doi:10.14720/aas.2020.116.1.1181 Original research article / izvirni znanstveni članek

Genetic studies of soybean [*Glycine max* (L.) Merr.] response to seed storage stress factors

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Received June 03, 2019; accepted June 03, 2020.
Delo je prispelo 03. junij 2019; sprejeto 03. junij 2020

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Abstract: Soybean [*Glycine max* (L.) Merr.] responded differently to storage stress factors. This research work aimed at assessing genetic potentials of fifteen soybean varieties to storage stress via accelerated aging technique and grouping them based on their levels of tolerance to storage stress using simple sequence repeat (SSR). Tolerance of the seed of 15 soybean varieties to storage stress was assessed by subjecting them to accelerated aging for 0, 6, 12, and 24 hours at 42 °C temperature and 100 % relative humidity (RH), after which their quality was assessed to determine their tolerance to storage stress. Same varieties were also stored for 6 months under ambient environment at 65 ± 5 % R.H and 25 ± 2 °C and they were genotyped with 19 simple sequence repeat (SSR) markers. The varieties were grouped on the basis of their levels of tolerance to storage stress. The principal components analysis (PCA) showed that germination rate index (GRI) and germination index (GI) were the major indices responsible for the significant variation in the seedling vigor characters. TGX1835-10E and TGX1448-2E were identified as varieties with good storage ability and therefore recommended for storage improvement in soybean breeding programs. Six SSR markers (Satt 565, Satt 175, Satt 281, Satt 600, Satt 160 and Satt 281) were identified as candidate markers for detection of alleles for tolerance to storage stress.

Key words: soybean; seed accelerated aging; principal components analysis (PCA); dendrogram; SSR markers; tolerance

Genetske raziskave soje [*Glycine max* (L.) Merr.] v povezavi s stresnimi dejavniki pri shranjevanju semena

Izvleček: Genotipi soje (*Glycine max* (L.) Merill) se odzivajo različno na stresne dejavnike med shranjevanjem semena. Namen raziskave je bil oceniti genetski potencial 15 sort semen soje na stres med shranjevanjem pri pospešenih tehnikah staranja. Genotipi so bili razvrščeni na osnovi njihove tolerance na stres med staranjem na osnovi enostavnih ponavljajočih se zaporedij (SSR) in agronomskih lastnosti. Toleranca 15 sort semen soje na stres med shranjevanjem je bila ocenjena z izpostavitvijo pospešenemu staranju za 0, 6, 12, in 24 ur pri 42 °C in 100 % relativni vlažnosti (RH). Nekatere od sort so bile shranjene tudi za 6 mesecev v razmerah 65 ± 5 % relativne vlažnosti in pri 25 ± 2 °C. Ti genotipi so bili ovrednoteni z markerji na osnovi19 enostavnih ponavljajočih se zaporedij (SSR). Sorte so se združevale na osnovi njihove tolerance na stres med shranjevanjem. Analiza glavnih komponent je pokazala (PCA), da sta bila indeks hitrosti kalitve (GRI) in kalitveni indeks (GI) najpomembnejša pokazatelja značilne variabilnosti v lastnostih, ki označujejo vigor sejank. TGX1835-10E in TGX1448-2E sta bili prepoznani kot sorti z dobro sposobnostjo shranjevanja in sta priporočeni v žlahtniteljskih programih soje za izboljševanje shranjevanja semena. Šest SSR markerjev (Satt 565, Satt 175, Satt 281, Satt 600, Satt 160 and Satt 281)je bilo prepoznanih kot primernih za ugotavljanje alelov odgovornih za toleranco na stres med shranjevanjem.

Ključne besede: soja; pospešeno staranje semen; analiza glavnih komponent (PCA); dendrogram; SSR markerji; toleranca

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1 INTRODUCTION

Soybean *(Glycine max* (L.) Merrill) is one of the oldest cultivated leguminous crops (Verma and Shoemaker, 1996). It is one of the most important economical legumes in the world, providing protein and oil to food and animal feed industries, as well as base ingredients for hundreds of chemical products (Hedley, 2001). It is grown for its beans, which have numerous uses (Camara, 2000). The beans can be processed into various meals, such as soy meat, cakes, baby foods, "tofu," and "dawadawa"- a local seasoning product for stews and soups (Abbey et al., 2001). The ability of the crop to increase dietary quality of resource-poor people all over the world (Hartman et al; 2011) has increased the demand for the beans with equivalent increase in its production. Provision of sufficient high-quality seed to farmers at an affordable price at the right time is a big challenge because the seeds deteriorate rapidly in quality during storage and therefore soybean farmers face inadequate supply of quality seed during the planting season (Dugjeet al., 2009). However, it has been reported that loss of germination potential of soybean seed is more acute in tropical and sub-tropical regions of the world when compared with temperate environments (Bhatia, 1996). This is because temperature, which is one of the major storage stress factor affects the storability of the seeds and also causes chemical changes, such as hydrolytic and oxidative rancidity (changes in oil quality) in the seeds, leading to reduced seed quality and subsequent loss of viability across time (Baskin and Delouche, 1973). This resulted into farmers planting available seed that often has low vigor, which in turn affects the yield (Nkang and Umoh, 1997). It has been proved that cultivars respond differently to environment situations due to their genetic variability (Flajšman et al., 2019; Badu-Apraku et al., 2017). Hence researchers have explored the use genetic variability among germplasms to solve genetic and agronomic problems.

Principal component analysis (PCA) and cluster analysis are methods of statistical grouping of germplasm, which provide information regarding the contributions of each character to total variation and group individuals based on the their genetic attributes respectively. These two techniques aid effective selection by plant breeders and or geneticists in crop improvement programs. In solving problem of soybean seed deterioration during storage, there is need to understand genetic traits responsible for tolerance to storage stress, hence this research work aimed at assessing genetic constituents of fifteen soybean varieties and their response to storage stress via accelerated aging technique with a view to grouping them based on their levels of tolerance to storage stress using Simple Sequence Repeat (SSR) prim-

Table 1: List of Fifteen varieties of soybean and their attributes

Serial Number	Varietal Name	Source	Seed colour	Days to maturity	Initial Germination %	Maturity group
1	TGX1990-52F	IITA	Milky White	95 - 102	97	I
2	TGX1989-48FN	IITA	Milky White	95 - 110	81	II
3	TGX1989-49FN	IITA	Milky White	95 - 110	95	II
4	TGX1835-10E	IITA	Milky White	81- 95	97	00
5	TGX1990-46F	IITA	Milky White	95 - 102	83	I
6	TGX1990-95F	IITA	Milky White	95 - 110	84	II
7	TGX1987-19F	IITA	Milky White	95 -110	89	II
8	TGX1990-78F	IITA	Milky White	95 - 105	96	II
9	TGX1989-53FN	IITA	Milky White	95 - 105	95	II
10	TGX1989-75FN	IITA	Milky White	95 - 105	83	II
11	TGX1990-114FN	IITA	Milky White	108 - 115	88	II
12	TGX1990-110FN	IITA	Milky White	108 - 115	97	II
13	TGX1440-1E	IAR&T	Creamy	90-100	89	I
14	TGX1448-2E	IAR&T	Creamy	108-115	89	II
15	TGX1740-1E	IAR&T	Creamy	90-100	79	I

IITA: International Institute of Tropical Agriculture
IAR&T: Institute of agricultural Training and Research
Maturity group classification was according to Yuesheng et al., 2006.

ers and identifying markers associated with tolerance or susceptible alleles in these soybean genotypes.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL MATERIAL AND SOURCES

Fifteen (15) soybean varieties were collected from International Institute of Tropical Agriculture (IITA) and Institute of Agricultural Research and Training, Obafemi Awolowo University (I.A.R & T), both in Ibadan, Nigeria (Table 1).

2.2 EVALUATION OF SEEDLING VIGOR CHARACTERISTICS

These soybean varieties were subjected to various seed quality assessment to determine their initial quality attributes. The tests conducted are**:**

Germination test: Fifty seeds in three replicates of each variety were planted in seed bowls filled with adequately moistened river sand. Germinated seedlings were counted daily as from 3rd to 6th day after sowing. Percentage germination (G %) was determined by finding the ratio of normal germinated seed at 6 days after sowing to total number of seeds planted according to the method suggested by ISTA (1996) using Equation 1 :

$$\%G = \frac{\text{No of normal seedlings that germinated}}{\text{Total number of seeds planted}} \text{ x } 100$$

(Equation 1)

Seedling vigor assessment: Seedling vigor of the germinated seeds was assessed using the seedling vigor parameters suggested by various past researchers using equations (2) – (5)

Coefficient of velocity of Germination (*CVG*): This is an estimate of the rapidity of germination of the seed lot and it was estimated according to the method described by Scott et al. (1884).

$$CVG = \frac{\Sigma Ni}{\Sigma NiTi} x100$$

(Equation 2)

Where: N is the number of seeds germinated each day and T is the number of days corresponding to N

Germination Index (GI): This is an index of the speed of germination. This was calculated based on method described by Akande et al. (2012).

$$GI = \frac{\Sigma \text{ (Nx)(DAP)}}{Total\ number\ of\ normal\ seedlings\ that\ emerged\ on\ final\ day}$$

(Equation 3)

where Nx is the number of normal seedlings that emerged on day x after seeding and DAP is days after planting

*Germination Rate Index (GRI)***:** This reflects the percentage of germination on each day of the germination period and was calculated according to the method of Olisa et al. (2010). Higher *GRI* values indicate higher and faster germination (Kedar, 2005).

$$GRI = \frac{G1}{x} + \frac{G2}{x} + \frac{G3}{x} + \cdots \frac{Gx}{x}$$

(Equation 4)

where, *G* = germination on each day after seed placement 1, 2, x = corresponding day of germination.

Seedling vigor Index (SVI): Seedling length was measured from five randomly selected seedlings of each replicate from the soil level at 3 and 6 days after planting. The SVI was then calculated based on method of Adebisi et al. (2004).

$$SVI = \frac{(Germination\ \%\ x\ Seedling\ length)}{100}$$

(Equation 5)

2.3 ACCELERATED AGING PROCEDURE, GERMINATION AND VIGOR TEST

Twenty five grams (25 g) of seeds in three replicates of the 15 soybean varieties were artificially aged using plastic boxes (11.0 × 11.0 × 3.5 cm) at 100 % relative humidity and 42 °C temperature for 6, 12 and 24 hours (Jagadishet al., 2013). Germination and seedling vigor attributes of the seed lots were determined after each aging period and compared to initial seed quality. The difference between the two quality factors determines their tolerance ability.

2.4 AMBIENT SEED STORAGE

Fifty grams (50 g) of clean seed of each variety was packaged into paper envelopes and stored under ambient environment with average relative humidity (RH) of 65 ± 5 % and temperature of 25 ± 2 °C in the laboratory of Grain Legumes Improvement Programme of IAR & T Ibadan for six months (Demir et al., 2008). Each variety was replicated three times and the envelopes were arranged in Completely Randomized Design (CRD). Samples of the stored seed were evaluated after six months of storage using procedure described above.

The germination percentage after accelerated aging and six months ambient storage was deducted from

initial germination percentage to estimate the resistance ability of each variety, which resulted to the percentage germination loss (G loss).

2.5 DNA EXTRACTION AND MOLECULAR ANALYSIS FOR STRESS TOLERANCE TRIAL

2.5.1 DNA extraction

Five grams of leave samples were harvested from the plant of each variety sown in the green house at about two weeks after planting and stored in ice, kept at -20 °C and later transported to Plant Science Laboratory of National Institute of Science Laboratory Technology, (NISLT) Ibadan, Nigeria for DNA extraction. Genomic DNA was extracted using the ZR Plant/Seed DNA Mini-Prep™ kit. DNA quantity and quality was measured using Nanodrop® (ND-1000 spectrophotometer) at A $260/_{280}$ absorbance. The ratio of A260/280 absorbance and values obtained ranged between 1.80 and 2.0, indicates good quality DNAs.

2.5.2 Polymerase Chain Reaction

The SSR amplification was carried out with 20 Simple Sequence Repeat (SSR) primers in 11 µl reaction mixture consisting of 2.0 µl of template DNA, 1.0 *µl each of forward and reverse primers, 1.0 µl dNTP mix, 6.0 µl* PCR assay buffer. The PCR reactions were performed in a thermo-cycler with initial denaturation at 94 *°C for five minutes followed by 35 cycles of denaturation at 94 °C for one minute, annealing for 30 seconds at 47 °C (56* °C for Satt 565 and extension for 30 s at 72 *°C with a final extension for seven minutes. The amplified products were separated on 6.0* % polyacrylamide gel. Gels were run for 3 h at 75 V in 1X TBE buffer. DNA fragments were visualized under UV light and photographed using gel documentation system. An identified band (allele) on gels was binary coded as 1 or 0 to indicate their presence or absence respectively in soybean for each SSR primer. The names and sequences of the SSR primers are presented in Table 2.

2.6 DATA ANALYSIS

Data obtained from accelerated aging and ambient stor-

Table 2: The sequence and annealing temperature of the soybean SSR primers

	Sequence		
Name of primer	Forward	Reverse	AT (°C)
Satt423	TTC GCT TGG GTT CAG TTA CTT	GTT GGG GAA TTA AAA AAA TG	47
Satt414	GCG TAT TCC TAG TCA CAT GCT ATT TCA	GCG TCA TAA TAA TGC CTA GAA CAT AAA	47
Satt434	GCG TTC CGA TAT ACT ATA TAA TCC TAA T	GCG GGG TTA GTC TTT TTA TTT AAC TTA A	47
Satt285	GCG ACA TAT TGC ATT AAA AAC ATA CTT	GCG GAC TAA TTC TAT TTT ACA CCA ACA AC	47
Satt154	AGA TAC TAA CAA GAG GCA TAA AAC T	AAA GAA ACG GAA CTA ATA CTA CAT T	47
Satt002	TGT GGG TAA AAT AGA TAA AAA T	TCA TTT TGA ATC GTT GAA	47
Satt160	TCC CAC ACA GTT TTC ATA TAA TAT A	CAT CAA AAG TTT ATA ACG TGT AGA T	47
Satt565	GCG CCC GGA ACT TGT AAT AAC CTA AT	GCG CTC TCT TAT GAT GTT CAT AAT AA	56
Satt281	AAG CTC CAC ATG CAG TTC AAA AC	TGC ATG GCA CGA GAA AGA AGT A	47
Satt233	AAG CAT ACT CGT CGT AAC	GCG GTG CAA AGA TAT TAG AAA	47
Satt600	GCG CAG GAA AAA AAA ACG CTT TTA TT	GCG CAA TCC ACT AGG TGT TAA T	47
Satt434	GCG TTC CGA TAT ACT ATA TAA TCC TAA T	GCG GGG TTA GTC TTT TTA TTT AAC TTA A	47
Satt285	GCG ACA TAT TGC ATT AAA AAC ATA CTT	GCG GAC TAA TTC TAT TTT ACA CCA ACA AC	47
Satt142	GGA CAA CAA CAG CGT TTT TAC	TTT GCC ACA AAG TTA ATT AAT GTC	47
Satt545	CAA TGC CAT TCC ATA TTT GTT	CAA TTG CCC TAG TTT TGA TAG	56
Satt389	GCG GCT GGT GTA TGG TGA AAT CA	GCG CCA AAA CCA AAA GTT ATA TC	47
Satt431	GCG TGG CAC CCT TGA TAA ATA A	GCG CAC GAA AGT TTT TCT GTA ACA	47
Satt354	GCG AAA ATG GAC ACC AAA AGT AGT TA	GCG ATG CAC ATC AAT TAG AAT ATA CAA	47
Satt175	GAC CTC GCT CTC TGT TTC TCA T	GGT GAC CAC CCC TAT TCC TTA T	47
Satt194	GGG CCC AAC TGA TAT TTA ATT GTA A	GCG CTT TGT GTT CCG ATT TTG AT	47

AT- Annealing temperature (°C).

age were subjected to analysis of variance (ANOVA) using SAS™ software package. Means were separated using Duncan Multiple Range Test (DMRT) at 5 % level of significance. Principal component analysis was conducted using standardized data obtained from the accelerated aging characters to determine factors contributing to the variance. Components with Eigen values > 1.0 were selected and factors with contributing characters values of > 6 were considered relevant for principal component (Matus et al., 1999). Varieties were then clustered into groups using hierarchical clustering based on squared Euclidean distance using PAST v2.17 software (Hammer et al., 2001). Pearson's coefficients of correlations between germination loss and seedling vigor characters were determined using STAR 2.0.1 software.Genetic diversity, Polymorphic information content (PIC), gene diversity, heterozygosity, percentage polymorphism, inbreeding coefficient and average number of alleles were estimated using Power Marker v3.0 software (Liu and Muse, 2005). Cluster analysis based on Euclidean distance coefficient was obtained with the unweighted pair-group method based on the arithmetic mean (UPGMA) to generate the dendrogram using PAST v2.17 software (Hammer et al., 2001).

3 RESULTS

3.1 RESPONSE OF SOYBEAN VARIETIES TO STORAGE STRESS

The mean square values for germination loss (G loss), coefficient of velocity of germination (CVG), growth rate index (GRI), germination index (GI) and seedling vigor index

Table 3: Mean square of soybean viability and other seedling vigor characteristics

SOV	df	Gloss (%)	GI	GRI	CVG	SVI
Rep	2	42.1	5.62	2.63	48.4	14.88
Variety (V)	14	1616.59**	5831.03**	662.92**	233.18**	219.15**
Aging Period (A)	4	28786.24**	50587.99**	4723.55**	633.39**	3302.44**
V x A	56	550.62**	1072.86**	114.07**	75.35**	47.64**
Error	148	60.91	96.76	10.54	36.95	11.1

*, **significant at p = 0.05 and p = 0.01, respectively
SOV: Sources of variation; df: Degree of freedom; G loss: Germination loss; GI: Growth index; GRI: Growth rate index; CVG: Coefficient of velocity of germination; SVI: Seedling vigor index

Table 4: Mean values of germination loss and seedling vigor parameters of fifteen soybean varieties

Variety	G. loss(%)	GI	GRI	CVG	SVI
TGX 1190-52F	32.00^{ab}	85.73^{fg}	25.43^{g}	37.32^{bcd}	15.08^{ef}
TGX 1989-48FN	15.20^{g}	87.67^{efg}	27.26^{efg}	39.65^{abcd}	16.07^{def}
TGX 1989-49FN	18.93^{efg}	103.13^{c}	31.48^{c}	41.27^{ab}	17.57^{bcde}
TGX 1835-10E	1.60^{h}	136.47^{a}	43.45^{a}	43.82^{a}	24.4^{a}
TGX 1990-46F	22.4^{efd}	82.07^{hg}	25.46^{g}	40.41^{abc}	14.99^{ef}
TGX 1990-95F	32.80^{ab}	63.73^{i}	18.26^{i}	30.65^{e}	10.29^{g}
TGX 1987-19F	29.60^{bc}	76.00^{h}	22.64^{h}	36.63^{bcd}	14.09^{f}
TGX 1990-78F	24.53^{cde}	94.87^{de}	28.92^{def}	35.96^{cd}	17.83^{bcd}
TGX 1989-53FN	26.67^{bcd}	88.53^{efg}	26.68^{fg}	39.38^{abcd}	14.95^{ef}
TGX 1989-75FN	37.60^{a}	53.07^{j}	14.84^{j}	29.21^{e}	8.51^{g}
TGX 1990-114FN	13.33^{g}	98.80^{cd}	29.31^{cde}	37.84^{bcd}	19.69^{bc}
TGX 1990-110FN	27.2^{bcd}	90.53^{ef}	25.46^{g}	35.08^{d}	14.03^{f}
TGX 1440-1E	16.53^{fg}	98.20^{cd}	29.92^{cd}	39.79^{abcd}	17.09^{cde}
TGX 1448-2E	4.80^{h}	114.80^{b}	35.08^{b}	40.24^{abc}	19.78^{b}
TGX 1740-1E	13.33^{g}	89.73^{efg}	27.88^{defg}	40.92^{abc}	16.14^{def}

Mean followed by the same alphabets are not significantly different at p = 0.05
G loss: Germination loss; GI: Growth index; GRI: Growth rate index; CVG: Coefficient of velocity of germination; SVI: Seedling vigor index

(SVI) of the fifteen soybean varieties as affected by accelerated aging and storage period are presented in Table 3. There were highly significant differences among soybean varieties in respect to their response to storage stress (temperature and pressure) as expressed by the germination loss and all the seedling vigor characteristics (Table 3).

There were significant differences among the soybean varieties with respect to their reactions to storage stress as measured by the germination loss and other seedling vigor characteristics at $p = 0.05$ level of significance (Table 4). Germination loss among the varieties ranged from 1.6 % to 37.6 % (as a measure of the difference in germination % between untreated seed and seed after 6 month of aging period). High germination loss (37.60 %, 32 %, 32.8 %) was recorded in 'TGX1989-75FN', 'TGX1190-52F' and 'TGX1190-95F' respectively while low germination loss was observed in 'TGX1835-10E' (1.60) and 'TGX1448-2E' (4.80). There was no significant difference in the germination loss of 'TGX1989-48FN' (15.20), 'TGX 1989-49FN' (18.93), 'TGX1990-114FN' (13.33), 'TGX1440-1E-1E' (16.53) and 'TGX1740-1F' (13.33) (Table 4). Germination loss (G loss) decreased with increasing aging period, for instance the seeds had lost germination ability of about 63.73 % as at 6 months of aging period. Other seedling vigor parameters (GI, GRI, CVG and SVI) decreased with increasing aging period (6 hours to 6 months) (Table 5).

3.2 CLASSIFICATION OF SOYBEAN VARIETIES BASED ON RESPONSE OF THE SEEDS TO STORAGE STRESS

The principal component analysis (PCA) based on response of the soybean varieties to storage stress revealed four component axes with Eigen values that were greater than 1.0. These accounted for 93.97 % of the total variation. PC1 accounted for 76.16 % of the variation with germination rate index and germination index being the major factor while the second principal component (PC2) was responsible for about 17.81 % of the variation and was associated majorly with coefficient of velocity of germination and germination loss (Table 6). Soybean varieties were clustered into two major groups (A and B) based on hierarchical clustering using squared Euclidean distance. Cluster 1 contained two soybean varieties ((TGX1448-2E and TGX1835-10E). Cluster 2 was

Table 5: Effect of accelerated aging period on soybean seed viability

Aging period	G loss %	GI	GRI	CVG	SVI
0 hour	0.00^{d}	111.11^{ab}	32.23^{b}	33.98^{c}	17.97^{b}
6 hours	8.00^{c}	113.42^{a}	36.54^{a}	44.14^{a}	27.63^{a}
12 hours	10.22^{c}	108.27^{b}	32.47^{b}	37.08^{a}	12.22^{b}
24 hours	23.56^{b}	87.93^{c}	25.87^{c}	37.08^{b}	12.22^{c}
6 months	63.73^{a}	33.71^{d}	10.52^{d}	36.88^{b}	4.33^{d}

Mean followed by the same alphabets are not significantly different at p = 0.05
G loss: Germination loss; CVG: Coefficient of velocity of germination; GRI: Growth rate index;
GI: Growth index; SVI: Seedling vigor index

Table 6: Characters with respect to its principal component (PC), Eigen values and variation based on response of the seed to storage stress

Characters	PC 1	PC 2
Germination loss	-0.47	0.27
Germination index	0.50*	-0.13
Germination rate index	0.51*	-0.022
Coefficient of velocity of germination	0.22	0.96*
Seedling vigor index	0.47	-0.01
Eigen value	3.81	0.89
% Variance	76.16	17.81
Cumulative	76.16	93.97

*component contributors

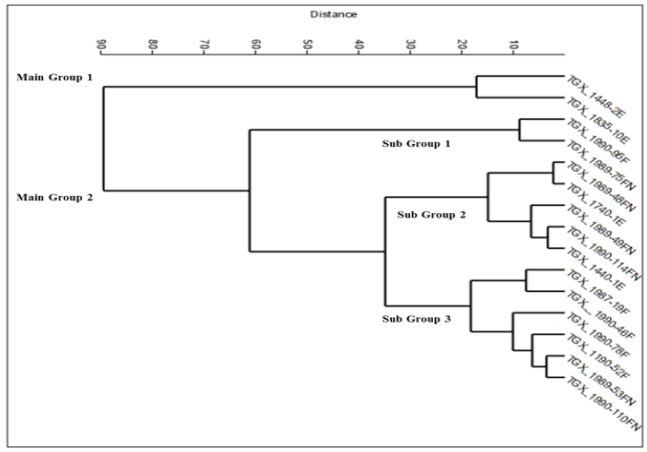

Figure 1: Dendrogram cluster grouping of 15 soybean varieties based on the response of the seed to storage stress

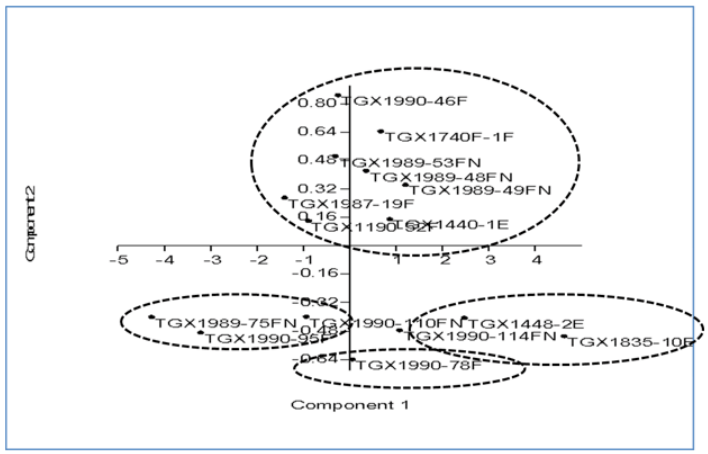

Figure 2: PCA scatter plot of soybean varieties evaluated based on the response of the seeds to storage stress on PC1 and PC2 axes

subdivided into three sub-groups. Sub-group 1 contained two varieties (TGX1990-95F and TGX1989-75EN), while sub-group 2 consisted of five varieties (TGX1989-49FN, TGX1740-1E, TGX1989-49FN, TGX1990-114FN and TGX1440-1E-1E) and sub-group 3 comprised six varieties (TGX1987-19F, TGX1990-46F, TGX1990-78F, TGX1190-52F, TGX1989-53FN and TGX1990-110FN) (Figure 1). The scatter plot of how the varieties are close to each other based on their response to storage stress shows that most of the varieties are similar to each other due to high germination loss after storage stress, hence they lied on the positive region of component 2. 'TGX 1448-2E', 'TGX 1990-114FN' and 'TGX 1835-10E' were similar to each other based on component 1, which is dominated by high germination indices after storage stress. 'TGX-1990-78F' was partially separated from other varieties along the lower negative region component 2 (Figure2).

3.3 CORRELATION BETWEEN PAIRS OF GERMINATION LOSS AND SEEDLING VIGOR PARAMETERS AT EACH AGING PERIOD

Correlation between pairs germination loss and seedling vigor parameters at each and across aging period showed that negative and significant association existed between pair of germination loss and seedling vigor parameters at 6 hours, 12 hours, 24 hours, 6 months and across aging period (-0.96** to -0.52*) (Table 7) Germination index (GI) correlated positively and significantly with germination growth index (GRI), coefficient of velocity of germination (CVG) and seedling vigor index (SVI), mostly at 12 hours, 24 hours, 6 months and across aging period (0.61* to 0.99**). Also, positive and significant relationship was recorded between pair of GRI with CVG and SVI, especially at 6 hours, 12 hours, 24 hours, 6 months and across aging period (0.50* to 0.99**). Similarly, CVG correlated significantly and positively with

Table 7: Pearson correlation between pairs of aging period and germination loss and seedling vigour parameters

	G loss %	GI	GRI	CVG	SVI
			Before		
Gloss	-	-	-	-	-
GI		-	0.93**	0.43	0.39
GRI			-	0.72**	0.29
CVG				-	-0.06
SVI					-
		6 hours			
	G loss	GI	GRI	CVG	SVI
Gloss	-	-0.52*	-0.88**	-0.79**	-0.86**
GI		-	0.47	0.61*	0.38
GRI			-	0.81**	0.96**
CVG				-	0.73**
SVI					-
		12 hours			
Gloss	-	-0.86**	-0.85**	-0.65**	-0.81**
GI		-	0.98**	0.72**	0.92**
GRI			-	0.79**	0.90**
CVG				-	0.61*
SVI					-
		24 hours			
Gloss	-	-0.96**	-0.95**	-0.68**	-0.92**
GI		-	0.99**	0.72**	0.96**
GRI			-	0.72**	0.96**
CVG				-	0.68*
SVI					-
		6 months			
	G loss	GI	GRI	CVG	SVI
Gloss	-	-0.85**	-0.96**	-0.42	-0.96**
GI		-	0.93**	0.51*	0.94**
GRI			-	0.50*	0.99**
CVG				-	0.52*
SVI					-
			Across		
Gloss	-	-0.80**	-0.89**	-0.88**	-0.89**
GI		-	0.85**	0.81**	0.82**
GRI			-	0.99**	0.97**
CVG				-	0.97**
SVI					-

* , **significant at p = 0.05 and p = 0.01, respectively
G loss: Germination loss; GI: Growth index; GRI: Growth rate index; CVG: Coefficient of velocity of germination; SVI: Seedling vigor index

SVI at 6 hours, 12 hours, 24 hours, 6 months and across aging period (0.52* to 0.97**).

3.4 CANDIDATE SSR MARKERS FOR DETECTION OF STORAGE STRESS TOLERANCE ALLELES IN SOYBEAN

3.4.1 Diversity studies

Eighteen out of the nineteen SSR primers (94.74 %) used in this study were polymorphic and were able to detect SSR markers linked with alleles associated with storage stress tolerance among soybean varieties. The polymorphic markers produced a total of 72 alleles in the 15 soybean varieties. The alleles ranged from two to seven per locus, with an average of four alleles per primer. Primers (Satt 389 and Satt 600) had the highest number of alleles (seven), and the rests had two to five alleles. Principal information content (PIC) values for the SSR markers ranged from 0.28 (SATT 160) to 0.81 (SATT 389) with an average of 0.57 (Table 8).

Five SSR markers (Satt175, Satt 600, Satt 190, Satt 565 and Satt 160) clearly discriminated the soybean varieties into tolerant and susceptible varieties. Satt 600 had two alleles (Satt 600_{100} and Satt 600_{140}) that linked with alleles in one of the tolerant variety (TGX1835-10E). Also, some other SSR primers like Satt 285 (Satt 285_{160} and Satt 285_{200}) were associated with alleles responsible for susceptibility to storage stress.

3.4.2 Genetic diversity of soybean varieties based on SSR molecular markers

Genetic distance among the 15 soybean varieties ranged from 0.069 to 0.514 in (TGX1989-75FN, and TGX1990-114FN) to (TGX1989-49FN and TGX1440-1E) respectively with a mean value of 0.265 (Table 9). The dendrogram grouped the soybean varieties into two main groups (A and B). The first main cluster (A) comprised 3 varieties (TGX1440-1E, TGX1448 and TGX1740) while the second main cluster (B) comprised twelve varieties, which was sub-divided into four sub-groups sub-cluster 2 had only one variety (TGX 1835-10E). Sub-cluster 3 comprised four varieties (TGX1190-52F, TGX1989-48FN, TGX1989-49FN and TGX1990-46F). Sub-cluster 4 had three varieties (TGX1990-46F, TGX1987-19F, TGX1990-78F) and sub-cluster 5 consisted of four vari-

Table 8: Major alleles and polymorphism information content of eighteen SSR markers tested on fifteen soybean varieties

S/No	Primer	MAF	Na	Na_{ave}	GD	He	MP	PP	%P	PIC	I
1	SATT423	0.40	4.00	2.07	0.66	0.80	0	4	100.00	0.59	-0.18
2	SATT414	0.50	3.00	2.40	0.62	1.00	2	1	33.33	0.55	-0.59
3	SATT434	0.54	3.00	2.47	0.57	0.93	0	3	100.00	0.49	-0.59
4	SATT285	0.50	3.00	3.00	0.50	1.00	3	0	0.00	0.38	-1.00
5	SATT154	0.38	3.00	1.47	0.66	0.69	0	3	100.00	0.59	-0.01
6	SATT002	0.50	3.00	2.20	0.58	1.00	2	1	33.33	0.49	-0.71
7	SATT160	0.83	4.00	1.07	0.30	0.25	0	4	100.00	0.28	0.20
8	SATT565	0.43	5.00	3.00	0.62	1.00	0	4	100.00	0.54	-0.60
9	SATT281	0.37	6.00	4.33	0.76	1.00	0	6	100.00	0.72	-0.29
10	SATT233	0.46	3.00	2.47	0.63	0.93	0	3	100.00	0.55	-0.46
11	SATT285	0.50	3.00	2.60	0.53	0.93	0	3	100.00	0.42	-0.72
12	SATT142	0.50	2.00	1.73	0.50	1.00	3	0	0.00	0.38	-1.00
13	SATT545	0.50	3.00	2.47	0.62	1.00	2	1	33.33	0.55	-0.58
14	SATT389	0.23	7.00	2.73	0.83	0.92	0	7	100.00	0.81	-0.07
15	SATT431	0.46	5.00	2.00	0.67	1.00	0	5	100.00	2.00	-0.46
16	SATT354	0.50	2.00	1.87	0.50	1.00	2	0	0.00	0.38	-1.00
17	SAAT175	0.40	6.00	2.07	0.69	1.00	0	6	100.00	0.64	-0.42
18	SATT600	0.33	7.00	1.80	0.76	0.67	0	7	100.00	0.73	0.16

MAF: major allele frequency; Na: number of observed alleles; Naave: average number of alleles; polymorphic alleles; % P: percentage polymorphism; PIC: polymorphic information content; I: inbreeding coefficient, He: heritability, MP: percentage monomorphism

Table 9: Distance indices among fifteen soybean varieties based on DNA analysis

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
V1	-	0.111	0.153	0.375	0.292	0.194	0.236	0.181	0.167	0.139	0.181	0.333	0.306	0.222	0.292
V2		-	0.125	0.403	0.264	0.25	0.208	0.153	0.194	0.194	0.236	0.333	0.361	0.278	0.347
V3			-	0.361	0.278	0.236	0.194	0.167	0.125	0.208	0.222	0.236	0.347	0.292	0.278
V4				-	0.389	0.403	0.389	0.389	0.403	0.375	0.361	0.375	0.514	0.347	0.472
V5					-	0.292	0.194	0.222	0.319	0.236	0.25	0.347	0.264	0.292	0.333
V6						-	0.236	0.236	0.222	0.194	0.181	0.333	0.389	0.278	0.347
V7							-	0.167	0.208	0.181	0.194	0.236	0.236	0.264	0.25
V8								-	0.208	0.125	0.194	0.292	0.375	0.292	0.389
V9									-	0.111	0.125	0.167	0.361	0.25	0.264
V10										-	0.069	0.222	0.306	0.25	0.319
V11											-	0.181	0.292	0.264	0.306
V12												-	0.333	0.361	0.347
V13													-	0.306	0.208
V14														-	0.236
V15															-

V1: TGX1990-52F; V2: TGX1989-48FN; V3: TGX1989-49FN; V4: TGX1835-10E; V5: TGX1990-46F; V6: TGX1990-46F; V7: TGX1987-19F; V8: TGX1990-78F; V9: TGX1989-53FN; V10: TGX1989-75FN; V11: TGX1990-114FN; V12: TGX1990-110FN; V13: TGX1440-1E ; V14: TGX1448-2E; V15: TGX1740-1F

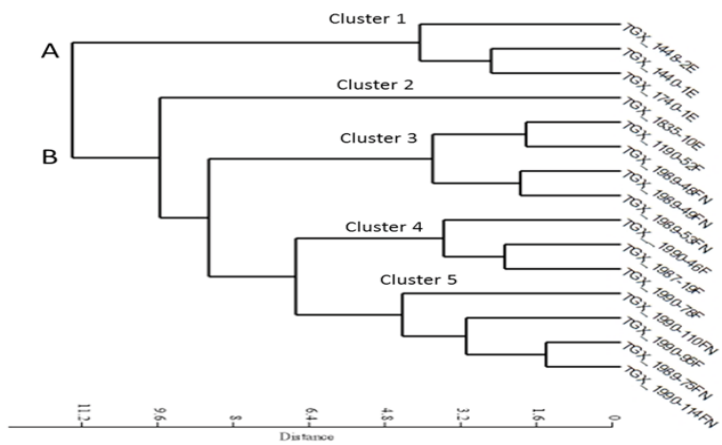

Figure 3: Dendrogram of 15 soybean varieties based on their genetic component identified using SSR markers

eties (TGX1990-110FN, TGX1990-95F, TGX1989-75FN, TGX1990-114FN) (Figure 3).

4 DISCUSSION

Seed storability is reported by Clerkx et al. (2004) to be a complex trait affected by environmental factors during seed formation, harvest and storage, and is usually controlled by several genes. The major storage stresses that seeds are exposed to are relative humidity and temperature. High germination loss and low seedling vigor parameters (GI, GRI, CVG and SVI) are indicators of low tolerance to storage stress imposed through accelerated aging and the ambient environment. Kehinde et al. (2013) found out that seed scientists have employed seeds viability and seedling vigor index to assess seed quality and the declined in these parameters have been associated with seed deterioration during storage. The significant variation observed in germination and other seedling vigor characteristics of the soybean varieties in

this study can be attributed to the genetic makeup of the soybean varieties.

There were variations in the rate of deterioration of soybean varieties used in this study. This variation increased as the ageing duration increased. Similar result was reported by Jagadish et al. (2013) in the aging of forty soybean genotypes. This implies that seed deterioration of soybean is inevitable; however, varieties differed in their responses to aging factor that had been imposed on the seed due to storage stress. Also, it could be deduced that the significant variation observed in germination and other seedling vigor parameters under artificial aging treatments and ambient storage is due to different genetic makeup of the soybean varieties. Therefore, the diverse genetic constituent of the soybean varieties makes selection for storage stress tolerance possible. Adebisi et al. (2004) reported that seed quality and longevity performance are components of genetic make-up in soybean. Germination rate index and germination index accounted for the major factor to classify the varieties based on their tolerance to storage stress. This suggests that germination percentage is not the only factor that determines seed quality; therefore attention should be paid to these seedling vigor characters.

Simple correlation analysis has been considered adequate as a rough guide to the magnitude and direction of the relationships between two traits (Adebisi et al., 2010). High magnitude of coefficient of correlation (r) obtained among pairs of seedling vigor index at 6 hours, 12 hours, 24 hours, 6 months and across aging period, shows a strong association among the parameters and each parameter could be used to improve seedling vigor in cowpea.This corroborates with the findings of Brown and Caligari (2008) who reported that high and positive association between characters suggests that each of two parameters pairs could be controlled by closely linked genes, same or similar genes or by genes with pleiotropic effects on these parameters. However, negative and significant association was obtained between pair of germination loss and seedling vigor parameters at 6 hours, 12 hours, 24 hours, 6 months and across aging period. This implies that lower percentage germination loss leads to higher seedling growth parameters.

The clustering pattern of SSR markers agrees with the patterns obtained from accelerating aging imposed storage stress as regards grouping of susceptible soybean varieties to storage stress. Highly susceptible varieties (TGX 1990-95F and TGX 1989-75FN) were clustered together in all the trials, therefore this information confirms that these varieties will require improvement, if their cultivation will continue. SSR markers (Satt 600, Satt 285, Satt 175, Satt 190, Satt 565 and Satt 160) clearly distinguished the varieties into tolerant and susceptible varieties. These markers could have close linkage with good storability. SSR primers that were specifically associated with tolerance alleles (Satt 600) and susceptible alleles (Satt 285, Satt 281) identified were considered as good candidate markers for screening soybean germplasm for the identification of tolerance and susceptible genotypes in soybean breeding programmes. The results corroborates the work of Jagadish et al. (2013), Singh et al. (2008) and Dargahi et al. (2014) that identified same SSR markers to have linkage with storability traits in soybean. Hence, these markers are considered good candidate molecular markers for identifying alleles linked with tolerance or susceptibility to storage stress in soybean.

5 CONCLUSION

Soybean varieties differ in their response to storage stress. 'TGX1835-10E' and 'TGX1448-2E' were regarded as tolerant soybean varieties to storage stress as evident in the low germination loss recorded after subjecting them to accelerated ageing procedure. This is attributed to inherent genetic potentials to withstand storage stress. SSR markers Satt 565, Satt 175, Satt 281, Satt 600, Satt 160 and Satt 281 associated with storage stress in soybean seed. These candidate markers can be further studied on large soybean varieties to confirm their linkage with tolerant genes. This will be of immense uses to ascertaining their ability to soybean germplasm screening for seed storage tolerance breeding programs.

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