Stability analysis to identify improved lines of cluster bean (*Cyamopsis tetragonoloba* L. Taub.)

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Abstract

To select a stable and best-performing cluster bean line over seasons, an experiment was carried out using five shortlisted advanced breeding lines of vegetable cluster bean in randomized block design with four replications in *Kharif* and *Summer* seasons of 2019–20 and 2020–21 at ICAR-Indian Institute of Horticultural Research, Bengaluru. Additive mean effect and multiplicative interaction analysis of variation indicated a significant genotype and environment (G × E) interaction for all the traits. A high environment effect of 33.73% of the total sum of squares was observed for the trait pods per cluster followed by yield per hectare, single pod weight, pods per plant and clusters per plant. The first two interactive principal component axes (IPCA) cumulatively contributed 87.9, 97.3, 94.6, 98.6 and 85.6% variations for yield per hectare, number of clusters per plant, pods per plant, pods per cluster and single pod weight, respectively, leaving a small but significant amount of variation in the third IPCA. A mean versus weighted average of absolute score (WAAS) biplot indicated that genotype IIHRCB 26-2-1 is stable and best for the trait clusters per plant (>20 clusters/plant) while, IIHRCB 22-1-1 is superior and stable (WAAS mean nearly 0.00) for pods per cluster with >5 pods/cluster, pods per plant with >90 pods/plant, single pod weight with >3.0 g and yield per hectare around 24 t/ha. For all the environments, genotype IIHRCB 22-1-1 was found 'all-time winner' for yield per hectare and single pod weight. Based on multitrait stability index, IIHRCB 22-1-1 was found the best performer and the most stable genotype.

Introduction

The ultimate aim of any breeding programme is to develop a stable performing line/hybrid for a targeted trait. The importance of phenotypic stability of genotypes in plant breeding has been widely advocated by several researchers so far. As the environment is highly dynamic in nature, it exerts the major effect on phenotypic expression of traits which result in the variation in the performance of a genotype across and within the location over the seasons (Koundinya *et al.*, 2019; Sharma *et al.*, 2020). Hence breeders always look for the genotypes with least interaction with the environment and exhibit a stable performance thereby optimizing the resource-use efficiency for better yields (Ahmad *et al.*, 2005). For this, breeders resort to multi-environment trials (METs) which help in estimating the effect of genotype × environment interaction (GEI) on the trait(s) and based on the degree of GEI, stable performing lines can be selected. The success of these METs depends on how accurately the effect of GEI has been analysed.

Different methods, viz. univariate and multivariate, have been proposed for the same, such as two-way analysis of variance (ANOVA), linear regression analysis, principal component analysis (PCA) and additive mean effect and multiplicative interaction (AMMI), GGE (Genotype + Genotype × Environment) model. The AMMI model revolutionized the GEI analyses by dividing the G × E component of variation into principal components (PCs) thereby making a graphical representation of the stability of genotypes (Zobel *et al.*, 1988). GGE biplots display the genotype and GEI at the same time besides finding the mega environments and identifying the genotypes which performed better in which environment (Yan and Tinker, 2006). To represent the entire variation in all the PCs, instead of PC1 alone as in AMMI, a new statistic weighted average of absolute score (WAAS) is developed which takes into account the total GEI and assign a score to genotypes. This WAAS is used in the *Y*-axis and mean values in the *X*-axis in the biplot to identify stable genotypes with better performance (Olivoto *et al.*, 2019a). In combination breeding programmes, breeders concentrate on multitrait improvement. But, these stability statistical models indicate genotype stability for only one trait (Koundinya *et al.*, 2021). Hence, a multitrait stability index (MTSI) is developed to identify stable and better performing genotypes based on all the traits under consideration (Olivoto *et al.*, 2019b). Previous literature review suggested that a few studies have been carried out on determining the phenotypic stability of seed yield and its attributing traits by Jain and

Patel (2012); Pawan *et al.* (2016) and Teja *et al.* (2022) using linear regression models but studies on improving and identifying stable vegetable podded cluster beans is scarce. Further, as said earlier linear regression models leave considerable variation without taking into account. In our experiment, the data were analysed by using advanced statistical tools, viz. WAAS and MTSI.

Cluster bean being an underutilized legume vegetable, native to north-west India and Africa has immense potential as a nutritious vegetable for diet diversification in the climate change scenario. It is also a drought-tolerant and green manuring crop adaptable to tropical climatic conditions. The vegetable cluster bean variety *Pusa Navbahar*, bred in the 1980s by Indian Agricultural Research Institute, New Delhi is still the ruling variety in India. Hence, to develop a high-yielding and nutritionally superior vegetable-type cluster bean we initiated our research work. Cluster bean germplasm collected from all over India were evaluated for their yield and horticultural traits and the genetic variability and character association for the quantitative traits were studied (Mishra *et al.*, 2020). Further, the divergence present in the germplasm was studied and genotypes were grouped into different clusters based on their genetic distance (Mishra *et al.*, 2019). It was observed that all the vegetable-type cluster beans were grouped into cluster I, characterized by fleshy, smooth and medium-long pods with slow-growing seeds. These vegetable-type lines were further evaluated for yield traits and five lines were shortlisted based on their performance. Pure line selection was continued in these shortlisted lines to achieve uniformity as they were local collections from different Indian states (online Supplementary Table S1). To identify a stable and best-performing line among them, the following investigations were carried out.

Materials and methods

Experimental material, experimental design and test location

Experimental materials include five advanced breeding lines developed in cluster bean i.e. IIHRCB 22-1-1, IIHRCB 23-1-2, IIHRCB 26-2-1, IIHRCB 27-1-3 and IIHRCB 32-1-3 (pedigree information is provided in online Supplementary Table S1) along with check Pusa Navbahar. The materials were grown at the Vegetable Research farm of ICAR-Indian Institute of Horticultural Research, Bengaluru (13°7′ N, 77°29′ E). The experiment was laid out in the field in randomized block design with four replications following 30 cm spacing between the rows and 10 cm spacing between plants. Total 40 plants per replication per genotype were maintained. The weather of the experimental site is sub-tropical climate. The experiment was laid out in two different seasons in two consecutive years, viz. *Kharif*, the rainy season and *Summer*, the dry and hot season. The weather parameters recorded during the experimental period are given in online Supplementary Table S2. The day temperatures during *Summer* in 2019 and 2020 were above 30°C, while the day temperatures were below 30°C in *Kharif* in 2019 and 2020. Rainfall was almost double during *Kharif* 2019 and 2020 when compared with the *Summer* 2019 and 2020. Humidity was also high during *Kharif* 2019 and 2020 than *Summer* 2019 and 2020.

Observations and statistical analysis

Observations were recorded on yield and yield attributing traits like number of clusters per plant (NCP), number of pods per cluster (NPC), number of pods per plant (NPP), single pod weight (SPW) and yield per hectare (YPH). NCP, NPC and NPP are measured as count data on 10 randomly selected competitive plants while SPW in g and YPH were calculated in t/ha. There were five harvestings in each season. The data over these five harvestings were added to obtain final NCP, NPC, NPP, SPW and YPH.

Statistical analysis

The data obtained from various parameters were subjected to the AMMI model analysis as per the statistical model given by Zobel *et al.* (1988). WAASs were calculated as per Olivoto *et al.* (2019a) by using the following formula. They were calculated from the interactive principal component axes (IPCAs) of G × E Interaction from AMMI ANOVA.

$$\mathrm{WAAS}_i = \frac{\sum_{k=1}^{P} |\mathrm{IPCA}_{ik} \times \mathrm{EP}_k|}{\sum_{k=1}^{P} \mathrm{EP}_k}$$

where WAAS_i = weighted average of absolute scores of the ith genotype or environment; IPCA_{ik} = absolute score of the ith genotype or environment in the kth IPCA and EP_{ik} = magnitude of the variance explained by the kth IPCA.

The nominal mean values were plotted against environmental IPCA1 to obtain *which-won-where* graphs. The nominal mean values were calculated as per the below formula:

$$\hat{Y}_{ij} = \mu_i + \mathrm{IPCA1}_i \times \mathrm{IPCA1}_j$$

where $\hat{Y}_{ij}$ = nominal mean for i^{th} genotype in the jth environment, μ_i = grand mean of the ith genotype, $\mathrm{IPCA1}_i$ = IPCA1 score of the ith genotype and $\mathrm{IPCA1}_j$ = IPCA1 score of the jth environment.

MTSI was proposed by Olivoto *et al.* (2019b). The scores obtained in factor analysis were used to calculate MTSI based on the genotype–ideotype distances. MTSI was calculated based on all the traits under the study, viz. NCP, NPC, NPP, SPW and YPH by using the following formula:

$$\mathrm{MTSI} = \left[\sum_{j=1}^{f} (F_{ij} - F_j)^2 \right]^{0.5}$$

where $F_{ij} = j^{\mathrm{th}}$ score of the ith genotype and $F_j = j^{\mathrm{th}}$ score of the ideotype.

Software

All the statistical analyses were performed using R open-source software 4.0.2 using the package Metan developed by Olivoto (2019).

Results

AMMI ANOVA

In the present experiment, it was observed from AMMI ANOVA that GEI is highly significant for all the traits (online Supplementary Table S3). Significant variation was observed for genotypes (G), environment (E) and their interaction (GEI) for all the traits under study. For the traits, yield per hectare and pods per plant, maximum variation was contributed by the genotypes which were 50.41 and 66.47% and for pods per cluster maximum variation was contributed by the environment (33.73%) while for the number of clusters per plant and single

pod weight, error component had more variation (37.41, 33.43%) than genotypes, environments and their interaction. The effect of the environment was found to be low for the number of clusters per plant (5.99%) followed by pods per plant (17.50%). The entire GEI variation was captured by the first three IPCAs depicting that there was no noise in the model. All the PCs were found to be significant for all the traits. The first two IPCAs contributed a maximum of 87.9, 97.3, 94.6, 98.6 and 85.6% variation for yield per hectare, number of clusters per plant, pods per plant, pods per cluster and single pod weight, respectively, leaving a small but significant amount of variation in the third IPCA.

Genetic parameters

The genetic parameters estimated are presented in online Supplementary Table S4. A moderate genotypic coefficient of variation (GCV) was observed for yield per hectare (21.14%) and pods per plant (20.40%) while very low GCV values were observed for number of clusters per plant (7.75%), pods per cluster (9.71%) and single pod weight (6.83%) implying that the extent of variation existed was low. This was due to the inclusion of only five accessions in the experiment. Similarly, moderate-to-low PCV values were observed for the traits as 21.89, 10.10, 20.92, 11.86 and 8.29% for YPH, CPP, PPP, PPC and SPW, respectively. A slightly higher PCV values indicated the less influence of environment on these traits. A very high heritability was observed for YPH (93.30%) and PPP (95.11%) while a moderate heritability was observed for CPP (58.96%), PPC (66.94%) and SPW (67.95%). Moderate-to-low genetic advance over mean (GAM) values were recorded for all the traits pointing that there would not be much improvement in mean value through selection. Lack of high heritability coupled with high GAM suggested that selection within these accessions for these traits would not be fruitful. High coefficient of variations and high heritability coupled with high genetic gain for number of pods per plant, number of clusters per plant and pods per cluster was observed by Mishra *et al.* (2020) in cluster bean when analysed with 38 accessions. The low genetic variation and genetic advance in present experiment was due to the use of the top five high yielding accessions selected from a diverse population. Hence, there would not be any significant further improvement through selection.

Mean versus WAAS biplot

The mean versus WAAS biplots for all the traits are presented in Fig. 1. The stable and fairly stable lines are given in Table 1. In our studies, the accession IIHRCB 27-1-3 was found to be stable for yield per hectare but its mean value was below average. Two accessions IIHRCB 22-1-1 and Pusa Navbahar have been considered fairly stable as their WAAS was near zero and had mean values more than average (>17.7 t/ha). Similarly, for single pod weight also IIHRCB 27-1-3 was observed as stable with a below-average mean value. The fairly stable accessions for this trait were IIHRCB 22-1-1 and IIHRCB 23-1-2 because of their near-to zero WAAS value and above-average mean values (>2.66 g). None of the accessions was qualified as stable for pods per plant. Although genotype IIHRCB 23-1-2 had the least WAAS, it was not recommended as it had below-average mean value (<80.01). For the number of clusters per plant, genotype IIHRCB 26-2-1 could be recommended as a stable genotype because of its near-to zero WAAS value combined with the above-

average mean value (>17.22). In the case of pods per cluster and the number of clusters per plant, the genotype IIHRCB 32-1-3 had near-to zero WAAS value yet was not qualified to be recommended as a stable genotype because of the below-average mean value (<4.68). The accessions IIHRCB 26-2-1 and IIHRCB 22-2-1 for pods per cluster and IIHRCB 22-2-1 and Pusa Navbahar for the number of clusters per plant were considered fairly stable. The genotypes present in the second quadrant of the biplot are suitable for favourable conditions; such genotypes for various traits were Pusa Navbahar for pods per plant, Pusa Navbahar and IIHRCB 32-1-3 for single pod weight and IIHRCB 26-2-1 for yield per hectare.

The WAAS values of environments indicated that *Summer* 2021 had least interaction with the genotypes while *Summer* 2020 had maximum interaction with the genotypes for cluster per plant, pods per cluster and single pod weight. *Kharif* 2021 had maximum interaction with the genotypes for yield per hectare while *Kharif* 2020 had maximum interaction with the genotypes for pods per plant. *Summer* 2020 and 2021 interacted similarly for yield per hectare whereas *Kharif* 2020 and 2021 interacted similarly for the number of clusters per plant. For the remaining traits, all four environments interacted differently. The *Summer* season in 2020 and 2021 had a negative effect on the environment except for the number of clusters per plant which was found more during *Summer*. *Kharif* 2021 was found to be a highly favourable environment for all the traits except for the number of clusters per plant. The low temperature and high rainfall during *Kharif* season promoted vegetative as well as reproductive growth. The temperatures >30°C during summer season negatively affect the pollination and fertilization which might be the reason for the highest pods per cluster, yield per hectare and pods per plant during *Kharif* season. The available information was not sufficient to explain the more number of clusters per plant during *Summer* season.

Which-won-where

In general, these plots are used for identifying the genotypes which performed better in which environment (Fig. 2). The accession IIHRCB 22-2-1 had maximum nominal mean value for yield per hectare, single pod weight and pods per cluster during *Summer* 2020 and 2021. For yield per hectare and single pod weight, during *Kharif* 2020 and 2021, IIHRCB 22-2-1 had maximum mean values suggesting that it was an all-time winner for these characters. In the case of pods per cluster, it had the maximum nominal mean value in *Kharif* 2021 and the second highest in *Kharif* 2020 following IIHRCB 23-1-2. IIHRCB 26-2-1 recorded the maximum nominal mean value for pods per plant in all seasons except for *Summer* 2021. It had the second-highest nominal mean value during the *Summer* 2021 following IIHRCB 22-2-1. Akin to it, IIHRCB 26-2-1 had the maximum number of clusters per plant during *Kharif* 2020, *Kharif* 2021 and *Summer* 2021 and was having the second-highest mean value during *Summer* 2020 following IIHRCB 23-1-2. The accessions IIHRCB 32-1-3, IIHRCB 27-1-3 and IIHRCB 23-1-2 had the lowest mean values for yield per hectare and pods per plant across all seasons.

Multitrait stability index

MTSI was calculated based on yield per hectare, single pod weight, pods per plant, pods per cluster and the number of
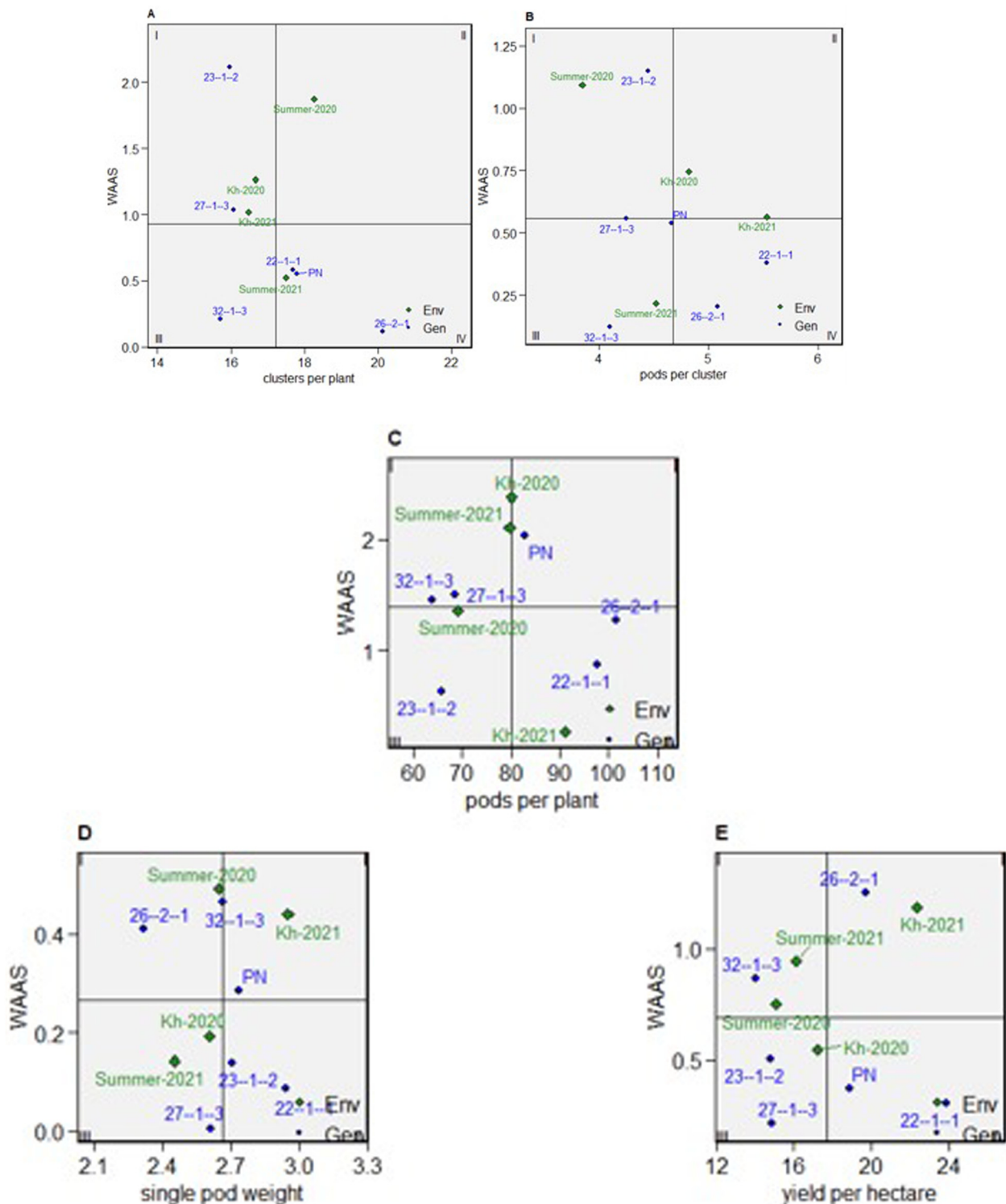
Figure 1. (a) Mean versus WAAS biplot for individual traits: (A) clusters per plant and (B) pods per cluster. (b) Mean versus WAAS biplot for individual traits: (C) pods per plant; (D) single pod weight and (E) yield per hectare.

clusters per plant. The factor analysis revealed that the first two PCs contributed 48.6 and 39.5%, respectively, which together explained 88% variation. The traits' number of clusters per plant, pods per plant and pods per cluster contributed 48.6% variation as they were associated with the first PC while yield per hectare and single pod weight contributed 39.5% variation (Table 2).

After varimax rotation, the communality ranged from 63.8% for pods per plant to 98.9% for single pod weight. Based on Fig. 3, the genotype IIHRCB 22-1-1 could be selected or recommended at 15% selection intensity. The selection of this stable and superior genotype would result in a selection differential of 2.68, 22.0, 18.2, 35.0 and 10.5% for the characters number of clusters per plant,

Table 1. Genotypes identified for various conditions based on mean versus WAAS biplot

Trait	Stable and better performer	Fairly stable and better performer	Better performer in favourable environment
Clusters per plant	IIHRCB 26-2-1	Pusa Navbahar IIHRCB 22-1-1	Nil
Pods per cluster	Nil	IIHRCB 22-1-1 IIHRCB 26-2-1	Nil
Pods per plant	Nil	IIHRCB 26-2-1 IIHRCB 22-1-1	Pusa Navbahar
Single pod weight	Nil	IIHRCB 22-1-1 IIHRCB 23-1-2	Pusa Navbahar IIHRCB 32-1-3
Yield per hectare	Nil	IIHRCB 22-1-1 Pusa Navbahar	IIHRCB 26-2-1

pods per plant, pods per cluster, yield per hectare and single pod weight, respectively.

Discussion

AMMI ANOVA

The principal objective of any experimental design is to assign experimental units to treatments for precise quantification of errors and achievement of accuracy which improves recommendations, repeatability, selection and genetic gains. For analysis of experiments, the ANOVA model is commonly used to compare the mean response value of treatments at different levels of factors. It further partitions the total variance among treatments into three sources, i.e. genotypic main effect (G), environmental main effect (E) and GEI effect but does not analyse the GEI. This GEI is very complex. Hence, when the GEI is significant, analysis and interpretation of GEI are crucial to identify the stable genotype. Out of the various methods used for the analysis of GEI, we used the AMMI model as it combines the ANOVA for genotype and environment main effect with PCA of the GEI effect.

The significant GEI for all the traits under study explained that all the traits were influenced by the environment. The effect of environment on the phenotypic expression of the traits was found to be high for pods per cluster, more than the genotype effect, suggesting that this trait was influenced more by the changes in the environment. The traits, number of clusters per plant and single pod weight had more variance from the error component indicating that there was significant noise in the model which was explained by the factors not under consideration. Although the amount of variation due to the environment was low, it had a significant effect on the number of clusters per plant and pods per plant, it had a significant effect. The first two IPCAs captured 80–99% variation for all the traits implying that maximum GEI variation was captured by them. Previously significant GEI was observed by Rashidi *et al.* (2013) and Shimray *et al.* (2022) in chickpea; Ashwini *et al.* (2021) in horse gram; Kvitschal *et al.* (2006) and Adjebeng-Danquah *et al.* (2017) in cassava.

Mean versus WAAS biplot

The AMMI1 biplot considers only the first PC leaving a significant amount of interaction variance in the remaining PCs.
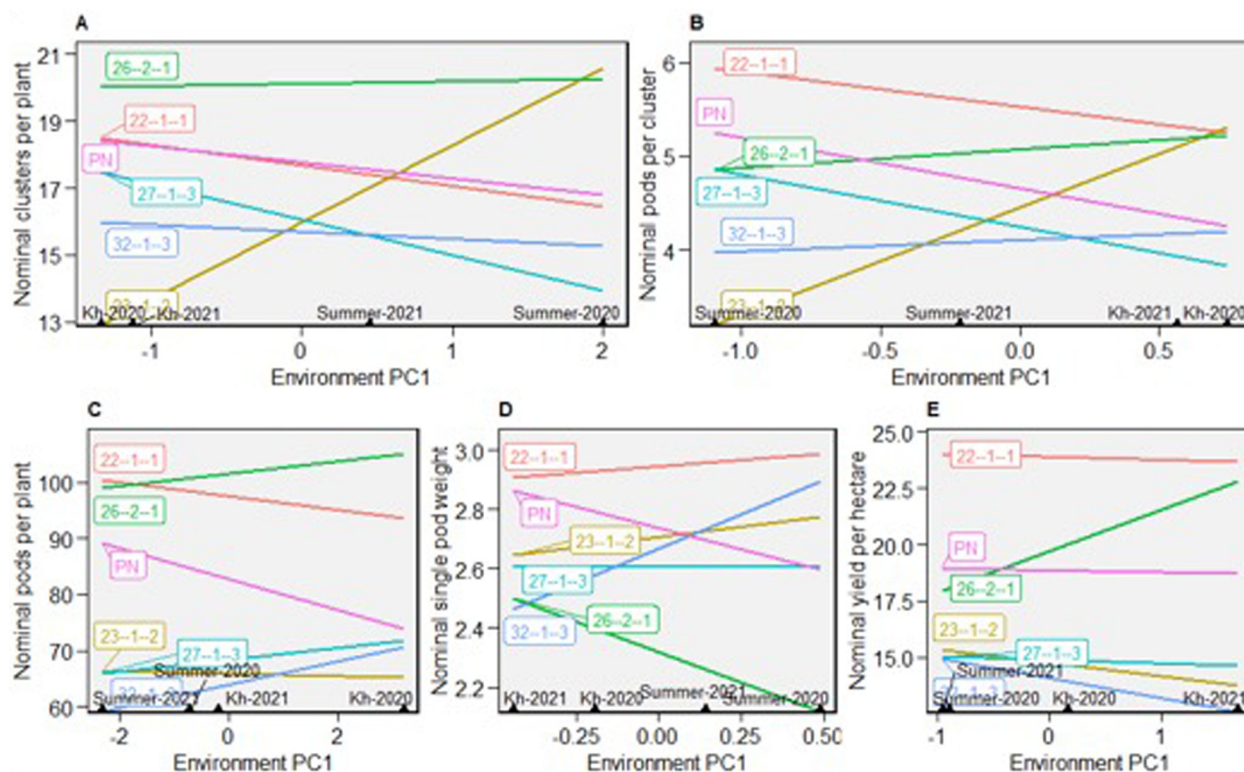

Figure 2. Which-won-where plots for individual traits in different environment: (A) clusters per plant; (B) pods per cluster; (C) pods per plant; (D) single pod weight and (E) yield per hectare.

Table 2. Communalities and selection differential of various characters based on MTSI

Sl. No.	Variable	Factor	Xo	Xs	SD	SD (%)	Communalities
1	cluster_plant	FA 1 (0.890)	17.2	17.7	0.462	2.68	0.920
2	pods_plant	FA 1 (0.735)	80	97.6	17.6	22	0.638
3	pods_cluster	FA 1 (0.967)	4.68	5.53	0.854	18.2	0.936
4	yield_ha	FA 2 (0.908)	17.7	23.9	6.2	35	0.918
5	singlepod	FA 2 (0.961)	2.66	2.94	0.28	10.5	0.989

Xo, mean of genotypes; Xs, mean of selected genotypes; SD, selection differential.

Hence, WAAS which is based on all the PCs in the AMMI ANOVA was used in the AMMI1 biplot. WAAS biplot between WASS and mean value (Y) facilitates the simultaneous selection for performance and stability for an individual trait. Stable genotype is those with a WAAS value of zero or near-to zero as their interaction with the environment is nil or very little. But mere stability is of no use unless it is coupled with high mean values. Therefore the genotypes with a WAAS value of zero or near-to zero with above average mean value are recommended for general cultivation (Koundinya et al., 2021). Based on the above-said criteria, only one accession was qualified for recommendation, i.e.

IIHRCB 26-2-1 for trait, number of clusters per plant. As the number of clusters is an important yield component, it may impart homoeostasis in yield over the seasons (Koundinya et al., 2019). The component compensation was previously explained by Eberhart and Russell (1966) who suggested selecting the genotypes which have stability for yield component traits. The biplots also helped in identifying the fairly stable genotypes with WAASs slightly higher than zero with above-average mean values. These genotypes could also be recommended for general cultivation as their interaction with the environment is less. Few genotypes interacted with the environment at a higher degree but
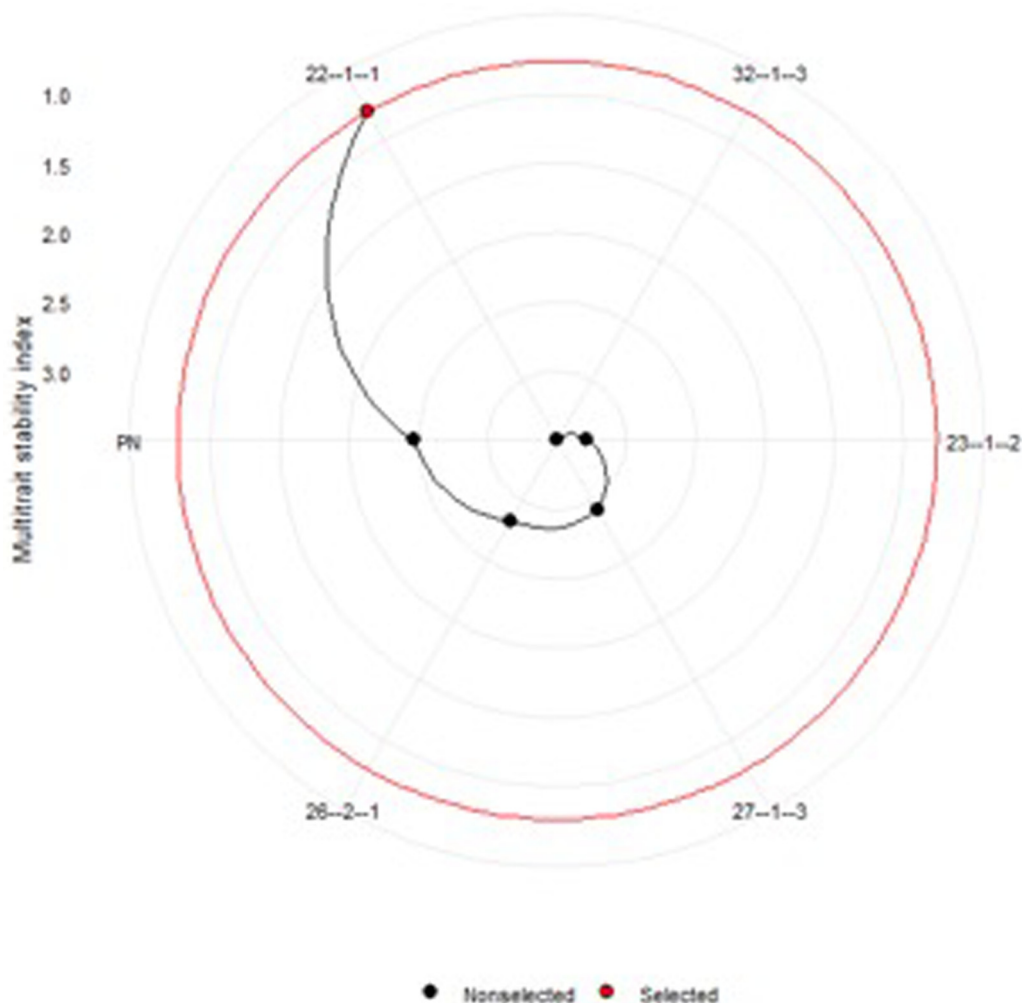
Figure 3. Selection based on MTSI.

had very high mean values. These genotypes are suitable for highly favourable conditions.

When two seasons are flocked together in the same quadrant, they are considered to have similar interactions with genotypes in both seasons for the trait under consideration. In the present experiment, there was no clear demarcation between the two major seasons, viz. *Kharif* and *Summer*. All four environments interacted differently with the genotypes for the majority of the traits. For clusters per plant, pods per cluster and single pod weight the *Summer* season interacted differently with the genotypes in 2020 and 2021. The effect of *Summer* season was found to be positive only for the number of clusters per plant when it had a negative effect on all the other traits which were favoured by *Kharif* 2021. Inferences on environment interaction with the genotypes based on WAAS versus mean biplot were previously studied by Koundinya *et al.* (2021).

Which-won-where

Olivoto *et al.* (2019a) provided a graphic interpretation by plotting the trait nominal means ($\hat{Y}_{ij}$) against environmental IPCA1 values. The accession which records the maximum mean value across all environments is called an 'all-time winner'. In our experiment, the genotype IIHRCB 22-2-1 had maximum mean values for yield per hectare and single pod weight in all seasons suggesting that it was an all-time winner for these characters. There was no all-time winner for other traits. The accessions IIHRCB 32-1-3, IIHRCB 27-1-3 and IIHRCB 23-1-2 had the lowest mean values for yield per hectare and pods per plant across all seasons suggesting their low productivity might be due to their genetic makeup. Based on these plots it is very difficult to differentiate *Summer* and *Kharif* as two mega environments as their interaction with genotypes was not in a specific pattern. Previously, Koundinya *et al.* (2021) identified genotypes for different environments and mega environments through these graphs.

Multitrait stability index

Olivoto *et al.* (2019b) proposed a tool MTSI for identifying stable genotype based on multiple traits. It is based on the genotype–ideotype distance using the scores obtained in factor analysis. The considered ideotype has the highest WAAS *Y* values for all the observed traits (Olivoto *et al.*, 2019b). Communalities indicated that single pod weight had maximum heritability (98.9%) followed by pods per cluster (93.6%) while pods per plant had the lowest heritability (63.8%). Lower MTSI values refer to the stable genotypes based on multiple traits. In this study, IIHRCB 22-1-1 obtained the lowest MTSI value indicating it as the most stable genotype among all the traits. It was supported by Table 1 as well as these genotypes were classified as a fairly stable and better performers for all the traits. The selection of IIHRCB 22-1-1 would improve the traits number of clusters per plant (2.68%), pods per plant (22.0%), pods per cluster (18.2%), yield per hectare (35.0%) and single pod weight (10.5%) over the ideal ideotype as they have higher selection differentials. Previously, Zuffo *et al.* (2020) also used MTSI to select stable soya bean genotypes under drought and salinity situations and Singamsetti *et al.* (2021) for selection of maize hybrids in different soil moisture regimes. All the previous studies stated that the MTSI is a useful tool for simultaneously selecting stable genotypes based on multiple traits.

Figure 4. Cluster bean line IIHRCB 22-1-1.

Conclusion

Stable performance of a genotype across environment/location/season is always the major criteria for promoting it as a variety/hybrid. However, estimation of this stability requires crucial analysis of GEI along with genotypic performance *per se*. Further, superiority in multiple traits across environment provides an added advantage to the grower. Hence, in this study using the AMMI model-based WAAS and MTSI tools, we identified cluster bean genotype IIHRCB 22-1-1 (Fig. 4) as the most promising and stable vegetable-type genotype for both *Summer* and *Kharif* cultivation in subtropical regions of India. Other important plant descriptor for this selected line includes, medium-tall, single stemmed plant with dark green, smooth, trifoliate leaves; pink flowers in racemose inflorescence develops into string-less, smooth, meaty, long pods (12–14 cm) borne in cluster at each node. Crop duration is 90–100 days.

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