for mean. The double arrowed ATC lines show the lowest *vs.* highest and stable *vs.* unstable genotypes (Fayeun et al., 2016). The stability was also explored by projection onto ATC vertical axis. For instance, G4, G12, G13, G23, G3, and G14 were strongly deviated from ATC line. These genotypes were unstable contributed high MS for GEI effect. The smaller distance between ATC line and genotypes markers also indicates high stability (Figure 13). For example, G5, G17, G25, G26, G15, G7, G16, and G8 were consistent for yield response showing slightly little projection. These shorter absolute deviations witnessed high stability (Melkamu et al., 2015; Fayeun et al., 2016; Ashraful et al., 2017). The term high stability is desirable only when associated with high means (Yan and Tinker, 2006). Moreover, the yield performance consists of both means and stability. Accordingly, the highest scores for PC1 (3.03, 2.25, 3.93, and 2.36%) and near zero absolute values for PC2 (0.77, -0.02, -0.93, and 0.13%) were recorded for G5, G17, G19, and G25, respectively (Table 16). The GGE biplot is effective to evaluate and rank the genotypes based on the means *vs.* stability (Yan et al., 2007; Amira et al., 2013; Pavel et al., 2015). The GGE

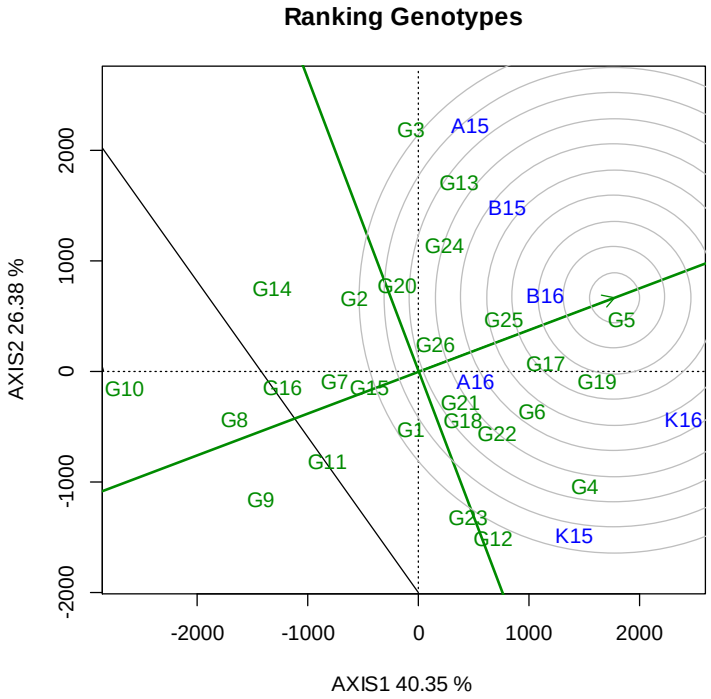

Figure 5. GGE Biplot for genotypes comparison (ranking) with reference to ideal genotypes in 2015 and 2016 at Assosa centre and Kamash, and Begi sub-center, Ethiopia.

biplot was also used to integrate both superior means *vs.* stability (Kang, 2002; Kang and Magari, 1996). According to Yan (2001), Yan and Hunt (2001, 2002) and Yan and Kang (2003), the first two PCs of GGE biplot are completely partitioning the GEI by visual displaying of G *vs.* GEI effects distribution, both poor *vs.* superior, and which-win-where *vs.* stability pattern for identifying and integrating of superior *vs.* stability as well as discriminating *vs.* representing ME mapping.

The ATC line drawn to pass via average genotypes *vs.* biplot origin serves as reference to compare the genotypes based on means and stability. This ATC performance line was used for genotypes ranking according to the mean and stability (Yan and Kang, 2003). The average means for genotypes were estimated by projection onto ATC horizontal axis (Figure 14). The projection is equal to the longest vectors of all genotypes. The center of concentric circles is showing the virtual ideal genotypes (Figure 15). The ideal genotypes could be high yielding and absolutely better for stability (Yan and Kang, 2003; Pavel et al., 2015). The smaller distance from ideal genotype indicates absolute stability. The highest yielding G5 following G19, G17, and G25 was high for both means and stability. The closely positioned genotypes were highly desirable due to high means and stability (Pavel et al., 2015; Richmond et al., 2015; Fayeun et al., 2016). The genotypes located near to ideal genotype were also highly productive and stable (Olayiwola et al., 2015; Ashraful et al., 2017; Massaine et al., 2018). The G10, G8, and G9 were highly projected from the center of concentric circles to unstable. Moreover, the G13 and G18 are not different from apparently inferior G20 and G1 (Figure 16). These highly projected genotypes were found to be the poorest and unstable (Edmore et al., 2015; Massaine et al., 2018). There were different genotypic groups observed from overall inter-relationship among all 26 tested genotypes (Figures 17 and 18). The G17, G19, and G25 were found to be positively and moderately correlated with most favorable G5 (Figure 5). There are high correlation among the best genotypes namely G5, G17, G19, and G25 (Figure 19). The G6, G19, G25, G26, and G21 had shown positively strong association with the most favorable genotypes (G17 and G5) (Figure 20). Similar results of strong correlation among the genotypes were reported by Ashraful et al. (2017). They confirmed that the genotypes being positioned close to each other on GGE biplot responding together similarly to the environments were found near to these genotypes.

The average environment coordination (AEC) line was passed via average environment *vs.* origin for ideal environment position delineation. The average means was estimated by projection onto AEC horizontal axis. The projection is equal to the longest vectors of all environments. The center of concentric circles shows virtual ideal environments. The deviation is zero

Table 7. IPCA1, IPCA2 scores, environmental index (effects), and %CV (scores) for six testing environments (Ls *vs*. Ys) across 26 soybean genotypes in 2015 and 2016 at Assosa centre and Kamash and Begi sub-center, Ethiopia.

ENV	Mean	Rank	DF	IPC1	IPC2	IPC3	IPC4	EI	%CV
A15	3136.4	2	29	67.87	8.10	-28.00	9.61	374***	-42.8
A16	1550.7	5	27	0.24	6.72	30.02	35.36	-1145.3***	0.99
B15	2607.7	4	25	37.76	-26.87	47.55	-7.19	-199.8^{NS}	-23.82
B16	1545.8	6	23	-1.83	53.96	20.02	1.00	-1036.6***	0.87
K15	3396.6	1	21	11.97	31.87	0.79	3.69	716.2***	34.96
K16	2947.7	3	19	10.69	16.85	11.71	-40.39	254.9^{NS}	30.67

^{NS} and *** were respectively non-significant and significant at 0.05 and 0.001 probability levels. ENV: Environments, grand mean=2530.79 kg/ha, R^2=0.78, and CV=26.58%.

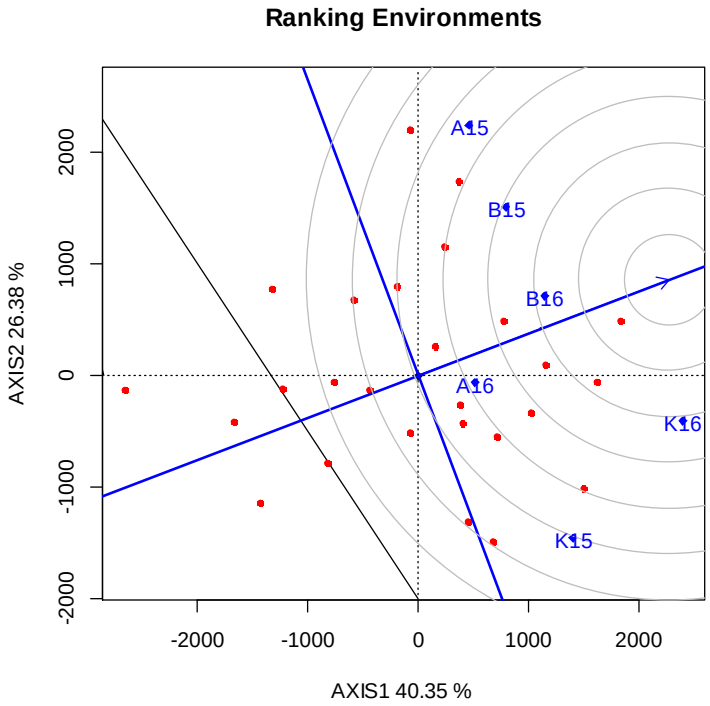

Figure 21. Ranking of environments with reference to virtual ideal environment according to discriminating ability *vs.* suitability of representation when plotted IPCA1 *vs*. IPCA2 of 26 genotypes in 2015 and 2016 at Assosa, Kamash, and Begi, Ethiopia.

indicating absolutely representative for average environments. The representing *vs.* discriminating ability was also explored by length of projection (Figure 22). The AEC concentric circle GGE biplot method is best to estimate the discriminating *vs.* representing ability for assessing the genotypes (Yan and Tinker, 2006; Yan et al., 2007; Atnaf et al., 2013). For instance, the suitability of B16 and K16 were high in representing all genotypes. The concentric circles nearest sites were high for their stability in representing the genotypes (Fayeun et al., 2016; Ashraful et al., 2017). The discriminating ability was significant and positive for A15 and K15 where they deviated strongly from the center of the concentric circles (Table 7 and Figure 23). The closer it is the better it will be as virtual ideal environment to all tested soybean genotypes. The K16 is highly favorable in representing all the tested genotypes considering both mean *vs.* stability. The ideal environments are close to ATC x-axis and zero projection onto ATC y-axis (Ashraful et al., 2017). Moreover, Blanche and Myers (2006) also witnessed the

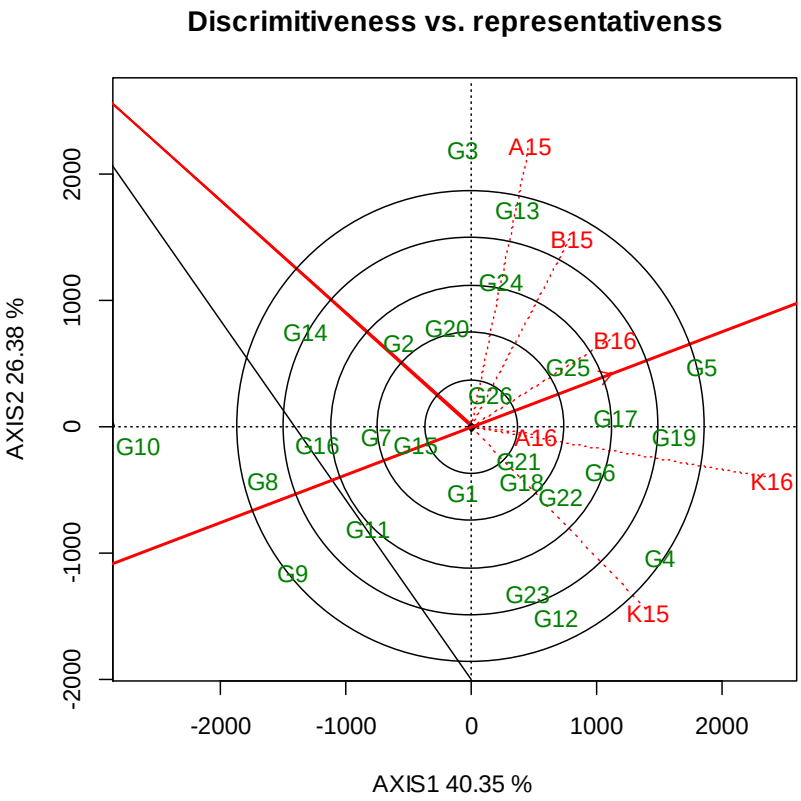

Figure 7. Discrimitiveness *vs*. representativeness for six testing environments of 26 genotypes in 2015 and 2016 at Assosa centre and Kamash, and Begi sub-center, Ethiopia.

efficiency of GGE for ideal genotypes *vs.* highly representing optimum environment identification for widely adapted genotypes selection.

The yield data *vs.* PC coordinates plotting for additive and interaction effects was done on the same biplot for yield variation efficiently partitioning. The A16 and B16 exhibited nearly additive effect on genotypes (Figure 24). The yield performance at A16 and B16 was associated with overall mean confirmed average responses to genotypes. The strongly projected sites were highly discriminated to all genotypes. The A15 and K15 were the longest which showed high yield variation for genotypes. The genotypes consistencies were better at A16 and B16 than inconsistent responses at A15 and K15 (Figure7). Environments with longer vectors are high in discrimination capability to all tested genotypes (Yan et al., 2007; Massaine et al., 2018). The results of the present study were strongly allied with Fayeun et al. (2016) who noticed little variation to genotypes for short vectors, while high variation for strongly projected testing sites. Similarly, the entries positioned near to the biplot origin were taken as an average means (Figure 25). For instance, G1, G18, G15, G21, and G26 showed average response for their yield means. G4, G5, G12, and G13 were the highest for their means being strongly projected from the center of the biplot. G3, G8, G9, and G10 were the poorest genotypes being deviated negatively (Figure 7). These polygon vertexes positively *vs.* negatively responding genotypes were unstable, adapted specifically at favorable environments (Melkamu et al., 2015). These two variables projection showed that the GEI which resulted from regressing of G over E as well as E over G contributed high MS for GEI variance. This resulted in inconsistency of the genotypes for mean performance due to strongly significant GEI effect in MEVT.

The angles present between the average tester axes *vs.* biplot vectors indicated the environmental stability (Figure7). The distance between the biplot vectors represented the similarity *vs.* differences in discriminating *vs.* representing to all tested genotypes (Fayeun et al., 2016). The environments near to AEC are high for their stability. Accordingly, K16, B16, and A16 were less than 90° that deviated little from AET axes (Figure7). These averagely responding environments were suitable for widely adapted soybean genotypes selection. Smaller angles for vectors showed strongly positive correlation (Massaine et al., 2018). The results of the present study were also in accordance with Marcin and Krzysztof (2016). A15 and K15 were highly projected from AET

showing high discriminating ability to all genotypes. Similar results were reported by Fayeun et al. (2016) and Bhartiya et al. (2017).

Conclusion

The pooled ANOVA showed strongly significant G, E, and GEI variances with CV of 26.58%, mean of 2530.79 kg/ha, and R^2 of 0.78%. The TTSS was partitioned into G, E, and GEI for MS contribution. The maximum was contributed by E (51.6%) followed by GEI (30.2%). The main variability is therefore due to the E and GEI. Genotypes G3, G5, G4, G12, G9, and G10 were located at corners of the polygon. Genotypes G3 and G12 were unstable significantly contributing to GEI due to high scores for PC2. Genotypes G5, G17, G19, and G25 were high for PC1 and near zero for PC2. This indicated high stability and heritability growing vigorously in producing maximum means might be due to broad sense genetic constituent for yield *vs.* stability so there are high selection probability and possibility for wide adaptability. These consistently performing ideal genotypes were proved for yield contributing desirable characters. Therefore, including these lines in future breeding programs would be advised in enhancing soybean productivity in Ethiopia. These two seasons *vs.* three locations data were used for GGE stability analysis; accordingly, further GGE *vs.* AMMI models GEI effect partitioning for G *vs.* L *vs.* Y should be considered with the objectives of promising (means *vs.* stability) genotypes exploring for both specific and broad adaptability.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Influence of environment on soybean [*Glycine max* (L.) Merr.] resistance to groundnut leaf miner, *Aproaerema modicella* (Deventer) in Uganda

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Groundnut leaf miner (GLM) [*Aproaerema modicella* (Deventer)] is a serious problem for soybean cultivation in Uganda causing yield losses of up to 100%. The use of soybean [*Glycine max* (L.) Merr.] cultivars resistant to GLM attack is an important strategy in the integrated pest management program. The aim of this study was to determine the environment × genotype interaction influence on the soybean resistance traits to GLM attack. Eighteen soybean genotypes were evaluated for resistance to GLM attack. The experiment was set up using randomized complete block design replicated three times under natural pest infestation in Budaka (Eastern) and Arua (Northern) districts in Uganda. Data were subjected to analysis of variance, Pearson's phenotypic correlation and cluster analysis. Highly significant ($p < 0.001$) differences among the genotypes were recorded for all the studied traits, except the number of pupae per plant which was significant ($p < 0.05$). GLM incidence and severity had significant negative correlations with rainfall and relative humidity. However, there were significant positive correlations between minimum temperature and GLM incidence as well as severity for most of the genotypes. Soybean genotypes VI046160 and VI046167 could be used as parents in breeding for resistance to GLM pest. Areas with high rainfall and humidity would be recommended for soybean production to minimize infestation by GLM.

Key words: Grain yield, Gelechiidae, incidence, Lepidoptera, natural infestation, severity, weather parameters.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is the most important oil crop in the world (Mattson et al., 2004; Bilyeu et al.,

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2010). Soybean provides the cheapest source of the highest protein content for both human and livestock diets and is an important industrial crop (Ramteke and Husain, 2008; Bhor et al., 2014). Soybean production constitutes 6% of all arable land in the world and has the highest percent increase in the area under production among crops annually (Bilyeu et al., 2010; Tukamuhabwa et al., 2012). In Africa, soybean is used mainly for making infant formula foods and animal feeds (Tukamuhabwa and Oloka, 2016). The crop is attacked by more than 273 species of insect pests (Shirale and Uttamrao, 2010) including groundnut leaf miner (GLM), *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae), which is one of the most devastating oligophagous pests (Shanower et al., 1993a; Praveena et al., 2011). The larvae feed on the leaf mesophyll between the two epidermis layers (Van der Walt et al., 2008b; Buthelezi et al., 2013; Kolhe et al., 2015). Heavy infestations cause leaves to turn brown, with subsequent loss of leaves which in turn reduces the photosynthetically active area leading to yield loss (Du Plessis and Van Denberg, 2011). Groundnut leaf miner causes yield loss of up to 100% on leguminous plants in the tropics (Cugala et al., 2010). In Uganda, the pest has been reported as an economic threat to soybean production in the Eastern and Northern regions causing 54% yield loss (Namara, 2015), yet about 91.2% of the crop is grown in these regions (UBOS, 2010). Chemical control measures are the most widely adopted among the management strategies of the leaf miner in soybean (Okello et al., 2013). However, many studies have shown that leaf miners can develop a high degree of resistance to a broad range of insecticides over a relatively short time (Mou and Liu, 2003; Anjani et al., 2007; Mou, 2008). Thus, the development of more reliable alternatives for managing the pest is needed. Host plant resistance offers the potential to reduce dependence on the chemical control which is expensive and environmentally unsound (Hesler and Tharp, 2005). The incidence of some pests and diseases is directly influenced by weather elements, principally rainfall, humidity and temperature (Rao, 2008; Moka et al., 2015). Heavy and persistent rains and high humidity has been shown to reduce GLM population in groundnut and soybean whereas dry weather with bright sunshine hours and occasional rains lead to a rapid buildup of the pest (Gadad et al., 2013; Moka et al., 2015). *A. modicella* is reported to be adapted in a number of agro-ecological areas that differ widely in climates (Buthelezi et al., 2013, 2016). However, information on the interactions between the environment and soybean genotypes on resistance to GLM is lacking. Yet, currently, there is an increasing need to develop resistant varieties that are adapted to a diversity of growing conditions. Recently, Namara (2015) identified 12 moderately resistant genotypes with relatively low severity scores, suggesting a potential to breed soybean for resistance to GLM. Therefore, this study was conducted to determine the influence of the environment on the soybean genotype resistance traits to GLM attack.

MATERIALS AND METHODS

Site characteristics

The study was carried out in Eastern and Northern Uganda. The first experimental location was at the District Agricultural Training and Information Centre, Iki-Iki sub-county in Budaka District (1° 06' N, 34° 00' E) located at an altitude of 1,156 masl. The location has sandy soil (Tukamuhabwa et al., 2012) and receives a mean annual rainfall of 1200 mm, with a mean annual temperature of 24.7°C (Obua, 2013). The second location was at Abi Zonal Agricultural Research and Development Institute in Arua District (3° 04' N, 30° 56' E) with an average altitude of 1215 masl. The area receives a mean annual rainfall of 1250 mm, and has a mean annual temperature of 24°C (Sserumaga et al., 2015); the soil is sandy clay loam (Fungo et al., 2011). These two areas are the hotspots of groundnut leaf miner in Uganda (Page et al., 2000; Epieru, 2004; Okello et al., 2010; Namara, 2015).

Soybean germplasms and experimental procedures

Eighteen soybean genotypes, obtained from the United States of America (USA), International Institute of Tropical Agriculture (IITA), Asian Vegetable Research and Development Center of Taiwan (AVRDC), and Uganda were used in this study (Table 1). These included five susceptible, four moderately susceptible, six moderately resistant, two tolerant and one resistant genotype to GLM.

The experiment was conducted twice during the second rains (September to December) in 2016 and in 2017. This was done because GLM is reported to severely inflict soybean during these periods in Uganda (Namara, 2015). Each plot consisted of a single row of two meters long. The distance between the rows was 60 cm with within row spacing of 5 cm. The plots were laid out in a randomized complete block design with three replicates and a distance of 1 m was kept between the different plots.

Data collection

The data was collected from 30 randomly selected plants per genotype on 40, 60 and 70th days after planting (Ramani and Lingappa, 1988) for GLM incidence and severity damage since GLM peak infestation was reported to occur during reproductive stage (Namara, 2015). Other traits recorded were the number of larvae per plant, number of pupae per plant and seed yield per plot. The GLM incidence (expressed as percent leaf damage) was made by counting the total number of leaves showing folding and mining symptoms from 30 randomly selected plants (Pavviya and Muthukrishnan, 2017). The GLM severity was scored using the standard scale of 1-5 as described by Praveena et al. (2011) in Table 2. The soybean grain yield of each plot was recorded in grams per plot and converted to kg ha^{-1} based on the average of the plots of each cultivar. Data on rainfall, humidity, and temperature for the corresponding months of the study were obtained from the Ugandan National Meteorological Authority.

Data analysis

The analysis of variance (ANOVA) was done for the combined locations using Genstat statistical software 12th Edition (Payne et

Table 1. Description of the soybean genotypes used in the experiment with groundnut leaf miner at District Agricultural Training and Information Centre, Iki-Iki sub-county in Budaka District and at Abi Zonal Agricultural Research and Development Institute in Arua District, Uganda.

Genotype	Pedigree	Maturity group	Origin	GLM reaction[1]
VI046160	CO-2	Early	AVRDC	T
VI046165	NRC-7	Early	AVRDC	T
VI046167	MACS-13	Early	AVRDC	R
BSPS 48C	-	Medium	Uganda	MR
NIIGC4.1-2	-	Medium	Uganda	MR
PI578457A	-	Medium	USA	MR
PI615437	-	Medium	USA	MR
PI605865B	-	Early	USA	MR
Maksoy1N	TGX 1835-10E	Medium	Uganda	MS
Maksoy2N	Maksoy 1N × Duiker	Late	Uganda	S
Maksoy3N	GC 00138-29 × Duiker	Late	Uganda	MR
Maksoy4N	Duiker × GC 00138-29	Late	Uganda	S
Maksoy5N	Nam 2 × GC 00138-299	Medium	Uganda	MS
Namsoy4M	Nam2 × GC00 139-29	Medium	Uganda	MS
Siesta	-	Medium	Uganda	S
Wondersoya	-	Medium	IITA	S
UG5	-	Medium	Uganda	MS
K-local	-	Early	Uganda	S

T: Tolerant; R: resistant, MR: moderately resistant, S: susceptible. The classification of GLM reaction followed Namara[1], (2015).

Table 2. Severity score and resistance category of groundnut leaf miner damage.

Foliage damage (%)	Severity score	Category
0	1	Immune
1-20	2	Resistant
21-40	3	Moderately resistant
41-60	4	Moderately susceptible
61-100	5	Highly susceptible

Source: Praveena et al. (2011).

al., 2009) to estimate the amount of variability for the traits. Prior to analysis, test for homogeneity of variance was applied on all traits. Square root transformation was applied on the number of larvae per plant and grain yield ha^{-1} to improve normality and reduce heterogeneity of the variances (Halverson and Handelsman, 1991). The analysis considered genotype as a fixed factor, and location, season and replication as random factors. Means were separated using the Fisher's protected least significant difference at 0.05% significance level. Pearson's phenotypic correlation estimates were computed for all the studied traits, and the relationship of the genotypes was determined using cluster analysis.

RESULTS

Groundnut leaf miner incidence and severity of soybean genotypes across seasons and locations

The results of ANOVA for GLM incidence and severity of damage on soybean genotypes across seasons and locations are presented in Table 3.

The GLM incidence showed highly significant ($p < 0.001$) differences among genotypes for all the three sampling dates across seasons and locations. The season as well as location effects were also highly significant ($p < 0.001$) for all the three sampling dates for GLM incidence. Similarly, the season × genotype interaction showed highly significant ($p < 0.001$) effects at 60 and 70 DAP for incidence whereas the location × genotype interaction had significant ($p < 0.01$) effect at 60 DAP only. The season × location interaction showed highly significant ($p < 0.001$) effects for all the sampling dates for GLM incidence, and the season × location × genotype interaction showed significant ($p < 0.05$ and $p < 0.01$) effects at 60 and 70 DAP, respectively for GLM incidence. Highly significant ($p < 0.001$) differences were

Table 3. Analysis of variance (mean squares) for groundnut leaf miner (GLM) incidence and severity at three recording dates across seasons and locations on 18 soybean genotypes in Uganda.

Source of variation	Df	GLM incidence			GLM severity		
		40 DAP	60 DAP	70 DAP	40 DAP	60 DAP	70DAP
Rep	2	470.88**	20.54	121.82***	2.86***	1.48***	1.32***
Genotype	17	430.64***	311.23***	378.87***	2.03***	1.70***	1.76***
Season	1	45501.60***	121431.14***	98976.35***	270.17***	383.96***	343.71***
Location	1	5060.76***	3669.70***	4608.61***	70.45***	38.16***	29.02***
Season × Genotype	17	132.72	153.85***	200.55***	1.04***	0.85*	0.66***
Location × Genotype	17	89.19	38.33**	23.2	0.78***	0.49***	0.34**
Season × Location	1	3622.98***	1438.77***	2058.36***	42.48***	13.89***	3.32***
Season × Location × Genotype	17	68.57	31.37*	32.07**	0.71***	0.51***	0.37***
Residual	142	89.1	15.78	14.2	0.24	0.14	0.15
Total	215	-	-	-	-	-	-
CV (%)	-	12.2	5.6	5.0	24.4	15.2	12.9

***, **, *Significant at $p < 0.001$, $p < 0.01$, $p < 0.05$, respectively, Df: Degrees of freedom, DAP: days after planting, CV: coefficient of variation.

observed among genotypes for GLM severity on all the three sampling dates. The environmental effects expressed by location and the seasonal effects were also highly significant ($p < 0.001$) for all the sampling dates for severity damage. Similarly, all the interactions showed highly significant ($p < 0.001$) effects for all the sampling dates across locations and seasons, except the genotype × season interaction which was significant ($p < 0.05$) at 60 DAP and location × genotype interaction which was significant ($p < 0.01$) at 70 DAP.

Mean groundnut leaf miner infestation of soybean genotypes across seasons

The results of GLM mean incidence and severity of evaluated soybean genotypes are presented in Table 4. The percentage of incidence for GLM among the genotypes ranged from 85.14 to 98.98% in 2016 and from 43.10 to 65.50% in 2017. The resistant genotype VI046167 recorded the lowest GLM incidence of 85.14% while the moderately resistant genotype K-local recorded the highest incidence value (98.98%) in 2016. In 2017, resistant genotype VI046167 recorded the lowest GLM incidence of 43.10% while the moderately resistant genotype Maksoy4N recorded the highest value (65.5%) across seasons. The mean performance of incidence across seasons and locations revealed that resistant genotype VI046167 recorded the lowest value of 64.12% while moderately resistant genotype Maksoy4N the highest value of 82.09%.

The mean scores for GLM severity ranged from 2.53 to 4.49 in 2016 and from 0.99 to 1.69 in 2017 across seasons. About 88.88% of genotypes exhibited moderate resistance level to GLM with the severity scores of less than 3. Imported genotypes from the Asian Vegetable Research and Development Center (AVRDC), VI046167 and VI046160 which had been reported to be resistant and tolerant to GLM attack respectively, exhibited resistance level with a severity score of < 2 whereas the reported tolerant genotype VI046165 was found to be moderately resistant to GLM attack across seasons and locations. Genotype K-local which was earlier reported as susceptible to GLM (Namara, 2015) had the highest severity score of 2.98 across seasons and locations and exhibited moderately resistance level to GLM attack, plant introductions PI615437, PI605865B and PI578457A which had been reported to be moderately resistant to GLM attack (Namara, 2015) showed moderate resistance level across seasons and locations. Commercial genotypes Maksoy2N and Maksoy4N which had been reported to be susceptible to GLM attack (Namara, 2015), exhibited moderately resistant level across seasons and locations whereas genotypes Maksoy1N, Maksoy5N and Namsoy4M which were known to be moderately susceptible to GLM attack showed moderately resistant level across season and location.

Number of larvae and pupae per plant and grain yield of soybean genotypes across seasons and locations

The ANOVA results for the number of larvae and pupae per plant and grain yield across seasons and locations are presented in Table 5.

Highly significant ($p < 0.001$) differences in the number of larvae and grain yield were observed among the genotypes across seasons and locations. A significant ($p < 0.05$) difference among genotypes for the number of pupae per plant was also observed. The seasons had highly significant ($p < 0.001$) effects on the performance of the genotypes for all the traits. The location had highly significant ($p < 0.001$) effect on the performance of the genotypes for number of larvae and pupae per plant and

Table 4. Mean of soybean genotype performance for groundnut leaf miner (GLM) incidence and severity across seasons and locations in Uganda.

Genotype	GLM Incidence		GLM severity		Average across seasons and locations			
	2016	2017	2016	2017	IN	FD	SS	GLMR
BSPS48C	96.38	48.68	4.15	1.17	72.53	26.6	2.66	MR
K-local	98.98	63.04	4.49	1.47	81.01	29.8	2.98	MR
Maksoy1N	96.36	55.49	4.21	1.36	75.92	27.9	2.79	MR
Maksoy2N	97.71	61.61	4.15	1.54	79.66	28.4	2.84	MR
Maksoy3N	97.7	51.06	4.06	1.05	74.38	25.6	2.56	MR
Maksoy4N	98.68	65.50	4.25	1.69	82.09	29.7	2.97	MR
Maksoy5N	98.0	53.17	4.05	1.25	75.58	26.5	2.65	MR
Namsoy4M	94.53	49.97	3.65	1.04	72.25	23.5	2.35	MR
NIIGC4.1-2	95.28	57.14	3.54	1.15	76.21	23.4	2.34	MR
PI578457A	94.4	47.32	3.69	1.11	70.86	24.1	2.40	MR
PI605865B	94.65	52.68	3.38	1.26	73.66	23.2	2.32	MR
PI615437	93.61	54.8	3.23	1.08	74.2	21.5	2.15	MR
Siesta	97.8	60.64	4.13	1.45	79.22	27.9	2.79	MR
UG5	95.1	48.99	3.73	0.99	72.05	23.6	2.36	MR
VI046160	88.01	50.74	2.53	1.1	69.38	18.2	1.82	R
VI046165	93.42	53.66	3.86	1.21	73.54	25.4	2.54	MR
VI046167	85.14	43.10	2.53	1.00	64.12	17.7	1.77	R
Wondersoya	96.81	62.89	4.27	1.62	79.85	29.5	2.94	MR
SEDM	2.22	4.31	0.43	0.89	3.3	-	0.29	-
LSD (0.05)	4.48	8.61	0.88	0.2	6.5	-	0.56	-

IN: Incidence (%), FD: foliage damage in % used to score the severity, SS: severity, GLMR: groundnut leaf miner reaction, MR: moderately resistant, R: resistant, SEDM: standard errors of differences of means, LSD: least significant differences of means.

Table 5. Analysis of variance (mean squares) for the number of larvae per plant, number of pupae per plant of groundnut leaf miner (GLM) and grain yield (kg ha^{-1}), across seasons and locations on 18 soybean genotypes in Uganda.

Source of variation	Df	NL	NP	GY
Rep	2	23.76	1.43**	46.04
Genotype	17	70.16***	0.53*	259.44***
Season	1	11462.79***	65.21***	29342.1***
Location	1	8358.47***	15.380***	183.92*
Season × Genotype	17	14.4	0.283	122.1***
Location × Genotype	17	50.1*	0.4911	32.64
Season × Location	1	8435.31***	115.88***	4042.14***
Season × Location × Genotype	17	54.63**	0.2857	87.56*
Residual	142	24.77	0.2938	43.21
Total	215	-	-	-
CV (%)	-	35.8	22.9	34.2

***, **, * Significant at $p < 0.001$, $p < 0.01$, $p < 0.05$, respectively, Df: Degrees of freedom, NL: number of larvae per plant, NP: number of pupae per plant, GY: grain yield in kg ha^{-1}, CV: coefficient of variation.

significant ($p < 0.05$) effect for grain yield. The location × genotype interaction was significant ($p < 0.05$) for the number of larvae per plant and non-significant ($p > 0.05$) for the number of pupae per plant and grain yield. The season × location interaction was highly significant ($p < 0.001$) for all the traits. There was a significant ($p < 0.01$) effect in the number of larvae per plant and significant ($p < 0.05$) effect in grain yield due to season × location × genotype interaction. The season × genotype interaction had highly significant ($p < 0.001$) effect for grain yield and

Table 6. Mean performance of 18 soybean genotypes for the number of larvae and pupae per plant of groundnut leaf miner and grain yield ha^{-1} across seasons and locations in Uganda.

Genotype	NL		NP		GY		YSL
	2016	2017	2016	2017	2016	2017	
BSPS48C	20.2	6.6	8.8	2.4	10.0	308.4	108.2
K-local	20.9	6.4	9.5	3.9	34.2	649.8	246.1
Maksoy1N	19.7	6.4	10.1	1.7	50.8	804.4	315.5
Maksoy2N	23.7	9.5	6.7	1.7	23.9	602.7	217.5
Maksoy3N	22.7	11.8	6.9	2.7	25.9	1428.6	461.3
Maksoy4N	27.4	11.9	9.5	2.8	3.4	444.2	133.6
Maksoy5N	19.8	4.8	7.5	1.6	30.1	995.7	343.8
Namsoy4M	25.9	5.4	7.4	2.9	48.1	1406.8	494.5
NIIGC4.1-2	24.6	7.6	5.3	3.1	106.3	1264.2	526.6
PI578457A	18.1	5.0	9.2	2.2	52.7	1100.6	409.5
PI605865B	20.5	6.3	9.8	2.4	45.2	951.3	353.6
PI615437	17.1	5.7	5.3	2.2	147.6	1629.5	690.2
Siesta	22.3	6.7	7.2	1.9	32.1	793.7	286.9
UG5	22.4	5.5	7.7	2.7	67.2	1551.4	566.9
VI046160	18.4	6.0	5.8	1.9	359.2	806.7	560.7
VI046165	17.1	2.4	4.1	1.1	109.1	1726.2	676.6
VI046167	19.5	4.5	8.1	1.2	146.4	753.1	391.1
Wondersoya	20.9	6.7	7.8	3.7	3.5	686.5	199.8
SEDM	5.6	1.9	0.6	0.3	2.8	6.8	5.4
LSD (0.05)	10.9	3.8	1.1	0.5	5.5	13.5	10.6

NL: Number of larvae per plant, NP: number of pupae per plant, GY: grain yield in kg ha^{-1}, YSL: yield across seasons and locations, SEDM: standard errors of differences of means, LSD: least significant differences of means. The number of pupae, grain yield and yield across seasons and locations are back-transformed values.

non significant ($p > 0.05$) effect for the number of larvae and pupae per plant.

Mean performance of various genotypes for different traits

The results of number of larvae and pupae per plant and grain yield in kg ha^{-1} of evaluated soybean genotypes are presented in Table 6.

The number of GLM larvae per plant among the genotypes ranged from 17.1 to 27.4 in 2016 and 2.4 to 11.9 in 2017 across seasons and locations with the moderately resistant genotype Maksoy4N having the highest number of larvae per plant (27.4) while the moderately resistant genotype VI046165 recorded the lowest value (2.4).

The number of pupae per plant ranged from 4.1 to 10.1 in 2016 and from 1.1 to 3.9 in 2017. The highest number of pupae per plant was recorded on the moderately resistant genotype Maksoy1N with a value of 10.1, while moderately resistant genotype VI046165 had the lowest value of 4.1 in 2016. In 2017, the moderately resistant genotype K-Local had the highest number of pupae of 3.9 per plant and the moderately resistant genotype VI046165 the lowest value of 1.1.

The mean performance for grain yield per hectare across seasons showed that the resistant genotype VI046160 recorded the highest soybean grain yield with 359.2 kg ha^{-1}, whereas the lowest grain yield ha^{-1} was recorded by the moderately resistant Maksoy4N (3.4 kg ha^{-1}) in 2016. In 2017, the moderately resistant genotype VI046165 recorded the highest soybean grain yield with 1726.2 kg ha^{-1} while the moderately resistant genotype BSPS48C had the lowest grain yield of 308.4 kg ha^{-1}. The results showed that the grain yield ranged from 108.20 to 690.2 kg ha^{-1} across seasons and locations with the moderately resistant plant introduction PI615437 recording the highest value of 690.2 kg ha^{-1}.

Relationships of different traits

The correlation coefficients among the studied soybean resistance traits to GLM are presented in Table 7. GLM incidence at 40 DAP was highly significantly ($p < 0.001$) positively correlated to the GLM mean severity ($r = 0.82$ and $r = 0.73$) at 40 and 60th DAP, respectively, significantly ($p < 0.01$) correlated to the GLM mean incidence ($r = 0.59$) at 60th DAP and GLM severity (0.62)

Table 7. Correlation matrix for different traits in soybean genotypes affected by the groundnut leaf miner (GLM) in Uganda.

Trait	IN40DAP	IN60DAP	IN70DAP	S40DAP	S60DAP	S70DAP	NL	NP
IN40	-							
IN60	0.59**	-						
IN70	0.58*	0.67**	-					
S40DAP	0.82***	0.73***	0.74***	-				
S60DAP	0.73***	0.66**	0.79***	0.96***	-			
S70DAP	0.62**	0.71***	0.91***	0.87***	0.89***	-		
NL	0.53*	0.52*	0.33	0.44	0.43	0.32	-	
NP	0.51*	0.15	0.25	0.40	0.45	0.29	0.43	-
GY	-0.38	-0.20	-0.36	-0.36	-0.42	-0.46	-0.42	-0.61**

***, **, * Significant at $p < 0.001$, $p < 0.01$, $p < 0.05$, respectively, NL: number of larvae per plant, NP: number of pupae per plant, IN: GLM incidence, S: severity score, DAP: days after planting, GY: grain yield in kg ha^{-1}.

at 70th DAP, and significantly ($p < 0.05$) correlated to GLM incidence (0.58) at 70th DAP, number of larvae per plant (0.53) and number of pupae per plant (0.51).

GLM incidence at 60 DAP was found to be highly significantly ($p < 0.001$) positively correlated to the GLM mean severity (0.73 and 0.71) at 40 and 70th DAP, respectively; significantly ($p < 0.01$) correlated to GLM severity (0.66) at 60th DAP, and incidence (0.67) at 70th DAP and ($p < 0.05$) to the number of larvae per plant (0.52). The GLM incidence at 70 DAP was highly significantly ($p < 0.001$) positively correlated to the severity ($r = 0.74$; $r = 0.79$ and $r = 0.91$) at 40, 60 and 70th DAP, respectively. The GLM severity at 40 DAP had highly significant ($p < 0.001$) positive correlation with the severity score (0.96 and 0.87) at 60 and 70th DAP, respectively. Similarly, GLM severity at 60 DAP had a highly significant ($p < 0.001$) positive correlation with the GLM severity at 70 DAP (0.89). A significant ($p < 0.01$) negative correlation was observed between the number of pupae and the grain yield (-0.61).

The correlation coefficients of soybean resistance traits to GLM with the weather factors are given in Table 8. There was a negative significant ($p < 0.01$) correlation between relative humidity in the morning (9 am) with GLM incidence ($r = - 0.99$) for the genotype Maksoy1N and ($P<0.05$) negative correlations (-0.99, -0.99, -0.99 and -0.99) for the genotypes BSPS48C, K-local, Maksoy3N and VI046167, respectively. The relative humidity in the evening was also negatively significantly ($p < 0.05$) correlated to the GLM incidence for the genotype Maksoy2N. A significant ($p < 0.01$) positive correlation between the minimum temperature and GLM severity for the genotype UG5 and significant ($p < 0.05$) positive correlations for all the studied genotypes were also observed except for Maksoy1N, Maksoy3N, VI046160, VI046167, VI046165 and Wondersoya. A significant ($p < 0.01$) negative correlation between rainfall and GLM incidence ($r = -0.997$) for Maksoy2N and significant ($p < 0.05$) for PI605865B was observed. Genotypes were grouped into two clusters. The first cluster comprised two sub-clusters A and B. Sub-cluster A consisted of eight genotypes from different origins, six Ugandan genotypes, one from IITA and one from the AVRDC Taiwan. Genotypes VI046165 from the AVRDC and the Ugandan genotype Maksoy4N had the same genetic distance and were genetically distinct from the rest in the same group. The second sub-cluster B had seven genotypes from different origins, four Ugandan genotypes and three plant introduction lines (PIL) from the USA. However, plant introduction PI615437 was found to be genetically distinct from others genotypes in the same sub cluster. The second-cluster C comprised two resistant genotypes from the AVRDC with the same genetic distance. These genotypes were independently distinct from the rest of genotypes.

Cluster analyses

The results of the cluster analysis performed using nine traits are as shown in Figure 1.

DISCUSSION

The results demonstrated highly significant differences among the genotypes for GLM incidence and severity, indicating genetic variability among the soybean genotypes for these traits, and suggesting that it is possible to genetically improve soybean for resistance to GLM (Table 3). Namara (2015) and Ptaware et al. (2001) identified soybean lines with varying degrees of resistance to GLM in Uganda and India, respectively. The highly significant season × genotype and location × genotype interaction effects observed across seasons and locations in sampling dates for GLM incidence and severity showed that the effect of GLM on genotypes was highly dependent on the seasons and environments. Liu et al. (2015) reported that the impacts of mining on plant leaves differ from season to season. Similarly, Buthelezi

Table 8. Relationships of groundnut leaf miner incidence and severity on 18 soybean genotypes in 2016 with weather factors in Uganda.

WP	BSPS48C		K_local		Maksoy1N		Maksoy2N		Maksoy3N		Maksoy4N		Maksoy5N		Namsoy4M		NIIGC4.1-2	
	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S
MaxT	0.76	0.64	0.82	0.62	0.78	0.6	0.93	0.66	0.75	0.61	0.99	0.64	0.72	0.64	0.75	0.65	0.63	0.66
MinT	0.99	0.99*	0.98	0.99*	0.98	0.99	0.89	0.99*	0.99	0.99	0.79	0.99*	0.99*	0.99*	0.99	0.99*	0.99*	0.99*
RHM	-0.99*	-0.98	-0.99*	-0.97	-0.99**	-0.96	-0.96	-0.98	-0.99*	-0.97	-0.88	-0.97	-0.99	-0.97	-1.00	-0.98	-0.97	-0.98
RHE	-0.96	-0.9	-0.98	-0.89	-0.97	-0.88	-0.99*	-0.91	-0.95	-0.88	-0.97	-0.9	-0.94	-0.89	-0.95	-0.9	-0.89	-0.91
R	-0.94	-0.87	-0.97	-0.86	-0.95	-0.85	-1**	-0.88	-0.93	-0.85	-0.98	-0.87	-0.92	-0.87	-0.93	-0.88	-0.86	-0.88

	PI578457A		PI605865B		PI615437		Siesta		UG5		VI046160		VI046165		VI046167		Wondersoya	
	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S	IN	S
MaxT	0.92	0.69	0.96	0.7	0.49	0.74	-0.77	-0.67	-0.62	-0.63	-0.37	-0.55	-0.63	-0.69	-0.5	-0.49	-0.57	-0.7
MinT	0.91	0.99*	0.86	0.99*	0.97	0.99*	0.97	0.99*	1**	0.99**	0.96	0.99	0.99*	0.99	0.99	0.98	0.99*	0.99
RHM	-0.97	-0.99	-0.93	-0.99	-0.92	-0.99	-0.93	-0.97	-0.98	-0.98	-0.99	-0.99	-0.98	-0.97	-0.99*	-0.99*	-0.99	-0.96
RHE	-1*	-0.93	-0.99	-0.93	-0.8	-0.95	-0.82	-0.89	-0.92	-0.92	0.96	-0.95	-0.91	-0.88	-0.97	-0.97	-0.94	-0.87
R	-0.99	-0.9	-0.99*	-0.91	-0.68	-0.88	-0.7	-0.79	-0.83	-0.83	-0.96	-0.88	-0.82	-0.78	-0.91	-0.91	-0.86	-0.77

**,* *Significant at $p < 0.01$, $p < 0.05$, respectively, IN: GLM incidence, S: GLM severity, WP: weather parameters, MaxT: maximum temperature, MinT: minimum temperature, RHM: relative humidity in the morning (0900 h), RHE: relative humidity in the evening (1500 h), R: rainfall.

et al. (2012) also reported that the epidemic of GLM is sporadic and its severity varies from location to location and from year to year. The variation among seasons and locations had predominant effects on the traits suggesting that soybean genotypes performed differently in different seasons and locations with high damage level observed in 2016 compared to 2017 which was a result of below normal rainfall of 28.6 to 78.2 mm during experimentation and high temperatures (29.3 to 33°C) recorded in 2016 compared to 2017, especially in Iki-iki (UNMA, 2016, 2017), that might explain the GLM population buildup and infestation. GLM incidence and severity are reported to vary depending on temperature, rainfall and humidity (Du Plessis, 2011; Arunachalam and Kavitha, 2012; Gadad et al., 2013) with an average of high GLM damage levels in Africa than in Asia where the pest originated due to environmental conditions (Du Plessis, 2011).

The study clearly showed a high significant season and location effects on GLM number of larvae and pupae per plant and significant season × location × genotype and location × genotype interactions for the number of larvae per plant. This indicated that the number of larvae and pupae per plant differed from season to season and from location to location with a high number of larvae and pupae per plant observed in 2016 compared to 2017. Fluctuations in *A. modicella* populations were reported to occur between locations, seasons and years by Logiswaran and Mohanasundaram (1986) and Shanower et al. (1992) in India, and in South Africa by Van der Walt (2007) and Buthelezi et al. (2016). The highly significant difference in the number of larvae among genotypes across seasons and locations (Table 5) could be attributed to the fact that the incidence of soybeans GLM is influenced not only by the performance of the genotype but also weather conditions (Du Plessis, 2011; Moka et al., 2015). Maksoy4N, a moderately resistant genotype to GLM recorded the highest number of larvae across seasons (27.4) while VI046167 resistant genotypes had the lowest number of larvae per plant 2.4 (Table 6). The number of larvae per plant was very high compared to the economic threshold level of 5-10 larvae per plant in Uganda and India (Kenis and Cugala, 2006; Van der Walt et al., 2008a). The high population levels in the areas of invasion suggested that this species was able to successfully adapt to the environmental field conditions, establish in the areas of soybean production in Eastern and Northern Uganda and confirmed the findings of Cugala et al. (2010) and Du Plessis (2011).

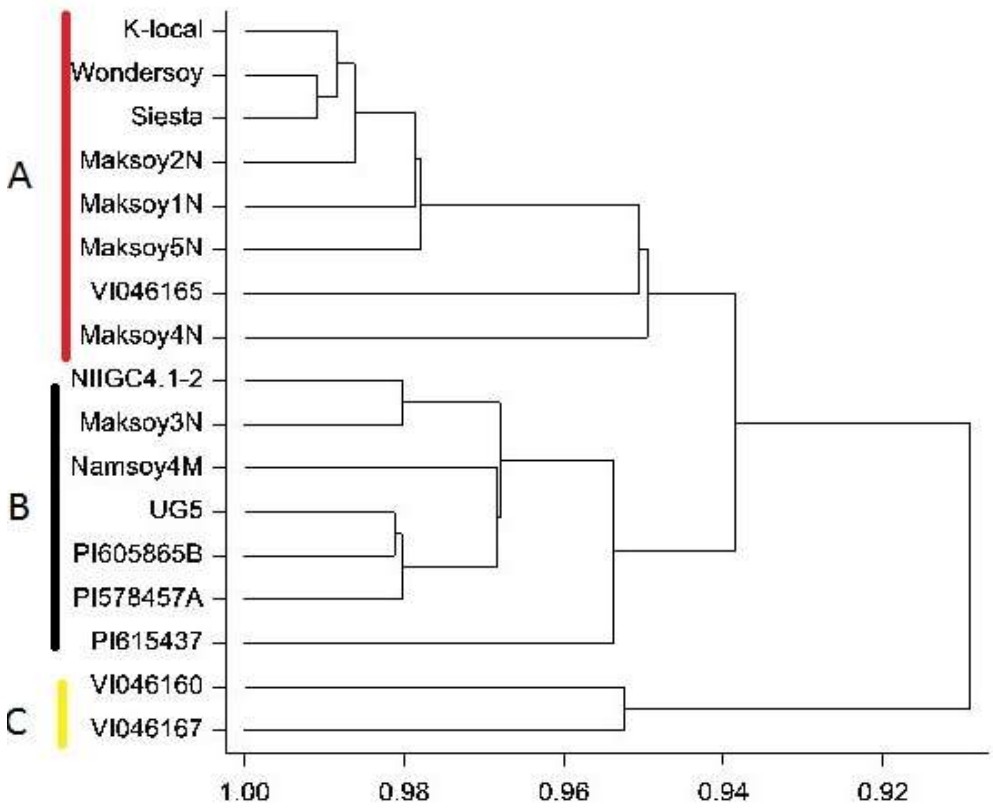

Figure 1. Ward's cluster dendrogram of the 18 soybean genotypes based on eight traits. A: Sub-cluster 1, B: sub-cluster 2, C: second cluster.

The study clearly showed that *A. modicella* is a priority pest and thus strategies for its effective management are pertinent. Cugala et al. (2010) reported an average of 29 to 38 larvae per groundnut plant in Mozambique. Van der Walt et al. (2008a) reported that in the absence of natural mortality factors, GLM numbers can increase by a factor of up to 20 per generation. With this increase, by the pod filling stage, the GLM has the potential to build up to a high population.

This study identified VI046160 and VI046167 as resistant genotypes across seasons and locations in terms of lower severity score (<2) and higher grain yield. Genotype PI615437 was the best performer in terms of grain yield recorded and recorded a relatively low severity score. The highly significant positive correlations observed between the GLM mean incidence and severity at different DAP suggested a linear relationship between these traits, implying that an increase in GLM incidence would lead to increased severity damage. However, a highly significant negative correlation (r = -0.61) observed between the number of pupae per plant and grain yield suggested that increase in pupae density would lead to lower grain yield. This arises because GLM pupation occurs in the webbed leaflets, and has the potential of decreasing the photosynthetic active leaf area affecting the growth and yield of the plant (Kenis and Cugala, 2006; Van der Walt et al., 2008b).

The results of this study clearly indicated that GLM incidence and severity depended on the environmental conditions. In this study, negative significant correlations were observed between morning and evening relative humidity with GLM incidence and severity, and significant negative correlations were observed between rainfall and GLM incidence along with severity for most of genotypes studied. Positive correlations were, however, recorded between the minimum temperature and the GLM incidence and severity (Table 8). Increased GLM incidence and severity with a rise in temperature and a decrease in relative humidity and rainfall was previously reported by Lewin et al. (1979), Joshi and Patel (2010), Arunachalam and Kavitha (2012) and Gadad et al. (2013). Shanower et al. (1993b) suggested that under mid-range temperature conditions (25 and 30°C), adults of GLM lived relatively longer and egg production was maximized. This could be the reason why *A. modicella* severely inflicts soybean during the second season (August to December) in Uganda (Namara, 2015), since the first rainy season (March to May) is less influenced by El Niño-Southern Oscillation (ENSO) phases, and records heavy and persistent rains and high humidity;

thus having lower inter-annual variability which tend to reduce GLM population compared to the second rainy season where dry weather with bright sunshine hours and occasional rains lead to a rapid buildup of the pest (Conway et al., 2005; Asadullah et al., 2008). The result from the cluster analysis showed that the geographical origins were not associated with the genetic diversity among genotypes. The sub-cluster A included the moderately resistant genotypes to GLM with high severity score close to 3 with low grain yield except for the genotype VI046165. These are mixtures of late and early maturity cultivars, suggesting genetic diversity among them. The sub-cluster B included genotypes with severity score less than 2.5 and therefore recorded as moderately resistant with medium maturing cultivars. The similarity of genotypes VI046167 and VI046160 suggested that these genotypes can equally be used as a source of resistant genes in the breeding program against leaf miner. Genetic patterns obtained from the cluster analysis would help soybean breeders make better choices when selecting among the available genotypes for parents.

Conclusion

Groundnut leaf miner incidence and severity depended on the temperature, relative humidity and rainfall. The study also indicated a possibility for improving soybean genotypes for resistance to GLM as a result of the genetic variability. The genotype VI046160 and VI046167 were recommended as resistant. It is recommended that a study be undertaken in order to understand the physical impact of rainfall on groundnut leaf miner eggs, larvae and on adult's emergency.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Curauá genetic diversity in germplasm banks and natural populations

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***Ananas comosus* var. erectifolius (L. B. Sm.) Coppens & F. Leal, popularly known as curauá, is a bromeliaceae, found in the Amazonian flora. It is of great commercial interest, mostly for the automobile industry. Despite the curauá's potential, little has been done to conserve its germplasm. For this, it's necessary to know its genetic variability. An efficient way of knowing it is to use molecular markers because they are polymorphic and not influenced by the environment. Thus, this work is aimed to evaluate the genetic diversity in curauá's access of different germplasm banks and its natural populations, using simple sequence repeat (SSR) and random amplified polymorphic DNA (RAPD) molecular markers. The similarity between the cultivars was calculated based on the Dice coefficient. From the similarity analysis, the cultivars grouping dendrogram was constructed using the unweighted pair group mean average method (UPGMA). High genetic similarity was observed between the individuals of each group and most of the variability found was between the groups. The low variability found within the groups is due to the way in which *A. comosus* var. *erectifolius* has been multiplied, through asexual reproduction in plantation areas. The data suggest that the conservation strategies of this species should focus on the largest possible number of collections in different geographic regions to increase the variability of the banks.**

Key words: *Ananas comosus* var. *erectifolius,* conservation, amazon plants, biodegradable products, genetic similarity.

INTRODUCTION

Ananas comosus var. erectifolius (L. B. Sm.) Coppens & F. Leal, known as curauá, is a fibrous plant belonging to the Bromeliaceae family occurring in the Amazon Forest (Ledo, 1967; Morais et al., 2016). The high-quality curauá fiber is in use in the automobile industry, where its value has already been recognized.

The value of this fiber in the fabrication of auto parts has made it commercially viable for companies to invest

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in larger-scale production. Moreover, curauá fiber has great potential in the production of cellulose nanofibers because of its high cellulose content. These nanofibers can be produced using different methods, given varying structures which includes nanowhiskers, nanofibers and/or nanofibrillated cellulose (Souza et al., 2015).

In recent years, genetic erosion of the genus *Ananas* has become increasingly severe, due to the cultivation of only a few varieties and the anthropization of their areas of native occurrence (Silva et al., 2016). This requires intensified efforts to preserve this germplasm, mainly to *A. comosus* var. *erectifolius*, considering that in the literature there is little information on its genetic variability and because the specie is subject to genetic erosion. This is due to the reduction of its cultivation by the aboriginal, owing to the replacement of its fiber by synthetic fibers in the utensils manufactured.

There are currently curauá germplasm banks located in several institutions, which vary in size and level of characterization. The maintenance of these collections can be optimized by better understanding the genetic variability contained in them, for example, duplicates can be identified and eliminated and collection enrichment decisions can be better targeted. According to the curauá potential economic and its risk of genetic erosion, this work aims to evaluate the genetic diversity between the accessions of curauá (*A. comosus* var. *erectifolius*) kept in the germplasm bank of Faculdade de Ciências Agronômicas de Botucatu (Unesp), Embrapa Amazônia Oriental and two cultivated regions (Arapiuns and Tapajós river).

MATERIALS AND METHODS

Plant material

For the plant material, 90 accessions of Faculdade de Ciências Agronômicas de Botucatu (Unesp) germplasm bank (60 of purple curauá - FCA_{PC} and 30 of white curauá - FCA_{WC}) and 26 accessions of the Embrapa Amazônia Oriental active germplasm bank, were evaluated; of which there are six white curauá from Bragança (B_{WC}), four white curauá from Ponta de Pedra (PP_{WC}), four purple curauá from Ponta de Pedra (PP_{PC}), six white curauá from Marilda (M_{WC}) and six purple curauá micropropagated of Embrapa (E_{PC}). We also evaluated 30 purple curauá individuals from Arapiuns river region (A_{PC}), and 15 purple curauá individuals of the Tapajós river region (T_{PC}).

Obtaining and analyzing SRH data

Genomic DNA was extracted from young leaf tissue. The amount and quality of DNA were estimated by comparisons with lambda DNA of known concentrations on 1% agarose gel, stained with ethidium bromide. The gels were visualized under UV light. Genomic libraries were developed from the protocol of enrichment for microsatellites developed by Billotte et al. (1999).

Plasmid extraction was performed by transferring 10 µL of a solution containing positive clones from ELISA plates to a Deep plate (Axygen) containing 1 mL of Circle Grow medium (4%) 0+30 µL of ampicillin (100 µg/mL) per well, being incubated for 22 h at 37°C in a stirrer at 250 rpm. The adhesive was changed and the material centrifuged for 6 min at 3000 rpm. The supernatant was discarded and 240 µL of GTE (Glucose-Tris-EDTA) was added to each sample. Plates were sealed and suspended in the vortex for 2 min and centrifuged for 6 min at 4000 rpm. The supernatant was again discarded and 60 µL of the material was transferred to 96-well PCR plates containing 5 µL of RNase (10 mg/mL). Additionally, 60 µL of 0.2 M NaOH – 1% SDS was added to each sample and then the plates were sealed and mixed 10 times by inversion. After incubation for 10 min at ambient temperature, the material was centrifuged at 1000 rpm.

Subsequently, 60 µL of 3M KOC was added to each clone and the plate was sealed and mixed 10 times by inversion. The adhesive was removed and the plate incubated in an oven at 90°C for 30 min. After cooling the plate on ice for 10 min, the material was centrifuged for 4 min at 4000 rpm at 20°C. The material was transferred to a filter plate (Axygen) and centrifuged for 6 min at 3000 rpm. Thereafter, 100 µL of isopropanol was added and the solution was mixed by inversion. The supernatant was discarded and 200 µL of 70% ice-cold ethanol was added. The solution was centrifuged for 5 min at 4000 rpm at 20°C, the supernatant was discarded, and the plate was inverted to dry for 60 min at ambient temperature. The material was resuspended in MilliQ water. Plasmid DNAs were confirmed by visualization on 1% agarose gel stained with ethidium bromide.

Sequencing reactions and conditions were described in the Big Dye Terminator Kit (Applied Biosystems, CA, USA), using the primer SP6 and an ABI Prism 3700 sequencer (Applied Biosystems) located at the Sylvio Moreira Center for Citrus Production in the Agronomic Institute of Campinas, Cordeirópolis, São Paulo State, Brazil. The obtained sequences were submitted to quality analysis and elimination of vector and adapter sequences using the software suite, Phred, Phrap, Consed, and Cross_Match (Laboratory of Phil Green; Genome Sciences Department, University of Washington; available on http://www.phrap.org/index.html).

Forward and reverse primers were designed using the software Primer 3, respecting the following criteria: Tm (annealing temperature) between 55 and 63 °C, maximum of 60% GC, fragment sizes between 150 and 350 bp, and at least 18 and at most 25 nucleotides without repetitive sequences. Synthesized primers were identified by the first letter of the genre in capital letters, followed by the first two letters of the species in lowercase letters and the number of the designed sequence.

The 19 pairs of synthesized primers were assessed using five purple curauá (*Ananas erectifolius*) individuals belonging to the UNESP Germplasm Bank. Amplification reactions were performed with 15 ng genomic DNA, 1 U Taq DNA polymerase (LGC Biotechnology), 1X PCR buffer (200 mM Tris pH 8.4, 500 mM KCl), 1.5 to 2.5 mM $MgCl_2$, 0.6 to 0.8 µL of Mix dNTPs (containing 2.5 mM of each dNTP), and 0.3 µM of each primer in a final volume of 10 µL.

Amplifications were performed in a PTC 100 thermocycler (MJ Research, Inc., Watertown, MA, USA). The programs followed the following conditions: 95°C for 5 min, 35 cycles of 94°C for 45 s, 60°C for 1 min, 72°C for 1 min and a final extension for 10 min at 72°C. Products received 5-µL loading buffer (95% formamide). After denaturation at 95°C for 10 min, they were immediately placed on ice and loaded with denaturing acrylamide gel (5%) preheated for 1 h. The process of separating the fragments by electrophoresis was performed with 1X TBE buffer for 2 h at 60 W. Fragments were visualized after staining with silver nitrate, which was performed according to the protocol described by Creste et al. (2001).

The pairs of primers with nonspecific band amplification were again tested at annealing temperatures between 61 to 65°C and at different $MgCl_2$ concentrations. In the absence of amplification of some genotypes, replications were performed to ensure no flaws in the reaction.

The transfer level of microsatellite loci was assessed by using 11 pairs of primers developed for *Ananas lucidus* in samples of *Ananas comosus* of the varieties Roxo-de-tefé (4 individuals) and Gomo-de-mel (4 individuals) from the germplasm bank of pineapples from the Agronomic Institute of Campinas (IAC/SP). Amplification reactions were performed according to those previously described for characterizing and detecting the polymorphism of *A. lucidus*.

In order to analyze the genetic diversity, the following parameters were estimated: average number of alleles/loci (A), percentage of polymorphic loci (P), observed average heterozygosity (H_o), and expected average heterozygosity (H_e). The average number of alleles per loci was obtained by the arithmetic mean of the total number of alleles divided by the total number of loci. Only the loci where the frequency of the most common allele did not exceed 95% were considered as polymorphic and took into account the percentage calculations.

The observed heterozygosity values were obtained by the average number of heterozygous genotypes in relation to the total genotypes of each locus. The expected heterozygosity was obtained by means of the average of H_e of all loci (estimate of multi-loci), calculated according to Nei (1978). Genetic similarity was estimated according to the Dice coefficient using the software NTSYSpc 2.01 (Rohlf, 2000). Based on the similarity analysis, a group dendrogram of cultivars was constructed using the UPGMA (Unweighted Pair Group Mean Average) method.

Obtaining and analyzing RAPD data

For RAPD (Random Amplification of Polymorphic DNA), 10 oligonucleotide primers were tested (OPG–03, OPG–17, OPJ–10, OPK–096, OPU–01, OPU–03, OPU–14, and OPZ–16 from the Operon Technologies, Inc.). The amplification test was performed using four individuals from each group (UNESP Germplasm Bank, Arapiuns River, Tapajós River, and Embrapa Germplasm Bank). The criteria for selection were the number of amplified loci, number of polymorphic loci, and amplified band intensity. The RAPD loci were analyzed for presence (1) or absence (0) of an allele in each of the individuals. The similarity between cultivars was calculated based on the Dice coefficient using the software NTSYSpc 2.01 (Rohlf, 2000). Based on the similarity analysis, a group dendrogram of cultivars was constructed using the UPGMA (Unweighted Pair Group Mean Average) method.

RESULTS

From the 46 sequences obtained from the sequencing of 131 clones from the enriched library for sequenced CT and GT, 22 of them contained SSR sequences formed by more than five replications (47.8%). Microsatellites with dinucleotide motifs accounted for more than half of those identified (69%), followed by trinucleotides (18%), and compounds (14%). From the assessed microsatellites, only two had CA sequences, one of them being a composite microsatellite formed by a replication (GA).

From the 22 sequences with microsatellites, primers were selected for 19, of which 11 (57.9%) allowed the amplification of clear bands and good repeatability, six (31.6%) amplified nonspecific bands, and two (10.5%) did not amplify any fragment. Table 1 shows the sequences of the 11 pairs of primers selected for the assessment of genetic variability in the germplasm of *A. comosus* var. *erectifolius*. Table 1 also shows the motifs, annealing temperatures, MgCl2 concentrations, and their respective allelic ranges.

Single fragments were considered as loci in homozygosity, whereas double fragments were considered as heterozygous. In the sample, 48 different alleles were observed for the set of 10 polymorphic loci and the number of alleles per locus ranged from three at the loci Alu 10, Alu 11, and Alu 12 to 10 at the locus Alu 05, with an average of 4.8 alleles per locus (Table 1).

Table 2 shows that the number of alleles/locus varied between 1.36 and 1.55 (~1.47), and polymorphic loci from 36.4 to 54.5% (~47.47%). The expected heterozygosity ranged from 0.181 to 0.272 (~0.237), while the observed went from 0.363 to 0.545 (~0.474). Also, the numbers of exclusive alleles were different among the nine accession groups, with the highest for white and purple curauá (six) from UNESP, and the lowest for individuals (three) from the rivers Arapiuns and Tapajós. Among the accessions from Embrapa germplasm bank, no exclusive alleles were observed. The genetic divergence (FST) observed among individuals composing the groups of *A. lucidus* was 0.5665, showing a genetic variability between groups of 56.6%.

A separation of individuals into two main groups was observed (Figure 1). The first group is subdivided into two subgroups, one of which includes the individuals of purple curauá from AGB–UNESP (FCA_{PC}) and individuals of purple curauá from Arapiuns River (A_{PC}), and the other group includes individuals of white (M_{WC}, PP_{WC}, and B_{WC}) and purple curauá (E_{PC} and PP_{PC}) from AGB–Embrapa.

In addition, the individuals of white curauá (FAC_{WC}) from AGB–UNESP and the individuals of purple curauá from the Tapajós River (T_{PC}) were included in the second group. The average distance between pairs of accession groups was 0.50 ± 0.24, with the highest value (0.82) between FCA_{WC} and the groups M_{WC}, PP_{WC}, and B_{WC}. However, the low genetic distances between accessions of purple (E_{PC} and PP_{PC}) and white curauá (M_{WC}, PP_{WC}, and B_{WC}) from Embrapa suggest a reduced variability between both individuals.

From the ten primers tested by RAPD, seven provided clear amplification and good repeatability products. The selected primers were OPG–03, OPG–17, OPU–01, OPU–03, OPZ–03, OPZ–14, and OPZ–16, which amplified fragments ranging from 300 to 4000 bp. These primers allowed the amplification of 35 RAPD fragments, 22 of them being polymorphic. The number of loci per primers ranged from two (for the primers OPG–03 and OPZ–14) to five (for the primers OPU–01 and OPZ–03), with an overall average of 2.9 loci per primer (Table 3).

The percentage of polymorphic loci per group ranged from 3.5% for FCA_{PC} to 17.5% for PC, with an average of 11.2%. Among banks, variability levels between the germplasm of purple and white curauá are similar, except for the accessions of white curauá from AGB–UNESP,

Table 1. Pairs of microsatellite primers developed for *Ananas comosus* var. *erectifolius* motif types, forward and reverse primers sequences, expected fragment sizes, observed allele amplitudes, annealing temperatures and magnesium chloride concentrations used for each primer pair.

Locus	Motif	Sequence of pairs of primers	Size of expected fragments	Allele range (bp)	Number of alleles/loci	Tm (°C)	$MgCl_2$ (mM)
Alu 01	$(AGA)_{10}$	F-5'TATTTGGCCATTTCACCCTC3' R-5'GATCCTCCACAAAGCTCCAA3'	193	197-209	4	60	1.5
Alu 03	$(CA)_{10}$	F-5'TGTTAGATTTGGGCCGTTTC3' R-5'GGCATCCCCATATCTTAGCA3'	260	260-280	4	60	1.5
Alu 04	$(CT)_{10}$	F-5'TAATTTGGTAGCACGGAGGC3' R-5'TCCCTTCATCCAAAAAGTCG3'	156	156	1	60	1.5
Alu 05	$(CT)_{19}$	F-5'TGATGGGAAATAGCTGAGCC3' R-5'AAAAACGAGCACAATCCCAC3'	266	240-280	10	60	1.5
Alu 08	$(TC)_{10}$	F-5'TTTCCTTTCCGCACAATTTC3' R-5'GTCGTGTGTGGAAACCACTG3'	219	220-250	5	60	1.5
Alu 09	$(AAG)_{10}$	F-5'ATGTAATTGACCCACCCCAA3' R-5'TTTCTATGCGGACTGAACCC3'	208	200 -230	4	60	1.5
Alu 10	$(TC)_{10}$	F-5'GGGTTCAGTCCGCATAGAAA3' R-5'CCAGTCCTGCAGTGACAATC3'	238	223-250	3	65	1.5
Alu 11	$(TC)_{16}$ $(AAG)_8$	F-5'ACAGCTTGCGAGAAACAAGG3' R-5'CAAGTTTTGCGACACCAATG3'	237	230-250	3	65	1.5
Alu 12	$(TC)_{16}$ $(AAG)_8$	F-5'GCCCTCCATTTCCACCTAAC3' R-5'GGTGGTATTGGTCGCTGTCT3'	169	169 -290	3	65	1.5
Alu 15	$(GA)_{13}$	F-5'AGGATACTCGATCTCCCGCT3' R-5'TCACCTGCAAAGGGAATAGG3'	203	260-300	5	65	1.5
Alu 17	$(CT)_{13}$ $(CA)_{10}$	F-5'GGAGCCATCTAATTGTTCCA3' R-5'ATATGCGACCAAGCACAACA3'	248	240-290	7	65	1.5

which present a higher percentage of polymorphic loci (12.3%) than those of purple curauá (3.5%), as shown in Table 4.

Two groups were formed, one consisting exclusively of purple curauá plants and the other of white curauá. Within groups of purple curauá, only A_{PC} and T_{PC} presented 100% similarity. The relationships between species were different from those established with microsatellites, which did not allow a clear distinction between purple and white curauá (Figure 2).

DISCUSSION

Crossing and molecular data of the seven species of the genus Ananas indicate their intercompatibility and fertility for hybrids. There is also molecular evidence on the close relationship between species of the genus Ananas in studies conducted by Ruas et al. (2001), which is considered to be phylogenetically close. Thus, the probability of transfer of primers between them is high. However, this study demonstrates that none of them has similarity to sequences of other species of Ananas, making the set of pairs of primers presented in this study an extra and new set for the assessment of *A. comosus* var. *erectifolius* and other species of Ananas. From the 22 sequences containing microsatellites, 19 pairs of primers (86%) were designed and the other three had an insufficient number of repetitions or the flanker sequences were very small, precluding the selection of primers meeting the established parameters.

Sequences formed by dinucleotides were the most frequent because of the use of CT and GT probes. Microsatellites with AG/CT and GA/TC motifs were more frequent than those with CA/GT. Oliveira et al. (2004) verified the higher frequency of GT and CT motifs for

Table 2. Diversity indices for the nine *Ananas comosus* var. *erectifolius* groups, where n: access sampled number; NA: total of alleles; P: polymorphic loci percentage; A: average number of alleles / lozenges; He: expected heterozygosity and Ho: observed heterozygosity, using 10 microsatellite loci.

Diversity indices	Population									Mean
	AGB–UNESP		Arapiuns/ Tapajós		AGB–Embrapa Amazônia Oriental					
	FCA_{PC}	FCA_{WC}	A_{PC}	T_{PC}	B_{WC}	PP_{WC}	PP_{PC}	M_{WC}	E_{PC}	
N	60	30	1	1	8	4	4	8	8	18.55
NA	15	16	15	15	16	16	14	16	14	15.2
P	45.5	54.5	45.5	45.5	54.5	54.5	36.4	54.5	36.4	47.47
A	1.45 −0.52	1.55 −0.52	1.45 −0.52	1.45 −0.52	1.55 −0.52	1.55 −0.52	1.36 −0.5	1.55 −0.52	1.36 −0.5	1.47 -
H_e	0.227 −0.261	0.272 −0.261	0.227 −0.261	0.227 −0.261	0.272 −0.261	0.272 −0.261	0.181 −0.252	0.272 −0.261	0.181 −0.252	0.237
H_o	0.454 −0.522	0.545 −0.522	0.454 −0.522	0.454 −0.522	0.545 −0.522	0.545 −0.522	0.363 −0.304	0.545 −0.522	0.363 −0.304	0.474 -

Note: The amount of expected heterozygosis was estimated according to Nei (1978); number in parentheses is the standard deviation.

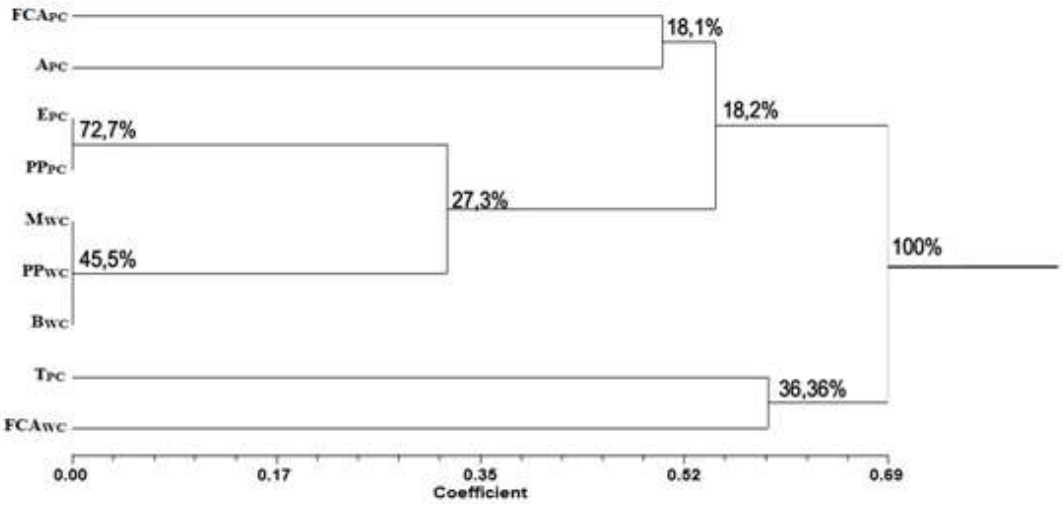

Figure 1. Dendogram showing the relationships among the nine access groups of *Ananas comosus* var. *erectifolius* evaluated. The distances between accesses were obtained by the UPGMA grouping criterion, based on the Dice coefficient Data from 10 microsatellite loci.

Table 3. Primers selected sequence for *Ananas comosus* var. *erectifolius* and number of loci obtained.

Primer	Sequence 5' – 3'	Number of loci	Number of polymorphic loci	Percentage of polymorphism
OPG–03	GAGCCCTCCA	6	2	33.3
OPG–17	ACGACCGACA	4	2	50
OPU–01	AGATGCAGCC	6	5	83.3
OPU–03	ACTGGGACTC	7	3	42.8
OPZ–03	CAGCACCGCA	7	5	71.4
OPZ–14	TCGGAGGTTC	2	2	100
OPZ–16	TCCCCATCAC	3	3	100
Total	-	35	22	-

Table 4. Percentage and number of polymorphic loci for the nine groups of *Ananas como*sus var. *erectifolius*.

Group	Curauá samples	Percentage of polymorphic loci (%)	Number of polymorphic loci
AGB–UNESP	FCA_{PC}	3.5	2
	FCA_{WC}	12.3	7
Arapiuns River	PC	17.5	10
Tapajós River	PC	12.3	7
AGB–Embrapa Amazônia Oriental	Bragança – WC	10.5	6
	Ponta de Pedra – WC	15.8	9
	Ponta de Pedra – PC	10.5	6
	Marila – WC	8.8	5
	Embrapa – micropropagated plant – PC	8.8	5
Average of groups	-	11.2	6.4

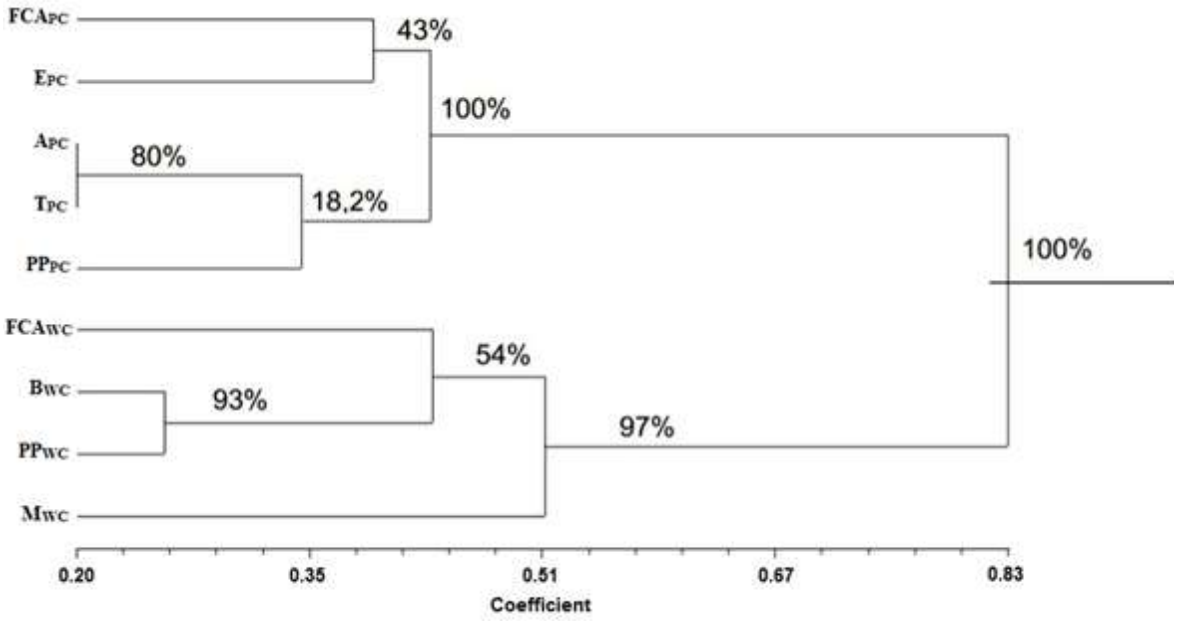

Figure 2. Relationship between the nine groups of *Ananas comosus* var. *erectifolius* individuals, defined by the UPGMA grouping criterion, based on the Dice coefficient.

Passiflora edulis when using the same enrichment protocol. In another study, GT/CA and GA/CT motifs occurred in a higher percentage for the genus Phaseolus (Campos et al., 2005). The frequency of microsatellite motifs is quite adjustable in plants. Once probes are used, the enrichment process are seen to have very similar annealing temperatures because they are composed of the same amounts of purines and pyrimidines, and if the possibility of technical problems with the GT probe is excluded, the data suggest the higher frequency of microsatellites with CT/GA motifs in curauá than GT/CA.

RAPD and SSR marker analyses showed a low genetic variability within each of the nine analyzed groups. The results were similar when compared to the observed indices for other asexual propagating species. The authors suggest that they are potentially duplicate cultivars, or at least a minimal genetic difference between them. In a study of genetic diversity of 16 accessions of the genus Ananas by means of 148 RAPD markers, Ruas et al. (2001) verified a great genetic similarity (80%) among five varieties of A. comosus and attributed this result to its asexual reproduction.

The low genetic variability observed within the

assessed curauá collections may be due to two main factors: 1 – collection formation from few individuals; 2 – low genetic variability in the collection regions. This second hypothesis seems to be less likely since Costa et al. (2002) found great genetic divergence among curauá accessions; they assessed 16 accessions using 104 RAPD markers (Active Germplasm Bank of Embrapa Amazônia Oriental) and found 79 (75.96%) polymorphic ones. Therefore, there is a slight relationship between geographical origin and pattern of genetic variability distribution.

Due to the cultivation method of curauá, there is a possibility of low genetic variability in the original regions of its cultivation, which can be due to two main factors: 1 – a reduction of variability resulting from the domestication process; and 2 – the genetic erosion caused by crop abandonment by indigenous people. Ferreira and Bustamante (2004) report that curauá is a relative of pineapple which have been domesticated by indigenous people a long time ago and probably can no longer survive in nature without human interference.

RAPD markers allowed the detection of variation within groups, but this variation was also, in general, quite low. The major differences observed among the nine groups of analyzed accessions, which were collected at six different locations, suggesting that a greater variability will be sampled if a larger number of sampling sites are sampled. In this case, there is no need to collect a large number of samples per site since the intra-site variability is generally low.

The data presented here regarding the genetic variability among accessions of the same region are divergent from those found by Costa et al. (2002), who used 104 RAPD markers and observed variability among accessions of some of the assessed locations. Among these accessions are white curauá samples from Bragança (B_{WC}) and Ponta de Pedra (PP_{WC}). In this study, no polymorphism was detected among the analyzed accessions from both sources with data from ten pairs of microsatellite primers. RAPD data demonstrate polymorphism between both groups; however, the genetic similarity between B_{WC} and PP_{WC} was quite high (76.6%), actually indicating 2 molecularly similar groups. The divergence between the data obtained in this study and that of Costa et al. (2002) is probably due to the difference of the assessed samples. There is no correlation between the degree of genetic similarity observed in genetic markers and the origin of accessions (Salla et al., 2002). On the other hand, our findings are in agreement with those of Costa et al. (2002), who demonstrated a high variability between accessions from different locations.

CONCLUSIONS

The greater genetic diversity will be sampled if a larger number of sampling sites are experimented, and there is no need to collect a large number of accessions per site since intra-site variability is low. Primers resulting from this study may decrease the time and expenses involved in the isolation of microsatellite markers, such as the construction of a genomic DNA library and DNA sequencing.

These primers are an extra and new set for assessing *Ananas comosus* var. *erectifolius* and will be useful for other species from the genus Ananas.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interest.

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