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Identification of stable sources for low phosphorus conditions from groundnut (*Arachis hypogaea* L.) germplasm accessions using GGE biplot analysis

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Abstract

Groundnut genotypes consisting of 83 Spanish and 76 Virginia germplasm accessions were evaluated under treatments having with and without phosphorus (P) application for two consecutive years and analysed using GGE-biplot analysis. The first two principal components (PC1 and PC2) of GGE-biplot explained 46.8% and 31.6% of variation respectively in Spanish and 59.1% and 27.3% of variation respectively in the Virginia group. Genotypes having PC1 scores >0 are adaptable and high yielding whereas those with PC1 scores <0 are non-adaptable and low yielding. Similarly, genotypes with PC2 scores near zero are stable and those with absolute larger PC2 scores are unstable. Treatment with phosphorus (MP) application was more discriminative while the treatment without P application was more representative and genotype NRCG-12296 (43) in Spanish and genotypes NRCG-11760 (9) and NRCG-14325 (27) in Virginia were ideal with high yield and stability across both with and without phosphorus (P) applications.

Keywords: Groundnut, germplasm, GGE-biplots, yield, stability

Introduction

The groundnut is a grain legume of the tropical world grown in about 25m ha area however its yield fluctuates from 300kg/ha to 1200 kg/ha (FAO 2015) depending upon the situation. The major factors affecting yield are climate, rainfall and soil fertility, particularly phosphorus (P) fertilizer. Most of the P supplied in the form of fertilizer gets converted into insoluble form and besides, there is imbalanced use of fertilizers particularly in dryland areas leading to reduced nutrient availability (Ajay et al. 2017) and low phosphorus availability in 30-40% of arable land (Runge-Metzger 1995). As a result of low nutrient availability yield in more than 50% of the groundnut growing countries of the world is less than 1000 kg/ha against the world average of 1650 kg/ha (FAO 2017). Hence, genotypes with the ability to acquire and use soil P more efficiently are desirable as they are economical, avoid soil-P depletion and stabilise yields (Singh and Basu 2005). This calls for screening groundnut genotypes for P use efficiency (Ajay et al. 2017) and screening groundnut germplasms under limited P availability conditions would provide stable sources for using them as donors in breeding programs.

Pod yield in groundnut is influenced by genotype (G) and environmental (E) main effects and genotype x environment interaction (GEI). The presence of GEI reduces the association between phenotype and genotype and influences the selection of genotype for the target environment. To exploit this GEI variation several stability measures have been proposed and the most widely used models being additive main effect and multiplicative interaction (AMMI) and GGE-biplot (Yan et al. 2000) models.

Evaluation of GEI among cultivars is performed through multi-season or multi-locational trials to identify high yielding genotypes for the target environment (Yan et al. 2001). In Multi-environment yield trials, the adaptability of genotypes is tested at several locations/environments. Identification of groundnut genotypes with an ability to grow and yield in low P as well as on medium P is an important goal in groundnut breeding

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(Singh et al. 2015).

GGE-biplot is a blend of two concepts, (i) GGE represents the main effect of genotype (G) and GEI influencing yield and (ii) graphical representation of GE by biplot technique of Gabriel (1971). GGE plots were drawn by subjecting yield variation to 'singular value decomposition (SVD) and then biplots are constructed using first and second principal components (PC1 and PC2). This is well suited for mega-environment analysis with packages such as Which-won-where plots, genotype evaluation via mean vs. stability plots and test environment evaluation (Amira et al. 2013). Taking advantage of this robust methodology field screening was undertaken to quantify the extent to which GEI influences pod yield of groundnut under with and without P application and to identify ideal genotype adapted to both the conditions.

Materials and methods

Field screening was conducted at ICAR-Directorate of Groundnut Research, Junagadh (lat 21°31'N, long 70°36'E), India during 2014 and 2015 summer seasons (February to May), in a medium black calcareous (17% $CaCO_3$) clayey, Vertic Ustochrept soil having moderately available phosphorus (15 kg/ha P), 7.5 pH, 0.7% organic C, 268 kg/ha N, 300-400 kg/ha K, 5 kg/ha available S and 1.6, 15, and 0.78 kg/ha DTPA extractable Fe, Mn, and Zn, respectively. A total of 83 Spanish and 76 Virginia groundnut germplasms were evaluated for two consecutive years under two levels of P i.e. without P application (LP) and with the application of 50 kg/ha P_2O_5 (as Di-ammonium phosphate) (MP) in Augmented design with SP-250A and ICGV 86590 as P use efficient checks (Ajay et al. 2014) and VRI 3 as P use inefficient check (Singh and Ajay 2013). A list of germplasm used in the study along with their codes are provided in Table 1 and

Table 1. Spanish and Virginia groundnut genotypes used in the study and their codes

	Spanish				Virginia		
Code	NRCG	Code	NRCG	Code	NRCG	Code	NRCG
1	101	43	12296	1	17	43	14376
2	168	44	12334	2	955	44	14378
3	201	45	12348	3	1913	45	14390
4	1086	46	12437	4	6064	46	14395
5	2538	47	12453	5	8956	47	14396
6	3491	48	12518	6	10191	48	14397
7	5007	49	12521	7	11679	49	14406
8	5360	50	12523	8	11693	50	14413
9	6236	51	12543	9	11760	51	14415
10	6331	52	12581	10	11942	52	14416
11	6937	53	12591	11	11981	53	14427
12	7175	54	12630	12	12049	54	14428
13	7249	55	12657	13	12065	55	14431
14	7306	56	12672	14	12109	56	14443
15	7443	57	12700	15	12138	57	14446
16	8428	58	12880	16	12174	58	14453
17	10388	59	12927	17	12177	59	14457
18	10496	60	14328	18	12297	60	14463
19	10541	61	14336	19	12393	61	14464
20	10572	62	14365	20	12423	62	14467
21	10807	63	14389	21	12431	63	14469
22	11088	64	14398	22	12478	64	14478
23	11126	65	14419	23	12713	65	14483
24	11154	66	14434	24	12732	66	14487
25	11157	67	14451	25	12858	67	14488
26	11175	68	14456	26	12968	68	14495
27	11179	69	14462	27	14325	69	14501
28	11228	70	14472	28	14327	70	11099
29	11236	71	14504	29	14330	71	12309
30	11275	72	7320	30	14331	72	12455
31	11289	73	12274	31	14338	73	12736
32	11551	74	12369	32	14340	74	12958
33	11651	75	12459	33	14342	75	12998
34	11874	76	12561	34	14347	76	14486
35	11990	77	12642	35	14354		
36	12069	78	12649	36	14355		
37	12148	79	12755	37	14356		
38	12256	80	12889	38	14358		
39	12272	81	12901	39	14359		
40	12273	82	12922	40	14371		
41	12290	83	13167	41	14372		
42	12294			42	14373		

further details about the qualitative and quantitative characters about these germplasm accessions could be obtained from the groundnut germplasm database (www.gnut.dgr.org. in). Nitrogen (as Urea) and potash (as muriat of potash) were applied at 50 kg/ha N and 60 kg/ha K_2O equally for both the treatments. The recommended crop management practices were adopted for raising a healthy crop. Pod yield recorded at maturity after drying the product under the sun for a week. GGE-biplot analysis was performed on pod yield separately for Spanish and Virginia groups using the 'GGEbiplotGUI' package (Bernal and Villardon 2016) in the R program (R core team 2018).

Results and discussion

Analysis of variance (ANOVA) was performed separately for Spanish and Virginia germplasm accessions in comparison to check genotypes for both the P treatments and are presented in Table 2. Significant differences were observed between checks for pod yield under both the P treatments for both Spanish and Virginia groups. Significant genotypic differences were also observed between germplasm accessions of both Spanish and Virginia groups for pod yield under both the P treatments. This indicates sufficient variability among genotypes of P use efficiency.

Table 2. Analysis of variance for pod yield in Spanish and Virginia groundnut genotypes under with (MP) and without (LP) P application during 2014 and 2015

Source	Df	LP14	LP15	MP14	MP15
Spanish					
Treatment (ignoring Blocks)	85	13.54 *	11.71 **	21.75 **	20.93 *
Check	2	10.35 *	7.76 **	33.28 **	146.18 **
Test genotype vs. Check	1	341.6 **	286.84 **	621.72 **	110.58 **
Test genotype	82	9.61 *	8.45 **	14.15 **	16.78 *
Block (eliminating Treatments)	2	3.24 ns	0.65 ns	2.02 ns	3.18 ns
Residuals	4	1.24	0.11	0.77	1.67
CV		10.19	4.59	8.03	11.73
Virginia					
Treatment (ignoring Blocks)	78	22.32 **	15.24 **	30.64 **	31.32 **
Check	2	10.35 *	7.76 **	28.13 **	146.18 **
Test genotype vs. Check	1	646.72 **	267.09 **	1018.08 **	140.52 **
Test genotype	75	14.32 *	12.09 **	17.54 **	26.8 **
Block (eliminating Treatments)	2	3.24 ns	0.65 ns	1.97 ns	3.18 ns
Residuals	4	1.24	0.11	0.77	1.67
CV		13.06	4.46	10.24	12.18

Both Spanish and Virginia groups differed with respect to pod yield under different environments. Spanish group had higher pod yield under MP15 and lowest under LP14 (Fig. 1) with a range of 1.5 (NRCG-14365) to 19.0 (NRCG-12296); 1.2 (NRCG-14365) to 16.7 (NRCG-12880); 3.0 (NRCG-14365) to 22.1 (NRCG-12069) and 1.7 (NRCG-14456) to 14.4 g/plant (NRCG-12296) under MP14, LP14, MP15 and LP15 respectively (Fig. 2). Among the Virginia group MP15 and LP14 had higher and lower average pod yield respectively (Fig. 1) with a range of 1.2 (NRCG-14396) to 18.8 (NRCG-14488); 1.6 (NRCG-14463) to 18.4 (NRCG-14478)' 2.7 (NRCG-14397) to 22.8 (NRCG-12423), 1.1 (NRCG-11981) to 15.4 g/plant (NRCG-14443) under MP14, LP14, MP15 and LP15 respectively (Fig. 3). Genotype NRCG-14478 from the Virginia group had a high pod yield when compared to all three check varieties under native P in both years. Among Spanish genotypes, NRCG-12880 and NRCG-12296 had high pod yield under LP14 and LP15 respectively. A decrease in pod yield was observed under low-P stress in both Spanish and Virginia groups (Figs. 2 and 3).

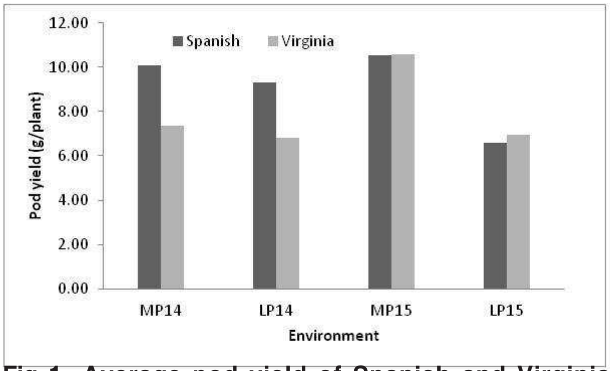

Fig. 1. **Average pod yield of Spanish and Virginia groundnut genotypes under different environments**

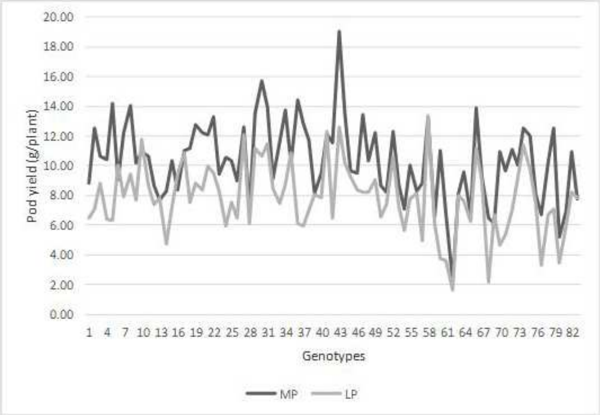

Fig. 2. **Average Pod yield (g/plant) among Spanish genotypes under with (MP) and without (LP) P application**

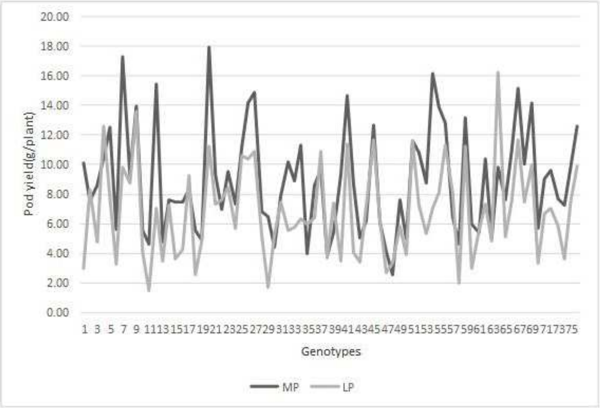

Fig. 3. **Average pod yield (g/plant) among Virginia genotypes under with (MP) and without (LP) P application**

Pod yield data from two P treatments over two years was subjected to singular value decomposition (SVD) and further GGE biplots were drawn using the first two interaction principal components (PC 1 and 2). GGE-biplot graphically explains genotype (G) main effects and genotype-environment interaction (GEI) effects. GGE-biplot is perfectly suited for analysis of data involving multiple environments (P treatments in the present study) with the help of packages such as which-won-where pattern; environmental evaluation using discriminating ability and representativeness; and genotypic evaluation using mean performance and stability across environments. The first two interaction principal components (PC1 and PC2) of GGE-biplot analysis explained 79 and 86% of total variation caused by G+GEI in Spanish and Virginia groups respectively and hence was considered satisfactory.

Which-won-where plot is depicted as a polygon and is helpful to estimate the presence of possible mega-environments (Yan and Tinker 2006). A polygon view of Spanish and Virginia groundnut genotypes respectively tested at four environments are presented in Fig. 4. In the Spanish group, all four environments i.e. two P treatments over two years fell into one sector whereas genotypes were grouped in seven sectors. Genotypes NRCG-12296, NRCG-12880, NRCG-11157, NRCG-14365, NRCG-14328 and NRCG-12069 were vortex genotypes and genotypes NRCG-12296 and NRCG-14434 were the winners in MP15 and LP 15 whereas genotype NRCG-12880 was the winner in MP14 and LP14 (Fig. 4). In the Virginia group, environments fell into two sectors whereas genotypes into seven sectors. Genotypes NRCG-12423, NRCG-14478, NRCG-12174, NRCG-14397, NRCG-14330, NRCG-14371 and NRCG-14347 formed the vortex genotypes and genotype NRCG-12423 was the winner in MP15 and LP 15 (Fig. 4) whereas genotype NRCG 14478 was the winner in LP14 and MP14.

Discriminative power vs representativeness of different environments (P treatments in the present study) was studied using GGE-biplot analysis separately for Spanish and Virginia groups and is presented in Fig. 5. Here, lengths of environmental vectors are proportional to the standard deviation of genotype yield in a corresponding treatment. Environments having long vectors classify genotypes more when compared to environments with very short vector (Yan et al. 2007). In the present study MP14 and MP15 are more discriminative compared to LP14 and LP15 in both Spanish (Fig. 5a) and Virginia (Fig. 5b) groups. The representativeness of environments is studied using the angle between environment vector and abscissa of average environment axis (dark thick line with an arrow). The smaller the angle, the more representative the test environment would be (Yan et

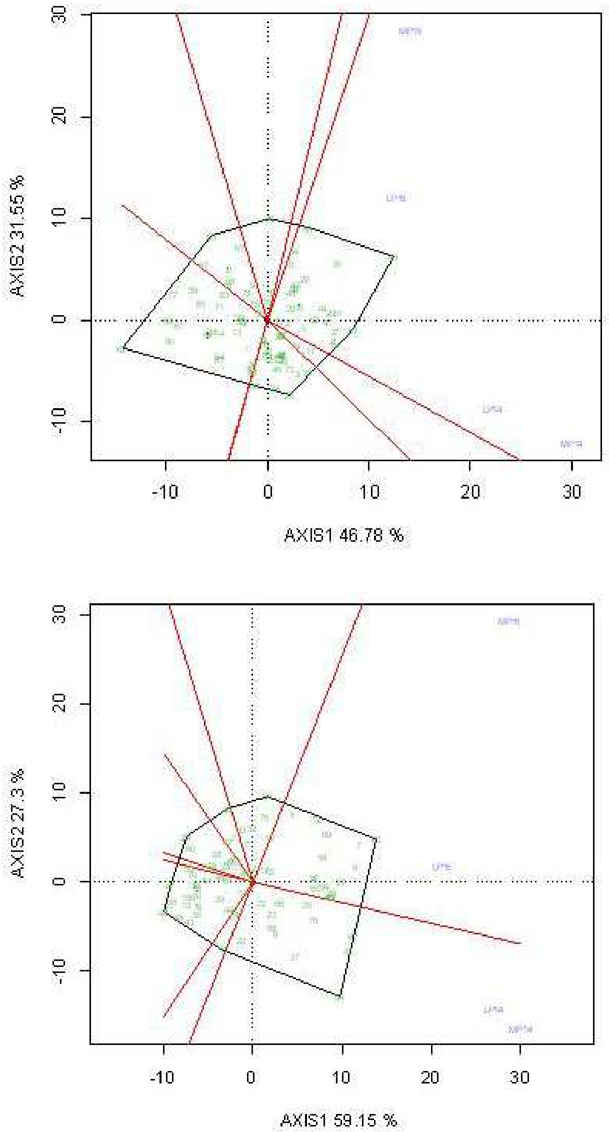

Fig. 4. **Which-won-where view of GGE biplot in Spanish and Virginia groundnut genotypes tested at four environments. LP14: without P application during 2014; LP15: Without P application during 2015; MP14: with P application during 2014; MP15: With P application during 2015**

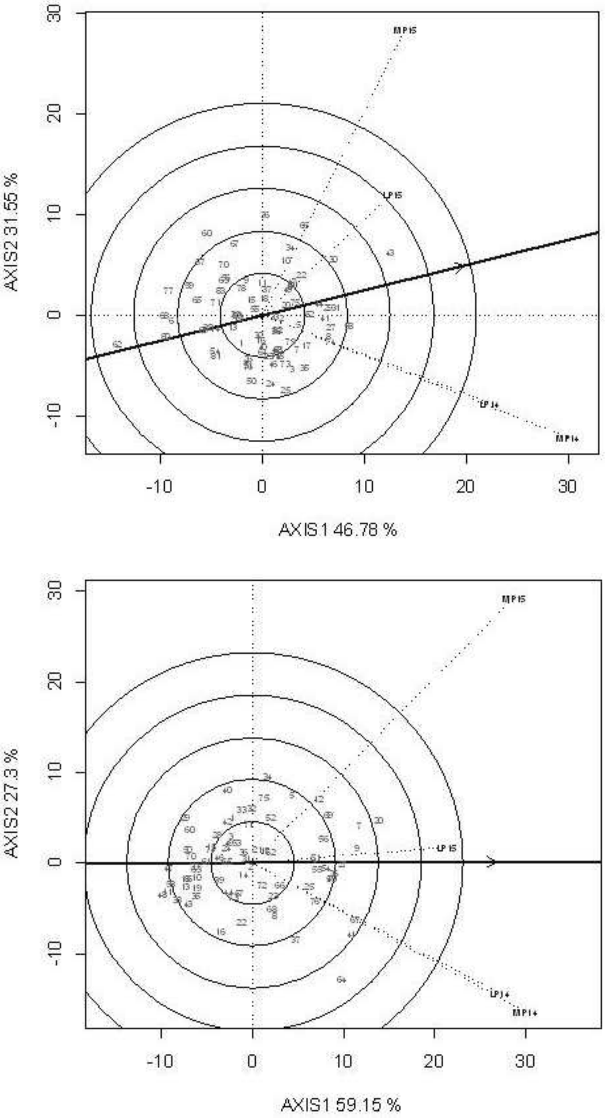

Fig. 5. **Discriminative power vs Representativeness view of GGE biplot in Spanish and Virginia groundnut genotypes tested at four environments. LP14: without P application during 2014; LP15: Without P application during 2015; MP14: with P application during 2014; MP15: With P application during 2015 (a) Spanish (b) Virginia**

al. 2007). Hence, LP15 followed by LP14 are more representative environments for groundnut.

The ranking of Spanish and Virginia groundnut genotypes for yield and stability is given in Fig. 6. There was a high correlation (r = 0.981 and r = 0.99 in Spanish and Virginia groups, respectively) between the genotypic PC1 scores and yield. Hence, genotypes having more than zero PC1 scores were regarded as higher yielders and less than zero were low yielders. In the Spanish group genotypes NRCG-12296, NRCG-11275, NRCG-12880, NRCG-11179, NRCG-11236, NRCG-11289 were high yielders and genotypes NRCG-14365, NRCG-12889, NRCG-14434, NRCG-14336 and NRCG-14419 were low yielders. Whereas, in Virginia group genotypes NRCG-12423, NRCG-11679, NRCG-11760, NRCG-14488, NRCG-14372, NRCG-14478 and NRCG-14428 were high yielders and genotypes NRCG-14396, NRCG-14397, NRCG-11981, NRCG-14453,

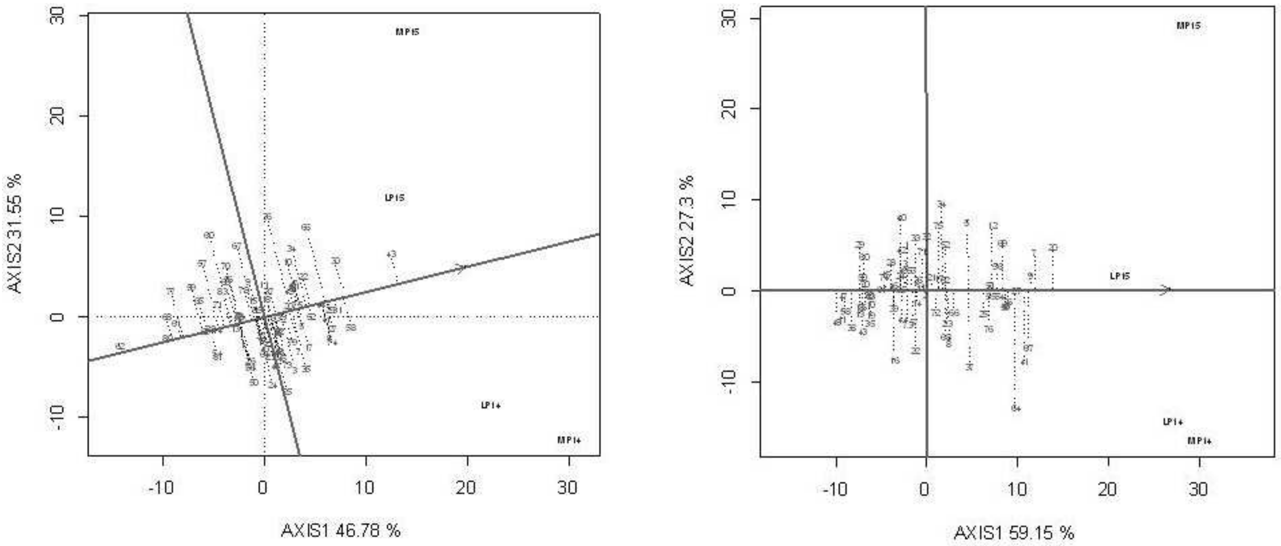

Fig. 6. Average environment coordination (AEC) views of the GGE-biplot based on environment-focused scaling for the means performance and stability of Spanish and Virginia groundnut genotypes. LP14: without P application during 2014; LP15: Without P application during 2015; MP14: with P application during 2014; MP15: With P application during 2015

NRCG-14358, NRCG-12065 and NRCG-14495 were low yielders. Unlike PC1, PC2 gives information about the stability of a genotype. Accordingly, in the Spanish group, genotypes NRCG-11236, NRCG-11289, NRCG-12290, NRCG-12334 and NRCG-12296 were stable and genotypes NRCG-14434, NRCG-12069, NRCG-11990, NRCG-11157 and NRCG-11874 were unstable. Similarly, in Virginia group genotypes NRCG-11760, NRCG-14325, NRCG-12968, NRCG-14428, NRCG-14487, NRCG-12455 and NRCG-12858 were stable and genotypes NRCG-14478, NRCG-14356, NRCG-14347, NRCG-8956 and NRCG-12998 were unstable.

Genotype with high average yield with relatively stable performance across environments are referred to as ideal genotypes and such genotypes are present at the centre of the concentric circle in GGE-biplot. In reality, a true ideal genotype may not exist but it can serve as a reference point for genotypic evaluation (Yan and Tinker 2006; Pavel et al. 2015). Genotype ranking based on mean yield and stability in comparison to ideal genotype is depicted in Fig. S1. The ideal genotype is situated in the centre of the concentric circle and has long vectors than another genotype with near-zero ATC ordinate. In the Spanish group, genotype NRCG-12296 located near to ideal genotype is desirable with high mean yield and stability across both with and without fertilized P conditions whereas genotype NRCG-14365 is far away from the ideal genotype and is the least favoured one (Fig. 5a). Among the Virginia group, genotypes NRCG-11760 and NRCG-14325 located near the centre of the concentric circle are desirable genotypes for both with and without fertilized P conditions as they have high mean yield and stable and genotype NRCG-14397 was considered as the least favoured Virginia genotype (Fig. 5b).

The present study demonstrates the robust methodology for selecting groundnut genotypes with high yield under low P environments and genotype NRCG-12296 (S. No. 43) in Spanish and NRCG-11760 (S. No. 9) in Virginia are the ones with high yield potential and adapted to low P fertilizer applications. These genotypes could be used for the development of new groundnut varieties suitable for low P stress conditions.

Authors contribution

Conceptualization of research (ABC, SKB, ALS); Designing of the experiments (ABC, NK, MCD); Contribution of experimental materials (ABC, ALS); Execution of field/lab experiments and data collection (HNM, ADM, MCD); Analysis of data and interpretation (HNM, ABC, NK); Preparation of the manuscript (ABC, SKB, ALS).

Declaration

The authors declare no conflict of interest

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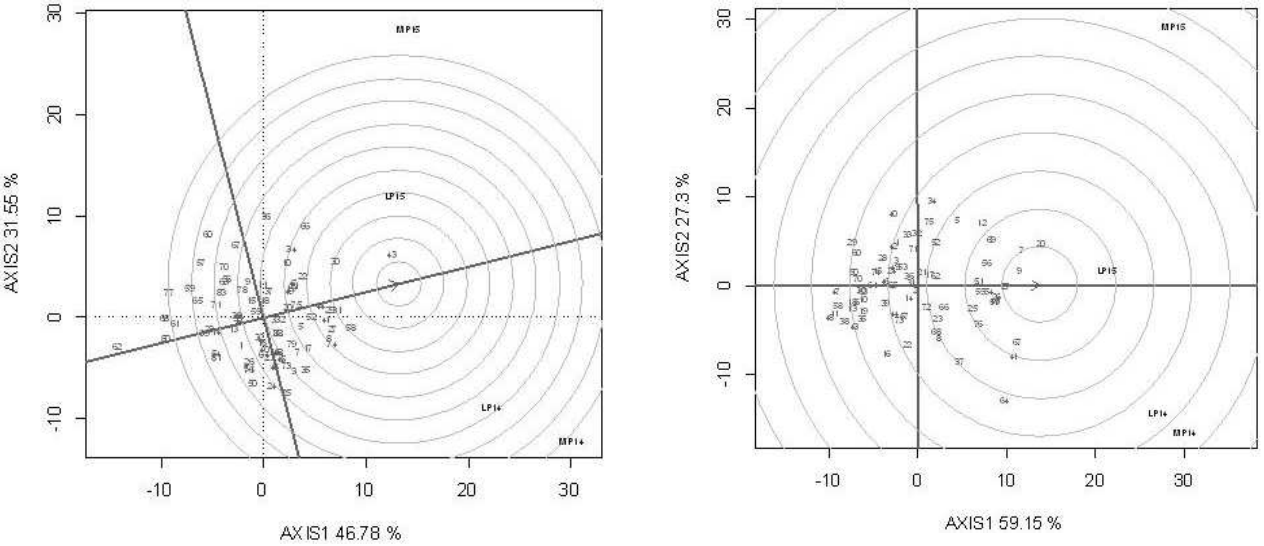
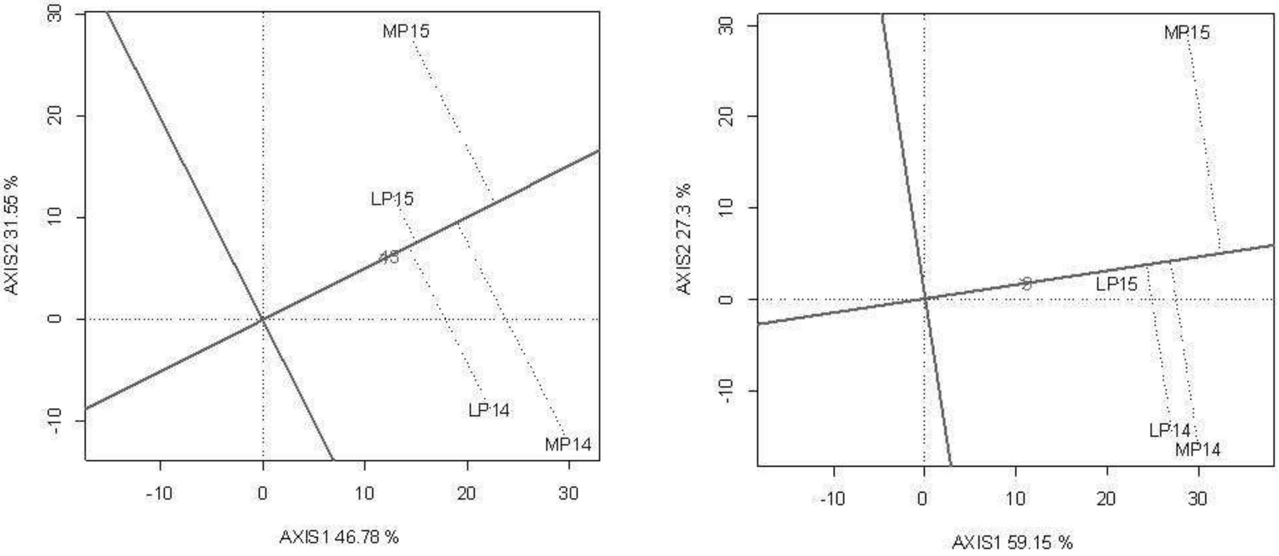


Supplementary Fig. S1. Ranking genotypes based on both mean and stability relative to an ideal genotype. Putting the ideal genotype at the center, concentric circles were drawn to visualize how far each genotype is from the ideal genotype



Supplementary Fig. S2. GGE-biplot showing the relative performance of NRCG-12296 (43) and NRCG-11760 (9) the highest yielding genotypes in Spanish and Virginia respectively at all test environment