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Deciphering G × E Interaction of Photo-insensitive Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] Mutants using AMMI and GGE Biplot Models

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ABSTRACT

Background: Photosensitivity in horsegram restricts horizontal expansion in its cultivable area. Using induced mutagenesis, photo-insensitive mutants of a popular variety PAIYUR 2 were evolved.

Methods: The mutants were experimented with across seasons and locations. All the experiments were conducted in the randomized block design with five replications. The AMMI and GGE biplot models were employed to tag the best-yielding and stable genotype(s).

Result: The ANOVA indicated significant effects of genotypes (G), environments (E) and their combined genotype × environment interaction (G × E) for all the experimental traits implying a large range of variation. The interaction effect in AMMI has been partitioned into several principal components. Of the six Principal Components (PC), the first PC explained the major variation. It is 60.20% for the number of clusters per plant, 99.60% for days to fifty percent flowering, 92.57% for the number of pods per cluster, 96.63% for the number of pods per plant, 99.66% for days to maturity and 96.34% for yield per hectare. AMMI and GGE biplot analyses helped to identify the best performing and stable photo-insensitive mutants TNAU-HG-DM-001 and TNAU-HG-DM-004 for further exploitation.

Key words: AMMI, GGE biplots, Horsegram, Photo-insensitivity, Stability.

INTRODUCTION

The food behavior of modern human narrowed the crop diversity. His food demands are catered by a few mega cereals. Accordingly, many crops despite their nutritional advantages are ignored consequently their presence either is recorded only in the germplasm records or cultivated in a packets. The lack of international market opportunities also limits their acreage expansion consequently named as underutilized crops. The concept of agriculture is changing, importance is given to crops with multiple utilities. Legumes are one such crop grown for food, fiber, fodder, oil and medicinal values. Horsegram is an important rainfed legume, grown under harsh environments. The research focuses on horsegram is attracting significance due to its nutritional significance (Sudhagar *et al*., 2023), stress tolerance ability (Sudhagar *et al*., 2022; Rajaprakasam *et al.,* 2023), medicinal and fodder values. Horsegram is predominantly cultivated during the *Rabi* season in southern India due to its photosensitivity. Its yield potential is reduced due to long duration and indeterminate growth habit (Priyanka *et al*., 2021). These problems can be overcome by evolving determinate and photo-insensitive cultivars.

Plants with determinate growth habits (DM) have a few advantages, suited for mechanical harvesting, increased productivity by increasing the number of plants and all-season cultivation. Genetic variability in horsegram is narrow (Chahota *et al*., 2013). The diploid status of horse gram makes induced mutation a viable breeding strategy to evolve variation (Priyanka *et al*., 2021). Two horsegram varieties PAIYUR 2 and CRIDA 1-18 R were mutated through a BRNS-funded project to evolve variations for growth habit and yield. A few DMs were evolved. Studies on the effects of genotypes (G), environment (E) and G × E (genotype by environment interactions) over different seasons and locations are to be estimated to select stable genotype(s). Among the statistical methods, the additive main effects and multiplicative interaction (AMMI) and genotype + genotype × environment (GGE) models stand out due to the large group of technical

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interpretations (Yan *et al*., 2000 and Balestre *et al.,* 2009). The utility of AMMI and GGE biplot models in the identification of stable genotypes is well documented (Kumar *et al*., 2023). Therefore, the present experiment is focused on identifying stable and good-yielding horsegram determinate genotype(s) using both AMMI and GGE biplot models.

MATERIALS AND METHODS

Genetic materials

The experimental material comprises six DMs and the check PAIYUR 2. The mutagenic generations (M_1 to M_3) were evaluated as per standard plant breeding protocols from the 2016 to 2019 cropping years. In the M_3 generation, the mutants with terminal flowering habits (determinate growth habit) were tagged, harvested individually and forwarded to the M_4 (2020 cropping season) on a plant-to-row basis. The homozygous lines were evaluated in the M_5 during the 2021 cropping year and the population was tested for the overall variability of economic traits and terminal flowering habit. Of the varied determinate populations evaluated, six DMs TNAU-HG-DM-001 (D1), TNAU-HG-DM-002 (D2), TNAU-HG-DM-003 (D3), TNAU-HG-DM-004 (D4), TNAU-HG-DM-005 (D5) and TNAU-HG-DM-006 (D6) were found promising and utilized for stability analyses. The generation advancement experiments were conducted in a randomized block design (RBD) with five replications. A standard package of practices was followed to realize healthy crop stands. The data on yield attributing traits were documented on a statistically sufficient number of plants at appropriate growth stages as per Mahajan *et al.* (2007). The data sets were analyzed using Microsoft Excel 2016 (Version 2306) and SPSS software (version 28.0.1).

Location, experimental design, data documentation and analyses

To ascertain the suitability potential of the determinate horsegram mutants, the yielding capability was tested in all three legume growing seasons (*kharif*, *rabi* and *summer*) of 2022 and 2023 cropping years as the DMs are photo-insensitive. The experiments in a season were conducted in two environments *i.e.,* Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu (11.02°N and 76.92°E) and Sugarcane Research Station, Tamil Nadu Agricultural University, Melalathur, Vellore (12.91°N and 78.87°E). Different sowing seasons were treated as environments. The environments, E1 represents *kharif*'22, Coimbatore; E2 indicates *kharif*'22, Melalathur; E3 denotes *rabi*'22, Coimbatore; E4 refers to *rabi*'22, Melalathur; E5 specifies to *summer*'23, Coimbatore; and E6 represents *summer*'23, Melalathur. The experiments were conducted in an RBD with five replications. Each experimental unit (plots) of a mutant was composed of seven rows measuring 5 m in length. The spacing pattern was 30 × 15 cm. Ten plants were randomly selected from each replication and data were recorded for days to fifty percent flowering (DFF) and days to maturity (DTM) at appropriate growth stages. The traits like the number of pods per plant (NPP), the number of pods per cluster (NPC) and the number of clusters per plant (NC) were documented at harvest. After post-harvest processing plot yields were worked out and accordingly seed yield per hectare (YDH) arrived.

The parent PAIYUR 2 is a photo-sensitive genotype; hence, its mean performance in the *rabi* season was only considered for comparison and excluded from further analyses. While the DMs are photo-insensitive and therefore their phenotypic performances in the 12 experiments were combined and utilized for arriving at ANOVA (analyses of variance) AMMI (Gauch and Zobel, 1990) and GGE biplot and graphical analyses (Yan *et al*., 2002). For graphical analysis, metan package of R Studio Team (2020) (https://cran.r-project.org) is used. The graphical outputs of GGE biplots and AMMI are utilized to illustrate the G × E interaction. The genotypes are ranked based on mean and stability values. The multi-environment data is used to generate a which-won-where pattern graph. The mean versus stability graph is drawn for the purpose of genotype evaluation. The test environments are ranked using discriminative versus representative graphs. The genotypes are ranked in increasing order with respect to the stability parameter. AMMI Stability value (ASV) is calculated as per Purchase (1997). The genotype selection index (GSI) is worked out as per Farshadfar *et al*. (2011).

RESULTS AND DISCUSSION

Photosensitivity in horsegram restricts its cultivation in all seasons. More acreage can be brought through photo-insensitive genotypes. Low variability for agronomically important traits in horsegram warrants opting for induced mutagenesis, as its potential is reported (Priyanka *et al*., 2021). Through a BRNS-funded project, from the photosensitive parent PAIYUR 2, a few DM were evolved. Determinate types are linked with photo-insensitivity (Ramtekey *et al*., 2019).

The rabi season yield performance of PAIYUR 2 is utilized for comparing the yield potential of the DM (Fig 1). The reasons for the abridged yield potential of the DM are analyzed (Fig 2). The DM grew to a maximum height of 35 cm because of which they had a lesser number of NC, NPC and NPP when compared to PAIYUR 2. The enhanced expression of these traits in PAIYUR 2 could be attributed to its indeterminate growth habit. Earlier, Singh *et al*. (2020) reported a positive linkage between these traits and yield. Though the yield levels of DM are comparatively low, owing to their 'photo-insensitivity' trait, we tried to identify the best among them since it would help for all-season horsegram cultivation which hitherto never existed. Further, the DM flowered and matured earlier than (15-20 days) PAIYUR 2 which can also be utilized either in the drought avoidance breeding programs or in the contingent cropping programs.

The DM TNAU-HG-DM-004 (819.55 kg/ha) and TNAU-HG-DM-001 (725.83 kg/ha) are identified as the best performers for YDH (Table 1). The ANOVA indicated significant effects of G, E and G × E for all traits (Table 2). For the traits, NC and NPC, the percentage of variation explained due to genotypes are noteworthy (96.19 and 36.87 respectively), indicating that the expression of these traits is genotype-dependent. This fact can further be explained by the fact that the DM grew ~35 cm. Therefore, they had less NC and NPC in all the seasons. Though they were grown in many

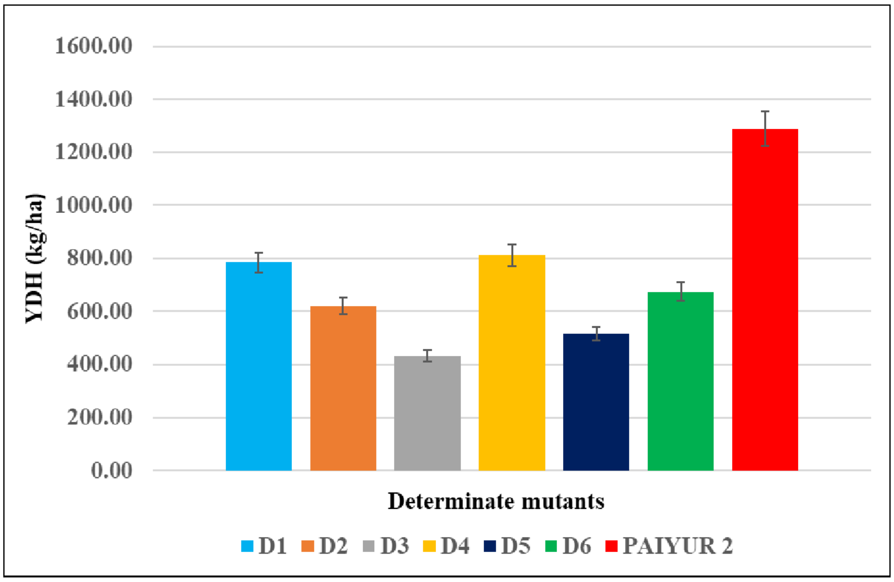


Fig 1: Comparison of yield potential of the determinate mutants with PAIYUR 2.

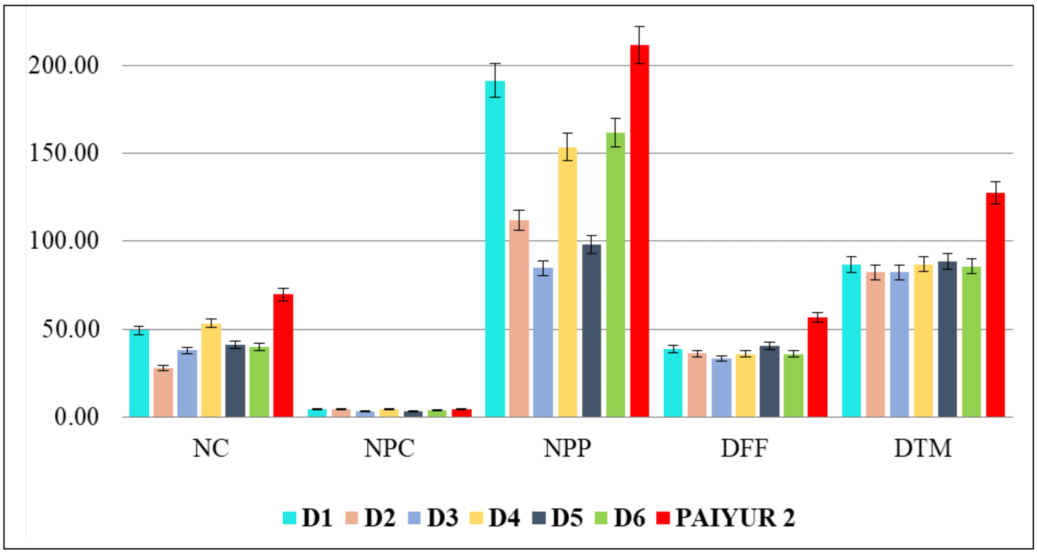


Fig 2: Comparison of yield attributing traits of determinate mutants with PAIYUR 2.

Table 1: Mean performance of horsegram determinate mutants for yield and its attributing traits (Average of three seasons and six locations±SE).

Mutants	NC	NPC	NPP	DFF	DTM	YDH	Rank
TNAU-HG-DM-001	45.80±1.81	4.81±0.09	154.82±19.55	38.54±0.87	87.38±2.19	725.83±63.12	2
TNAU-HG-DM-002	26.48±0.89	4.08±0.16	90.05±11.97	35.8±1.09	83.58±1.67	504.21±65.62	4
TNAU-HG-DM-003	35.98±1.19	3.29±0.05	78.28±3.84	32.94±0.59	84.22±2.24	390.48±20.84	6
TNAU-HG-DM-004	49.87±2.15	4.44±0.07	149.61±6.95	36.14±0.48	89.38±1.27	819.55±18.54	1
TNAU-HG-DM-005	37.32±3.20	3.35±0.11	89.86±4.98	40.72±0.53	88.01±0.78	473.85±28.12	5
TNAU-HG-DM-006	37.04±1.46	3.59±0.22	121.88±21.45	36.66±0.51	88.59±1.40	532.96±79.08	3

seasons and locations, the trait expression did not change, explaining the genotypic significance. While the traits, DFF, DTM and NPP are highly influenced by the G × E interaction (59.38%, 37.67% and 55.47%). These findings can further be supported by the facts that variations in soil moisture, photoperiod, dew and temperature (environmental factors) modify the tendrilling habit (plant height) thereby altering the flowering behavior and seed yield. Similar results were also reported by Ngalamu *et al*. (2023) in soybean.

The interaction effect in AMMI has been partitioned into six PCs. However, the first PC explained the major variations. It was 60.20%, 99.60%, 92.57%, 96.63%, 99.66% and 96.34% for NC, DFF, NPC, NPP, DTM and YDH respectively. Similar results were obtained by Sharma *et al*. (2022) in cluster beans where the first PC explained 50.7% of total variation.

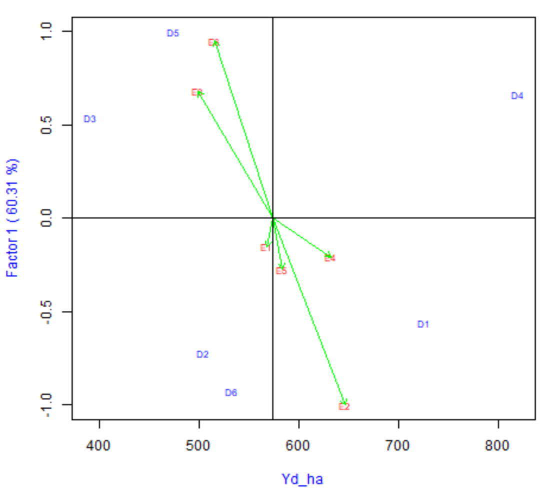
Interpretation of biplots

The AMMI-I and AMMI-II biplots were produced to illustrate both genotype and environmental influences simultaneously. In the biplot, the genotypes on the vertical line have higher main effects (genotypes or environments). The genotypes or environments that align horizontally have similar interaction patterns (Yan, 2011). The contribution of PCA 1 was 60.3% and PCA 2 was 30.77% for YDH. The YDH vs. PC1 biplot (Fig 3) shows that E2, E4 and E5 expressed the highest main effect. Similarly, Sharma *et al.* (2022) classified genotypes and environments based on main and interaction effects in cluster beans.

The environments displaying PCA scores in close proximity to the origin indicate minimal or negligible interaction. Notably, the PC1 score for E5 approaches zero, making it the most desirable environment. The D1 and D4 demonstrated higher main effects for YDH (Fig 3). With respect to the AMMI 2 biplot (Fig 4), the D3 was found to have an interaction effect. For the trait DTM, PCA 1 explained about 78.22% of the variation whereas PCA 2 explained about 20.85% (Fig 5). The AMMI 1 biplot (Fig 5) indicates that E2 and E4 expressed the lower main effect for the trait DTM. The D2 is located near the environment and has a less interacting effect (Fig 6). Similar results were obtained by Silva *et al*. (2016) in soybean.

A genotype with an ASV closer to zero is considered as stable (Table 3). Accordingly, D3 is ranked first for YDH because of its lower ASV. Similarly, for DTM, D5 is the stable genotype. The mutants D4, D5 and D3 are identified for DFF. For NPC, D1, D5 and D4 are ideal.

The lower value of GSI describes the better performance of a genotype for a trait. The order of better performers for YDH is D1 and D4 (Table 3). Similarly, for DTM: D5 and D6, for DFF; D5 and D4, for NC and NPC; D1 and D4, for NPP; D4 and D2 are identified. By considering the above orders, the mutants D1 and D4 are selected for further yield improvement programs. The mutants D5 and D6 are earmarked for utilization in the maturity group improvement programs. Such findings based on GSI were also reported by Simion *et al*. (2018) in cowpea.



*Factor 1 and 2 indicates PCA1 and PCA 2

Fig 3: AMMI 1 biplot for YDH.

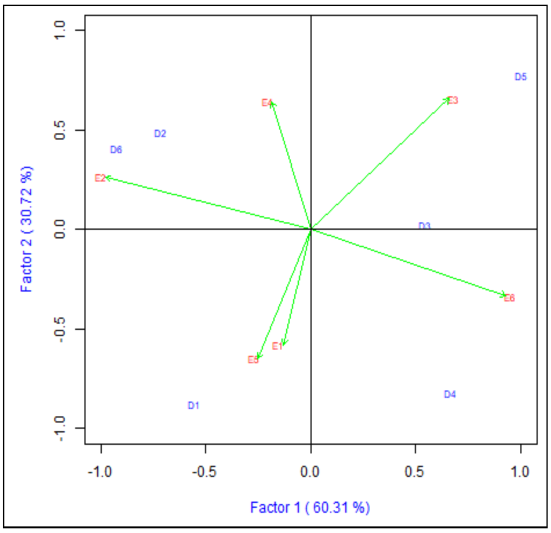


Fig 4: AMMI 2 biplot for YDH.

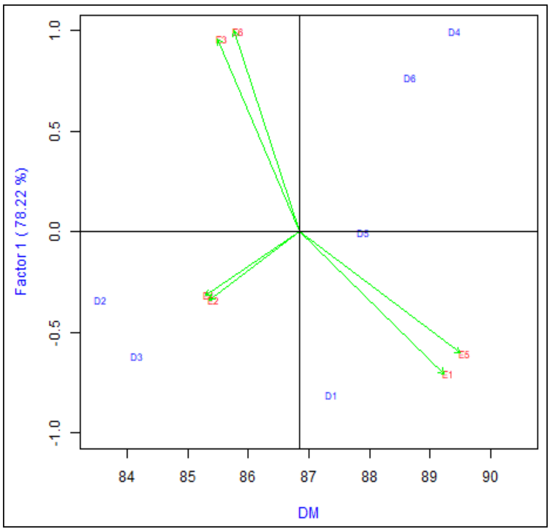


Fig 5: AMMI 1 biplot for DTM.

Table 2: AMMI-derived analysis of variance.

Source	df	NC		DFF		NPC		NPP		DtM		YDH	
		MS	VE (%)	MS	VE (%)	MS	VE (%)	MS	VE (%)	MS	VE (%)	MS	VE (%)
Genotypes	6	6000.72**	96.19	1566.69**	30.36	36.87**	61.54	35719.97**	37.21	8573.31**	31.97	679.84**	29.26
Environments	5	178.92**	2.39	634.60**	10.25	6.52**	9.07	28915.42**	25.10	2569.23**	7.98	624.65**	22.53
Interactions	30	17.69**	1.41	612.68**	59.38	3.52**	29.38	7230.87**	37.67	3219.06**	60.03	259.94**	55.47
PCA1	10	31.96**	60.20	1830.80**	99.60	9.78**	92.57	20963.68**	96.63	9624.67**	99.66	749.24**	96.34
PCA2	8	25.95**	39.10	8.39**	0.36	0.53^{ns}	4.05	905.75**	3.34	39.55**	0.32	30.53**	2.91
PCA3	6	0.36^{ns}	0.40	0.65^{ns}	0.02	0.47^{ns}	2.67	6.21^{ns}	0.01	1.09^{ns}	0.00	9.14^{ns}	0.46
Residuals	144	4.45^{ns}	0.00	1.26^{ns}	0.00	0.42^{ns}	0.00	14.80^{ns}	0.00	1.75^{ns}	0.00	6.13^{ns}	0.00

df = Degree of freedom, M.S = Mean sum of square, VE% = variability explained %, ** indicates significance at 1% level.

Table 3: Assessment of stability of horsegram determinate mutants using AMMI analysis.

Traits	NC					DFF					NPC					NPP					DTM					YDH				
Mutants	Mean	ASV	RA	RM	GSI	Mean	ASV	RA	RM	GSI	Mean	ASV	RA	RM	GSI	Mean	ASV	RA	RM	GSI	Mean	ASV	RA	RM	GSI	Mean	ASV	RA	RM	GSI
D1	45.87	2.20	1	2	3	38.70	5.11	4	2	6	4.83	0.20	1	1	2	154.87	31.29	5	1	6	87.37	5.27	5	4	9	725.90	12.43	2	2	4
D2	26.50	3.14	4	6	10	35.80	7.03	5	5	10	4.10	1.26	6	3	9	90.03	6.01	1	4	5	83.57	2.23	2	6	8	504.13	13.25	3	4	7
D3	31.17	2.83	2	5	7	32.80	1.66	3	6	9	3.30	1.17	5	6	11	78.23	28.06	4	6	10	84.17	4.13	3	5	8	390.47	9.40	1	6	7
D4	49.90	2.89	3	1	4	36.20	0.36	1	4	5	4.47	0.68	3	2	5	150.53	21.88	2	2	4	89.40	6.51	6	1	7	819.50	13.69	4	1	5
D5	37.43	13.90	6	3	9	40.80	1.03	2	1	3	3.33	0.62	2	5	7	89.90	25.74	3	5	8	87.90	1.69	1	3	4	473.87	18.72	6	5	11
D6	37.20	9.03	5	4	9	36.63	12.79	6	3	9	3.60	1.03	4	4	8	121.90	37.89	6	3	9	88.67	5.02	4	2	6	532.93	16.56	5	3	8

*AMMI stability value (ASV), Rank of the ASV (RA), Rank of trait mean (RM) and Genotype selection index (GSI).

GGE Biplots for YDH and DM

GGE biplot utilizes a scatter plot to visually represent both the genotypes and environments for identifying the mega environments, ranking the genotypes and determining stable environments (Yan *et al*., 2007). The GGE biplot discriminativeness vs. representativeness graph demonstrates the superior environment with an excellent discriminative capacity to differentiate genotypes (Kumar *et al*., 2023). In the present study, for the traits YDH and DTM, environments E5, E3 and E6 have the capacity to discriminate the genotypes (Fig 7, 8).

The mean vs. stability biplots show the genotypes' mean performance across the environments. The ideal environment and stable genotypes can be identified using the average environmental coordinates (AEC) and average environment axis (AEA) respectively. The D1 and D4 are stable for YDH (Fig 9). For the trait DTM, D3 is stable and productive (Fig 10).

The polygon view of the GGE biplot is a simple way to understand the performance of genotypes in specific environments and estimate their interaction. In the polygon for the trait YDH, mega environment (ME) I, is formed by E2 and ME II is formed by environments E4, E1, E5, E3 and E6 (Fig 11). The vertex (better performing) genotypes for ME I and II are D1 and D4 respectively. Similarly, for DTM, MI is formed by E1, E2 and E4 and E5, E3 and E6 are located in ME II (Fig 12). The mutants D4 and D6 are at the vertex of ME II, indicating their long duration. The mutants D2, D3, D1 and D5 are placed in areas where there were no associated environments, implying their early to mid-maturity habits.

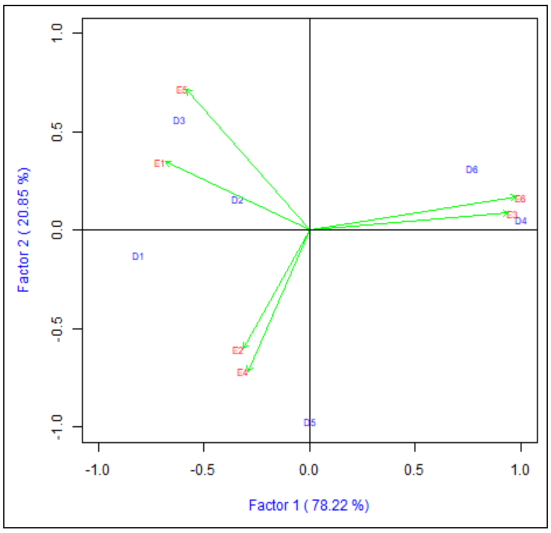


Fig 6: AMMI 2 biplot for DTM.

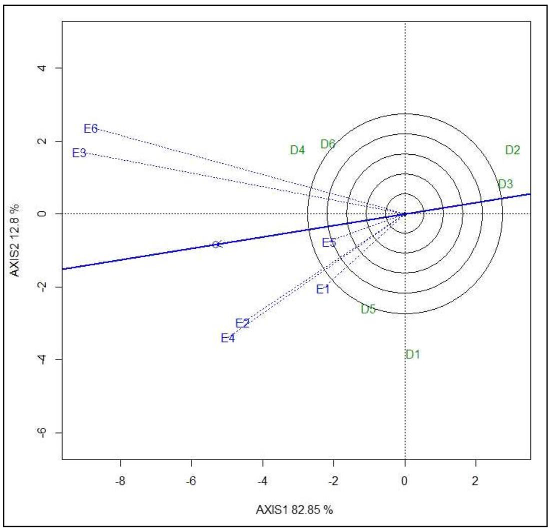


Fig 8: Discriminativeness vs. representativeness for DTM.

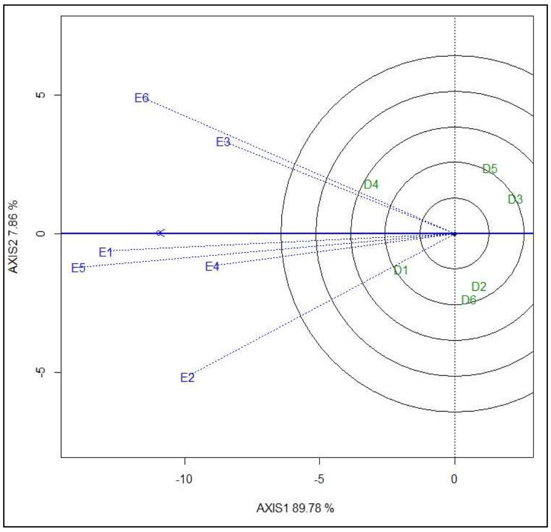


Fig 7: Discriminativeness vs. representativeness for YDH.

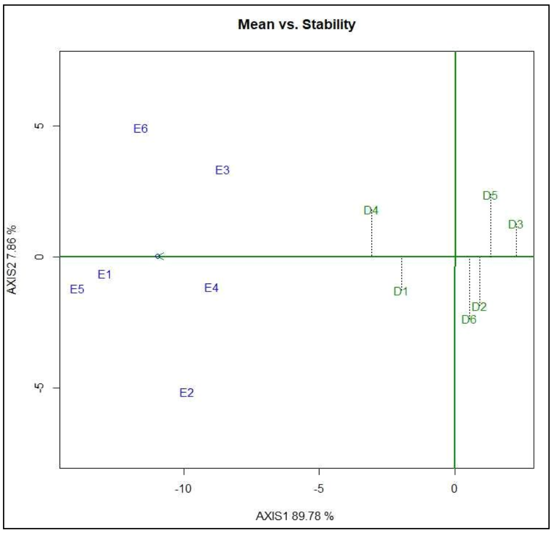


Fig 9: Mean vs. stability biplot for YDH.

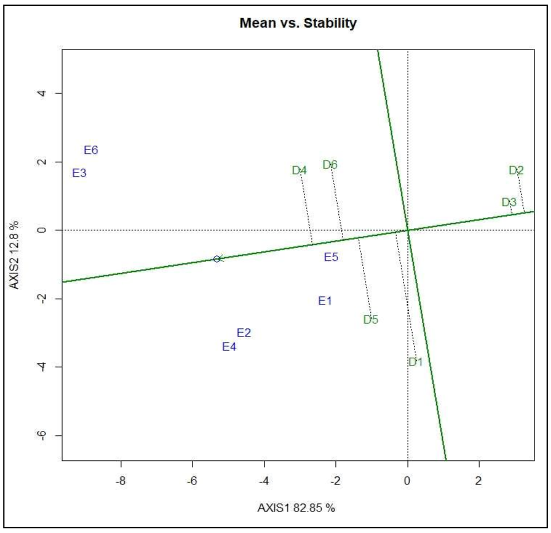


Fig 10: Mean vs. stability biplot for DTM.

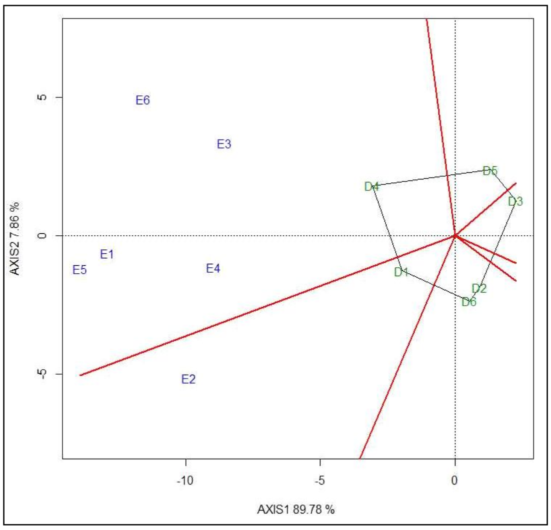


Fig 11: **'**Which won where' biplot for YDH.

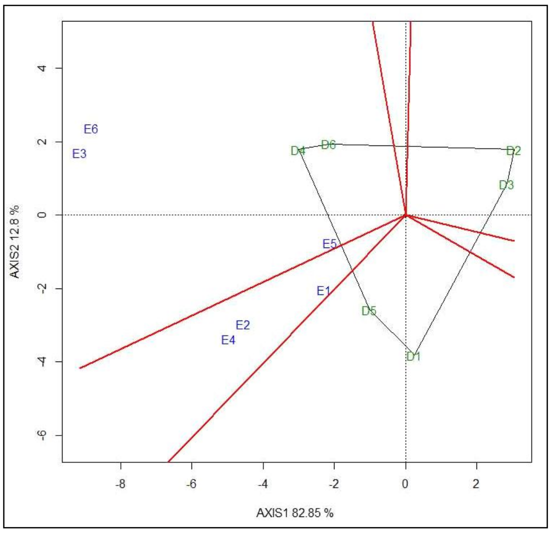


Fig 12: 'Which won where' biplot for DTM.

CONCLUSION

Stable performance and better adaptability are the primary factors considered while selecting the ideal genotype(s). In the present study, the genotype, environment and interactions are highly significant for yield-attributing traits, implying that there is a large range of variation. The DM, TNAU-HG-DM-001 (D1) and TNAU-HG-DM-004 (D4) are identified as stable for YDH. For DTM, the TNAU-HG-DM-002 (D2) and TNAU-HG-DM-003 (D3) are stable. These mutants, therefore, be utilized either (1) directly for a variety release for precision farming/high-density planting situations or (2) as a donor for photo-insensitivity in horsegram improvement programs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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