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Multi-seasons evaluation of Spanish bunch advanced breeding lines for fresh seed dormancy in groundnut (*Arachis hypogaea* L.)

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Abstract

A study was conducted to identify sources of highly stable fresh seed dormancy with Spanish descent in groundnut. Pooled analysis of variance revealed highly significant differences between genotypes and genotype environment interactions for fresh seed dormancy at weekly intervals, suggesting differential behavior of genotypes for fresh seed dormancy in response to environmental conditions. Based on duration of dormancy, four advanced breeding lines (PBS 16023, PBS 15014, PBS 14064, and PBS 11077) were identified as new sources with fresh seed dormancy of 21 days in Spanish background. These genotypes can be used in regions where in-situ germination is problematic. In addition, they can be incorporated into local genetic improvement to develop high-yielding genotypes with two to three weeks of fresh seed dormancy to prevent losses caused by in-situ germination.

Key words: Groundnut, Stability, Fresh seed dormancy, Spanish, GGE biplot

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop farmed in 117 countries with varying agro-climatic conditions between latitudes 40°N and 40°S. It is grown on 30.4 million hectares of land worldwide, with a total production of 51.5 million tons and a productivity of 1694 kg/ha from 2018 to 2020 (triennial average) (FAO, 2022). After China, India has the second largest acreage and production of groundnuts. It is grown on around 6.09 million ha of land in India, with production and productivity of 10.21 million tons and 1676 kg/ha (DES 2021). Groundnut productivity in India is relatively low due to various biotic and abiotic factors. Under rainfed production system Spanish (subsp. *fastigiata* var. *vulgaris*) types are most preferred owing to their short life cycle and easy harvesting, but they lack fresh seed dormancy (Kulheri and Sikarwar, 2019). Lack of seed dormancy is a serious issue in Spanish bunch types, leading to 20-50% reduction in yield due to in-situ germination caused by erratic rains during crop maturity (Kumar *et al*., 2019). To avoid crop losses under such conditions, at least 2-3 weeks of fresh seed dormancy would be required.

Seed dormancy is the failure or delayed germination of mature and viable seed under germination-friendly conditions. In the most basic terms, it refers to the nongermination or low germination of freshly collected seeds. It is a stage of seed growth or physiological activity that has been halted. It is an adaptive feature that permits the seed to remain viable for an extended period

and to survive in difficult climatic and seasonal settings (Patro and Ray, 2016). Dormancy is advantageous because it prevents preharvest sprouting of seeds in the standing crop when rain falls during the maturity stage.

Phenotypes are the manifestations of Genotype (G), Environment (E), and their interactions. The most important sources of variation for quantitative traits are the interaction of genotype with environment (GEI). Significant year-to-year variations in fresh seed dormancy have been observed due to genotypic differences, and GEI is most common because of the differential expression of genotypes across environments, which may complicate the selection process of a genotype for a target trait. In such cases, stability analysis provides an excellent solution for the relative performance of genotypes across seasons. Plant breeders commonly apply multi-environment trials (METs) to examine the relative performance of genotypes across environments, and such MET data is subjected to AMMI or GGE biplot analysis to determine the best adapted genotype (Kumar *et al*., 2019).

The GGE biplot stresses the notion that G and GE are the two sources of variation that are essential for genotype evaluation and must be addressed concurrently to identify optimal genotype across test conditions (Yan and Tinker, 2006). GGE biplot analysis is a data imaging method that depicts a GE interaction in a two-way table graphically. It is a very effective technique for mega environment analysis (which genotypes won where), in which specific genotypes can be recommended to specific mega environments (Yan and Tinker, 2006), genotype evaluation (mean performance vs. stability), and environmental evaluation (power to discriminate among genotypes in target environments). GGE biplot analysis is an effective approach for comprehensively exploring multi-environment trials that is based on principal component analysis (PCA). It enables visual analysis of the correlations between test settings, genotypes, and genotype-by-environment interactions. As a result, the current study was carried out to examine genotype-environment interactions (GEI) on the intensity of fresh seed dormancy and to identify the most stable groundnut genotypes with 2-3 weeks of fresh seed dormancy in a Spanish background.

Materials and method

Fourteen advanced breeding lines were evaluated for fresh seed dormancy for four seasons *viz.,Kharif* 2019, *Kharif* 2020, Summer 2020 and Summer 2021 along with four released varieties *viz*., TAG 24, Dh 86, Girnar 3 and TG 37A as checks. These genotypes were harvested at maturity as indicated by blackening of inner parenchyma of the pod (Miller and Burns, 1971). To study fresh seed dormancy, a sample of mature pods were randomly selected and shelled immediately after harvesting of groundnut and precaution was taken to prevent any damage of the testa, cotyledons and embryo while removing seeds from pods. The mean pod moisture content of summer harvested produce was 38 % and *kharif* season harvested pods had 32 % moisture content. Evaluation for fresh seed dormancy was conducted under both laboratory and field conditions.

To assess fresh seed dormancy in laboratory, freshly harvested pods were shelled and mature seeds from test genotypes were tested. The seeds were kept on germination paper in petri plates and regularly watered. The data on number of seeds germinated were recorded at weekly intervals for up to three weeks. The seeds were surface sterilized before keeping them for germination to prevent possible fungal infections. When seeds don't germinate, they tend to decay after a week. Therefore, after first and second week, new set of seeds were kept for germination from the same seed lot. Percent germination was calculated and the genotypes which showed dormancy for a period of two weeks or more were selected.

To further validate the laboratory tests, freshly harvested pods were used for germination tests. Seeds from fresh pods were treated with carbendazim (3g/kg of seeds) fungicides to protect from soil-borne diseases. A total of eighteen groundnut genotypes were evaluated at ICAR-Directorate of Groundnut Research, Junagadh, Gujarat, India (Lat. 21°31' N, long. 70°36' E) in medium black calcareous soil. The data of maximum and minimum temperature (^{0}C), relative humidity (%) and solar radiation (W/m^2) during

the observation period was recorded. The experiment was laid out in a randomized complete block design with three replications. Each replication consisted of 50 freshly harvested seeds sown at 2 to 3 cm deep for each genotype. The seeds of each genotype were sown at 45 cm spacing between rows and 10 cm between plants. The soil moisture was maintained at field capacity during the growth period up to 35 days after sowing (DAS). The observations were recorded on number of seeds germinated at weekly interval until the end of experiment.

The percentage of germinated seeds for each entry at a given date was calculated by the following formula: Germination (%) = Number of germinated seeds*100/Total number of seeds sown. Fresh seed dormancy is characterized by its duration and intensity. These two parameters were studied in the present investigation for all the genotypes.

Duration of fresh seed dormancy was measured by days taken to attend 50 per cent germination by a genotype and intensity of fresh seed dormancy was measured as percentage of non-germinated seed at each date of observation. These parameters were estimated using the method suggested by Kumar *et al.,*(1991). Degree of dormancy was classified according to the scale devised by Landfort *et al.,*(1965).

To confirm the existence of genetic differences among genotypes and to determine the significance of the main effects and interactions effects an initial pooled analysis of variance was performed using DSAASTAT software. Genetic components *viz.,* coefficient of variation (CV), and heritability (h^2) were also estimated from data obtained from the analysis of variance. The data were also graphically analyzed to interpret the G × E interaction using the GGE biplot software. GGE-biplot analysis was performed in R (R core team, 2021).

Singular value decomposition (SVD) of the first two principal components was used to fit the GGE biplot model (YAN, 2002),

$$Y_{ij} = \mu + \beta_j + \varepsilon_1 \hat{\imath}_{i1} \varsigma_{j1} + \lambda_2 \hat{\imath}_{i2} \varsigma_{j2} + \varepsilon_{ij}$$

where, Y_{ij} is the trait mean for genotype *i* in environment *j*, $\grave{\imath}$ is the grand mean, $\hat{a}j$ is the main effect of environment *j*, $\grave{\imath} + \hat{a}_j$ being the mean yield across all genotypes in environment *j*, $\ddot{e}_1$and $\ddot{e}_2$ are the singular values (SV) for the first and second principal components (PC_1 and PC_2), respectively, $\hat{\imath}_{i1}$ and $\hat{\imath}_{i2}$ are eigenvectors of genotype *i* for PC_1 and PC_2, respectively, $\varsigma_{j1}$and ς_{j2} are eigenvectors of environment *j* for PC_1 and PC_2, respectively, $\mathring{a}_{ij}$ is the residual associated with genotype *i* in environment *j*. In GGE biplot analysis, scores of PC_1were plotted against PC_2 (Yan and Tinker, 2006). GGE explains two most important sources of variation *i.e*., genotype main effect (G) and genotype × environment (GE) interaction effect. The GGE analysis was considered satisfactory as the first two principal components (PCA1 and PCA2) of the GGE explained most of the variation prevailed. GGE biplot is perfectly suited for analysis involving multiple environments which is based on genetic correlation between environment and the which-won-where pattern; evaluation of environment based on discriminating ability and representativeness; and evaluation of genotype based on mean performance and stability across environments. The GGE biplot graphically displays G + GE of the MET data in a way that facilitates visual variety evaluation and mega-environment identification (YAN *et al*., 2007). The GGE biplot analyses were performed using package "GGEBiplotGUI" (in R statistical software, version 3.4.1 (R core team, 2021).

Results and discussions

Intensity of fresh seed dormancy

Pooled analysis of variance for germination per cent at weekly intervals revealed highly significant genotypic differences for fresh seed dormancy followed by genotype × environment interactions and environment had the least influence. Large sum of squares for genotype showed that there was sufficient genetic variability among all the genotypes for intensity of dormancy at different weekly intervals (Table 1). The higher genotypic variation relative to environmental counterpart is also consistent with the high autogamous nature of groundnut (Nath and Alam, 2002). Significant effects of genotype found in this study agree with other authors (Kumar *et al*. 2017, 2018a,b). Analysis of variance for intensity of dormancy indicated that 83.65 to 88% of the total sum

Table 1. Pooled analysis of variance for germination percentage at weekly intervals averaged over four seasons viz., *Kharif* 2019, *Kharif* 2020, Summer 2020, Summer 2021

Source of Variation	df	7 DAS		14 DAS		21 DAS	
		MSS	% ss	MSS	% ss	MSS	% ss
Rep	1	206.05	0.18	44.28	0.03	131.05	0.09
Environment (E)	3	160.71**	0.43	263.66*	0.60	158.81	0.32
Genotype (G)	16	5915.88**	83.65	7191.71**	88.00	7787.69**	82.72
E*G	48	274.56**	11.65	204.41**	7.50	373.09**	11.89
Residual	67	69.21	4.10	75.28	3.86	112.10	4.99
Total	135	838.21		968.58		1115.78	
CV		9.57		10.22		13.02	

*Significance at P< 0.05 level, **Significance at P< 0.01 level

of squares was attributable to the genotypes (G), 7.5 to 11.9% of total variance attributed to GEI and less than 1% of total variance was attributed to environmental variances. The small proportion of environment indicated least environmental influence on intensity of dormancy. However, magnitude of GEI was very much smaller than that for the genotype SS, it is indicating that the differences in the response of the genotypes across environments were not much large. Present findings agree with the earlier works (Kumar *et al*., 2017, 2018a, b)

Intensity of dormancy is defined as the percentage of seeds not germinated even after specified period after the harvest (Kumar *et al*., 1991). From practical point of view, high intensity of dormancy (>90 %) for 2-3 weeks duration is very important (Kumar *et al*., 2017). The grand mean values for intensity of dormancy ranged from 9.4% in TG 37A to 100 % in PBS 15014, PBS 14064, PBS 16023, PBS 11077, PBS 14060, PBS 14068, PBS 15028, and PBS 16044 over the seasons during 2019 to 2021 at 7 DAS. At 14 DAS intensity of dormancy ranged from 3.1 (TG 37A) to 100% (PBS 14060, PBS 14064, PBS 15014, PBS 16023, PBS 16044, and PBS 11077). Further from pooled mean data at 21 DAS revealed PBS 15014, PBS 11077, and PBS 16023 (100 %) and PBS 14064 (99.2 %) with highest intensity of dormancy and TG 37A (3.1%) with the least (Table 2). Results revealed that three advanced breeding lines PBS 15014, PBS 11077 and PBS 16023 had an average 100% intensity of fresh seed dormancy followed by two advanced breeding lines PBS 14064 (99.2 %) and PBS 15028 (99.1%). Three commonly cultivated varieties *viz.* Dh 86, TG 37A and TAG24 had dormancy of 11.5, 3.1 and 37.3 respectively at 21 DAS. This large variation in intensity of dormancy could be due to genotypic differences among the genotypes and environmental factors which affect dormancy by their effect on mother plant and seeds during storage. These findings agree with the results of several researches published earlier (Kumar *et al*., 1991; Faye *et al*., 2009; Naganagoudar *et al*., 2015; Kumar *et al*., 2017; 2018a,b).

Table 2. Average intensity of fresh seed dormancy of 14 advanced breeding lines along with check varieties across the seasons

SN	Genotypes	Intensity of Dormancy (%)		
		7 DAS	14 DAS	21 DAS
1.	Dh 86	24.8	14.0	11.5
2.	PBS 11092	97.9	97.9	95.4
3.	PBS 14060	100.0	100.0	96.7
4.	PBS 14064	100.0	100.0	99.2
5.	PBS 14068	100.0	98.8	97.3
6.	PBS 15014	100.0	100.0	100.0
7.	PBS 15022	99.4	98.8	98.8
8.	PBS 15027	97.5	97.5	95.0
9.	PBS 15028	100.0	99.2	99.1
10.	PBS 15056	94.6	91.1	79.0
11.	PBS 16022	97.5	97.5	95.8
12.	PBS 16023	100.0	100.0	100.0
13.	PBS 16033	92.5	91.3	85.8
14.	PBS 16044	100.0	100.0	94.6
15.	PBS11077	100.0	100.0	100.0
16.	TAG 24	72.5	63.4	37.3
17.	TG 37 A	9.4	3.1	3.1
18.	Girnar3	97	96	91

DAS – Days after sowing

GGE biplot analysis

Biplot is a 2D visualization matrix that has two axes, first data was centered afterward

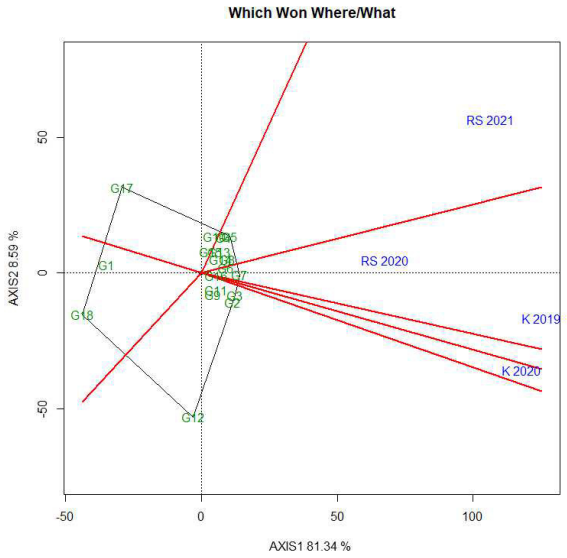

Fig. 1. The ''Which-Won-Where'' GGE biplot for groundnut genotypes evaluated for intensity of FSD in four environments.

Genotype code G1: Dh 86, G2: Girnar 3, G3: PBS11077, G4: PBS 11092, G5: PBS 14060, G6: PBS 14064, G7: PBS 14068, G8: PBS 15014, G9: PBS 15022, G10: PBS 15027, G11: PBS 15028, G12: PBS 15056: G13: PBS 16022, G14: PBS 16023, G15: PBS 16033, G 16: PBS 16044, G17: TAG 24, G18: TG 37A

sectionalizing the singular value (SV) into GE scores for individual principal components *viz.*, PC1 and PC2 followed by intriguing the PC1 scores contrary to the PC2 scores to create a biplot. The greater PC1 value indicates greater yielding ability whereas the lower PC2 value signifies stability. Three major components can be elucidated using the biplot such as (a) 'which-won-where' pattern or MET, proposed by Yan *et al*. (2000) is an effective approach to visualize the pattern of GEI based on the correlation between G and E; (b) stability *vs* mean performance over the environment for genotype evaluation; (c) ranking of genotypes. The main effect of genotype (G) plus G × E interactions is the principal source of variation in the assessment of the genotype's performance under multi-season trials.

Mean performance and stability of eighteen genotypes of groundnut was analyzed by GGE biplot. Cumulative variance of first principal component (PC1) and the second interaction principal component (PC2) respectively clarified 81.34 % and 8.59 % for intensity of FSD. So, in the present study, GGE analysis was considered satisfactory as the first two principal components of the GGE explained highest (89.83 %) intensity of FSD. The greater PC1 value (81.34 %) indicates greater dormancy whereas the lower PC2 value (8.59 %) signifies stability.

The Fig. 1 present a polygon view of spanish bunch groundnut genotypes evaluated for fresh seed dormang at 21 DAS. Accordingly except PBS 15056, other advanced lines were best performing genotypes for FSD.

A biplot is made up of an asymmetrical polygon with stripes or lines running vertically from the biplot's center to the polygon at a right angle. All the genotypes that are apart from the biplot center are linked with the polygon thus covering all genotypes in the polygon marker. The vertical stripe that runs perpendicular to the polygon from the center of the biplot represents an expected environment in which the two genotypes on opposite sides of the polygon are expected to behave similarly. Furthermore, it divides the biplot into different parts, each with its own enticing or winning genotypes (Oladosu *et al*., 2017). The genotype should have a high IPCA1 score and IPCA2 score close to zero (more stable) (Yan, 2001; Yan and Tinker, 2006). The axis of average environment coordination (AEC) abscissa is a single arrowed line that passes through the biplot origin with an arrow indicating to the direction of the best performing genotypes (highest yield). The axis of AEC ordinate is the line that passes through the biplot origin and is perpendicular to the AEC abscissa. The AEC ordinate approximates the genotypes contribution to the G×E interaction indicating that more the closest genotype to the AEC abscissa, the more is consistent or stable in the test environments (Yan, 2001; Yan and Tinker, 2006). Accordingly, the GGE biplot of genotype-focused scaling for fresh seed dormancy showed clustering of genotypes except Dh 86, TAG 24, TG 37A, and PBS 15056. Among these, PBS 15056 had good dormancy intensity but was unstable across seasons and Dh86 though stable across seasons had poor intensity of dormancy. Genotypes PBS 11077, PBS 16023, PBS 15014, and PBS 14064 with high intensity of dormancy were also stable across seasons (Fig. 2).

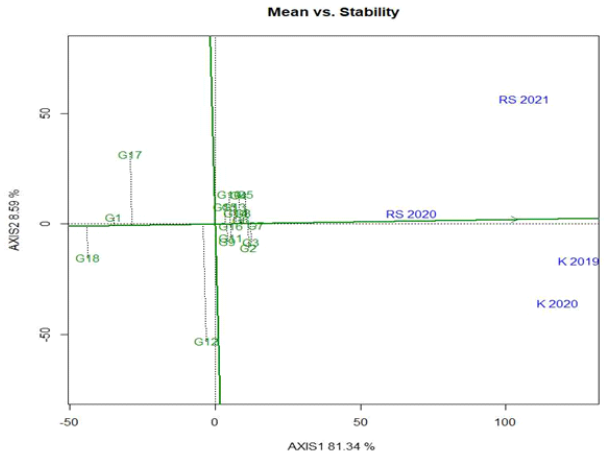

Fig 2. The 'Mean vs. Stability' pattern of GGE biplot illustrating interaction effect of 18 genotypes under four seasons for intensity of FSD. The biplots were created based on Centering = 0, SVP = 2, Scaling = 0.

Genotype code G1: Dh 86, G2: Girnar 3, G3: PBS 11077, G4: PBS 11092, G5: PBS 14060, G6: PBS 14064, G7: PBS 14068, G8: PBS 15014, G9: PBS 15022, G10: PBS 15027, G11: PBS 15028, G12: PBS 15056: G13: PBS 16022, G14: PBS 16023, G15: PBS 16033, G 16: PBS 16044, G17: TAG 24, G18: TG 37 A

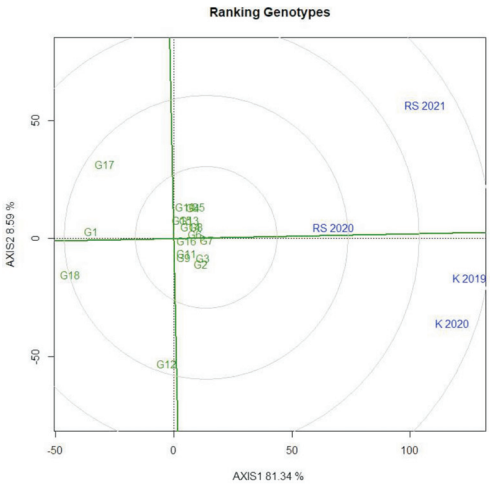

Fig 3. The GGE biplot 'genotypes ranking' pattern for genotype comparison with ideal genotype showing G + G × E interaction effect of 18 genotypes under four seasons for intensity of FSD. The biplots were created based on Centering = 0, SVP = 2, Scaling = 0.

Genotype code G1: Dh 86, G2: Girnar 3, G3: PBS11077, G4: PBS 11092, G5: PBS 14060, G6: PBS 14064, G7: PBS 14068, G8: PBS 15014, G9: PBS 15022, G10:PBS 15027, G11: PBS 15028, G12: PBS 15056: G13: PBS 16022, G14: PBS 16023, G15: PBS 16033, G 16: PBS 16044, G17: TAG 24, G18: TG 37 A

Through the genotype ranking biplot (Fig. 3) we can detect an ideal genotype in contrast to other genotypes evaluated.Majority of genotypes except Dh 86, TAG 24, TG 37A, and PBS 15056 could be noted as the best ideal genotype for fresh seed dormancy. Commonly, an ideal genotype is always placed into the innermost circle and relatively nearer the head of the arrow at the center of the circular ring. The genotype located in the inner circle is highly desirable compared to the genotypes of the outer circle. However, in some cases when no genotype was positioned inside the inner circle, consequently, genotypes next closer to the inner circle are an ideal one. Consequently, genotypes were regarded as ideal genotypes across the tested environment if they were positioned closer to the center of the biplot origin, indicating that they are stable genotypes. For an effective selection, an ideal genotype should have both high mean and stability properties.

Conclusion

Genotypes PBS 16023, PBS 15014, PBS 14064, and PBS 11077 had high intensity of dormancy at 21 DAS and were stable across environments. These newly identified sources for fresh seed dormancy under spanish background can be used in areas were in-situ germination is problematic during harvest period. In addition, they can be integrated into local breeding programs to develop high yielding genotypes with 2-3 weeks of fresh seed dormancy to avoid losses due to in-situ germination.

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