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Evaluation of Genetic Diversity of Advanced Pigeonpea [*Cajanus cajan* (L.) Millspaugh] Interspecific Derivatives Using Mahalanobis D^2 and Selection of Elite Lines Using Multi-Trait Genotype-Ideotype Distance Index

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ABSTRACT

The experiment was conducted during *kharif* (May–November, 2022) season at the field experimental area of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana and at Regional Research Station, Faridkot, Punjab, India to assess the genetic diversity of 40 advanced BC_1F_8/F_9 pigeonpea interspecific derivatives derived from a cross between *Cajanus scarabaeoides* (L.) Thouars (ICP 15683) and *C. cajan* (L.) Millspaugh (ICPL 20329) using Mahalanobis D^2. The data for 11 key productivity traits was statistically analysed using R software. The derivatives were grouped into five clusters. Intra-cluster distances varied from 0.00 (Cluster V) to 107.40 (Cluster IV), while inter-cluster distances ranged from 123.49 (Cluster II and V) to 511.00 (Cluster IV and V). The derivatives in the present study were mostly divergent for harvest index, pods plant^{-1}, primary branches and seed yield plant^{-1}. The MGIDI index was remarkably efficient in selecting superior derivatives, resulting in desired gains across numerous traits. The MGIDI index highlighted six high-performing genotypes—AL 2581, AL 2611, AL 2577, AL 2613, AL 2606 and AL 2597. This research laid the groundwork for forthcoming pigeonpea hybridization initiatives, enabling the selection of parents based on both diverse genetic backgrounds and specific traits. The study suggested a targeted screening of lines within promising clusters for utilization in ongoing breeding programs, facilitating the development of valuable populations and hybrids.

KEYWORDS: Pigeonpea, cluster, mahalanobis, genetic diversity, interspecific derivatives, MGIDI

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1. INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millspaugh), is the foremost legume to get its complete genome sequenced with genome size of 833.1 Mbp (Singh et al., 2012, Varshney et al., 2012). It is an important part of the human diet in impoverished countries, especially in tropical and subtropical regions (Miano et al., 2020). It belongs to the *fabaceae* family and is one of the oldest grain legumes (Torres et al., 2007; Varshney et al., 2011). India is recognized as the primary center of origin and diversity for pigeonpea (Vavilov, 1926; Van der Maesen, 1990). Around 5.3 mt of pigeonpeas are produced annually on 6.0 mha of land worldwide. Asia, Africa and America share 85.7%, 13.6% and 0.7% of the total world production of pigeonpea, respectively. India, Myanmar, Malawi, Tanzania and Kenya are the leading producers of pigeonpea in the world (Anonymous, 2022). India accounts for 79% of global pigeonpea production and ranks as its second most important legume after chickpea, with 4.90 million hectares under cultivation (Sharma et al., 2020; Anonymous, 2022).

Pigeonpea contains 20–22% protein, 65% carbohydrates, 1.2% fat and is rich in lysine, dietary fiber, vitamins and minerals, offering 2–3 times more protein than cereals (Sun et al., 2020; Atuna et al., 2023; Talari and Shakappa, 2018). Compared to other legumes, pigeonpea has a significant potential to improve the lives of the impoverished due to their higher production in harsh environmental circumstances like heat, drought and low soil fertility (Negi et al., 2021). Pigeonpea are therefore a desirable legume for farmers with limited resources. Deep taproot of pigeonpea aids in nitrogen fixation and soil erosion reduction and it is commonly cultivated as an intercrop (Namuyiga et al., 2022; Simion et al., 2022). Pigeonpea, a crop often subject to cross-pollination, has seen commercial exploitation of hybrid vigour. Heterosis, influenced by genetic diversity and gene action (Bowman and Falconer, 1960), manifests through hybridization of genetically diverse parents, yielding heterotic or transgressive recombinants. Enhanced parental diversity correlates with increased potential to improve economically valuable traits in offspring. Accumulating germplasm and conducting genetic analyses facilitate the identification of genotypes with superior yield or other desirable attributes. Establishing genetic diversity within a crop at a specific centre requires years of dedication. However, leveraging this genetic variance for crop enhancement necessitates morphological characterization of these genotypes, as plant breeders select diverse inbred lines as parents based on their morphological performance. In the case of pigeonpea, distinct local preferences exist for colour, shape, taste and other agro-morphological traits. The assessment of genetic diversity in pigeonpea is crucial to broaden the genetic base of cultivated varieties and germplasm conservation (Gowda et al., 2013).

The Mahalanobis D^2 statistic (Mahalanobis, 1936) is suggested as a useful approach for clustering similar genotypes in crops based on their morphological traits (Bhandari et al., 2017). This method utilizes multivariate analysis to quantify the genetic divergence among genotypes and is commonly and consistently applied to assess genetic diversity in various crops (Sharma et al., 2018; Chaudhary et al., 2021; Kaur et al., 2021; Deepashree et al., 2023; Mahla et al., 2024). Breeders frequently seek to generate an ideotype, which is a genotype that combines many traits for optimal performance (Donald, 1968). The goal of ideotype design is to improve crop performance by taking into account multiple attributes at the same time while selecting genotypes (Olivoto and Nardino, 2021). Keeping this in view, the present experiment was taken up to study genetic diversity among 40 advanced pigeonpea interspecific derivatives and selection of derivatives using Multi-Trait Genotype-Ideotype Distance Index (MGIDI) selection index for their utilization in future crop improvement programme.

2. MATERIALS AND METHODS

2.1. Plant materials

The material used for conducting this experiment included 40 advance BC_1F_8/F_9 interspecific derivatives of *C. scarabaeoides* (L.) Thouars (ICP 15683)×*C. cajan* (ICPL 20329). The derivatives were planted at the field experimental area of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30°54'41.4"N 75°47'08.4"E) and at Regional Research Station, Faridkot (30°40'31.6"N 74°44'56.6"E), Punjab, India during the *kharif* (May–November, 2022) season. They were sown in paired row plots of four meters length with inter and intra row spacing of 50 cm and 25 cm, respectively and were subjected to recommended agronomic practices and replicated twice in randomized complete block design (RCBD).

2.2. Recording of data

Observations were recorded for each derivative line in each replication for various productivity traits *viz*., days to 50% flowering, days to maturity, plant height (cm), primary branches plant^{-1}, secondary branches plant^{-1}, number of pods plant^{-1}, seeds pod^{-1}, 100-seed weight (g), seed yield plant^{-1} (g), biological yield plant^{-1} (g) and harvest index (%) as per Anonymous (1993).

The harvest index was computed using the formula:

Harvest index=(Grain yield/Biological yield)×100

2.3. Statistical analysis

The data collected for different traits from both the

locations was pooled to perform statistical analysis using R software (version 4.3.1; Anonymous, 2023). D^2 statistics (Mahalanobis, 1936) was applied to morpho-physiological data to study genetic divergence. As per the Tocher's method given by Rao (1952), the clustering of derivatives, calculation of intra cluster and inter cluster distances and mean values of clusters were done. The method proposed by Singh (1981) was employed to determine the relative significance of characters contributing to genetic divergence. The Multi-Trait Genotype-Ideotype Distance Index (MGIDI) was analysed using the R Package 'metan' (version 1.18.0; Olivoto and Lúcio, 2020). Normalisation, Factor Analysis, Ideotype Planning and Computing Genotype Distance to Ideotype were the four procedures used to create the MGIDI index (Olivoto and Nardino, 2021).

3. RESULTS AND DISCUSSION

The ANOVA indicated significant differences among the derivative lines for all the traits studied indicating the presence of sufficient genetic variability (Chauhan, 2023). The existence of significant genetic variability for these different traits in pigeonpea was also reported earlier by Yohane et al. (2020) and Sharma et al. (2023). Cluster analysis was employed to examine the genetic diversity among a group of genotypes. This approach facilitated the grouping of genotypes based on their similarities and dissimilarities for various traits. It also aided in identifying diverse genotypes suitable for use in breeding programs to create genetic variation.

3.1. Distribution of interspecific derivatives into different clusters

The assessment of genetic diversity using Mahalanobis D^2 analysis categorized the test derivatives into five clusters (Table 1), indicating the presence of genetic diversity among them. Cluster I contained the highest number of genotypes (29), followed by Cluster II and Cluster III with four genotypes each, Cluster IV with two genotypes and Cluster V with a single genotype.

The varying number of accessions across different clusters indicated a broad spectrum of genetic diversity both within and among the clusters. A limited range of genetic variation was present among the genotypes that belonged to the same cluster. Selecting such genotypes often led to ineffective breeding. Moreover, genotypes in isolated clusters exhibited distinct characteristics, making them more diverse. Factors such as isolation, restricted gene flow and natural or human selection could have contributed to the formation of these clusters. The evaluated derivatives demonstrated high genetic diversity, making them well-suited for selecting improved lines with desirable traits for pigeonpea breeding programs.

Table 1: Cluster information of pigeonpea interspecific derivatives

Cluster	Interspecific derivatives	No. of derivatives
I	AL 2575, AL 2578, AL 2580, AL 2581, AL 2582, AL 2583, AL 2584, AL 2586, AL 2587, AL 2588, AL 2589, AL 2590, AL 2591, AL 2592, AL 2593, AL 2594, AL 2596, AL 2598, AL 2599, AL 2601, AL 2602, AL 2603, AL 2604, AL 2605, AL 2606, AL 2608, AL 2609, AL 2612, AL 2614	29
II	AL 2576, AL 2579, AL 2595, AL 2607	4
III	AL 2577, AL 2585, AL 2597, AL 2611	4
IV	AL 2610, AL 2613	2
V	AL 2600	1

From the study, it was inferred that although the derivatives were developed from the same region and parents, they were distributed across various clusters. Based on this clustering pattern, it was concluded that there was no relationship between genetic diversity and geographical origin. As a result, selecting parents solely based on regional diversity proves to be ineffective. The results were in line with the previous findings (Nag and Sharma, 2012; Kaur et al., 2023).

It is anticipated that crosses between members of these clusters will result in significant heterosis and a diverse array of genetic recombinants in the F_2 generation, displaying desirable traits. The findings of the current study was consistent with those of previous researchers who grouped pigeonpea germplasm into various clusters: 6 clusters with 49 genotypes (Pushpavalli et al., 2018), 7 clusters with 68 genotypes (Ranjani et al., 2021) and 4 clusters with 50 genotypes (Naing et al., 2022).

3.2. Identification of diverse derivatives

Distribution of various derivatives into different clusters indicated the presence of variation among them. However, this distribution does not reveal how much variation exists among them. The intra and inter-cluster distances (Figure 1), in addition to the mean values for the various observed traits, helped to clarify the degree of distinction. The intra-cluster distance revealed variation in morphological traits among the genotypes within the same cluster.

In the fifth Cluster, the intra-cluster distances were minimal (0.00) due to the presence of only single genotype. Conversely, fourth cluster exhibited the highest intra-cluster distance (107.40). Lower intra-cluster distances

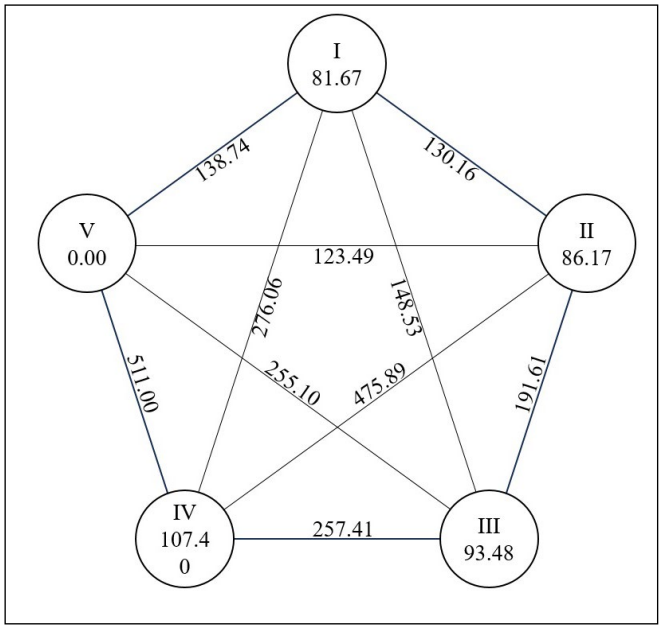

Figure 1: The intra and inter cluster distances of interspecific derivatives

indicated more uniformity among genotypes within clusters, whereas higher values suggested greater diversity among clusters. Reduced intra-cluster distances implied limited genetic variation within a cluster, potentially impeding the generation of desirable recombinants within the cluster. In this study, the fourth cluster, which had the highest intra-cluster distance, showed the most significant heterogeneity among the genotypes analysed.

The genotypes included in the Cluster IV and V (511.00) displayed maximum diversity among each other that was followed by Cluster II and Cluster IV (475.89), Cluster I and Cluster IV (276.06), Cluster III and Cluster IV (257.41), Cluster III and Cluster V (255.10). The prevalence of high genetic variation between the members of these clusters could be exploited for a hybridization program to get better recombinants in the segregating generations.

On the other side, the lesser inter-cluster distances between Cluster II and Cluster III (191.61), Cluster I and Cluster III (148.53), Cluster I and Cluster V (138.74), Cluster I and Cluster II (130.16), Cluster II and Cluster V (123.49) enlightened the lower degree of divergence and close genetic makeup of these genotypes.

The high inter-cluster distances compared to intra-cluster distances indicated greater genetic variation among derivatives. For optimal results, it is recommended to use derivatives from clusters with the greatest inter-cluster distance for hybridization, this would increase the likelihood of identifying best segregants in segregating generations. These results were in agreement with the earlier work reported by Muniswamy et al. (2014) and Kaur et al. (2023).

3.3. Cluster means for different morphological traits

There was significant difference between clusters for all the characters. It was clear from the cluster means (Table 2) that each cluster differed from other clusters in some way. Previous studies (Pandey et al., 2016; Pushpavalli et al., 2017; Singh et al., 2020; Sharma et al., 2023) have linked the high yield potential in pigeonpea with factors such as the number of primary and secondary branches plant^{-1}, pods plant^{-1} and 100-seed weight. In this study, genotypes in the fourth cluster exhibited favourable characteristics, including significant number of branches, pods, seed yield, biological yield and taller plant stature, which could contribute to improving seed yield and overall plant vigour. Conversely, genotypes in the second and third clusters showed traits such as early maturation and short stature. Despite differences, all clusters had statistically equal number of seeds pod^{-1}, with the third cluster displaying a higher harvest index.

To enhance yield potential, a hybridization program could leverage the genetic diversity present in these clusters to produce highly desirable recombinants in the F_2 generation. Parents with dwarf stature, early maturation, increased branching and higher pod numbers were identified as potential candidates for breeding genotypes suitable for mechanical harvesting and multiple cropping systems. Genotypes exhibiting greater biomass and taller stature were considered desirable for applications in feed and fuel production, whereas those with increased seeds pod^{-1}

Table 2: Mean performance of clusters for some important pigeonpea traits in interspecific derivatives

Cluster	DFF	DTM	PH	PBPP	SBPP	PPP	SPP	HSW	BYPP	HI	SYPP
I	97.59	143.88	225.95	8.90	12.82	218.73	4.38	7.10	218.37	22.25	48.50
II	90.37	135.66	174.48	7.36	12.01	201.85	4.14	5.97	199.57	22.07	44.00
III	90.67	137.71	207.85	8.76	11.80	251.24	4.29	7.55	179.41	30.03	53.61
IV	100.75	146.55	233.65	11.06	13.81	306.26	4.19	7.33	269.79	24.63	65.54
V	101.68	150.35	251.51	6.43	12.08	171.94	4.15	6.29	253.32	17.41	42.57

DFF: Days to 50% flowering; DTM: Days to maturity; PH: Plant height (cm); PBPP: Primary branches plant^{-1}; SBPP: Secondary branches plant^{-1}; PPP: No. of pods plant^{-1}; SPP: Seeds pod^{-1}; HSW: 100: Seed weight (g); BYPP: Biological yield plant^{-1} (g); HI: Harvest index (%); SYPP: Seed yield plant^{-1} (g)

and higher seed weight were ideal for breeding programs aimed at enhancing yield. Moreover, targeted selection for particular traits could facilitate the development of inbred lines with specific high-yielding attributes. Including AL 2596 from the first cluster as a parent could contribute to enhancing earliness in F_2 recombinants.

To create superior recombinants with elevated heterotic values for yield and associated traits, hybridization between parents spanning different clusters is essential. Parents should be selected from clusters demonstrating moderate to high D^2 values to ensure a wide spectrum of variability or segregation for the desired traits. Initially, clusters exhibiting the target traits need to be pinpointed and the most promising genotypes within these clusters should be prioritized for inter-crossing to achieve optimal combinations in subsequent generations.

3.4. Contribution of different traits towards total diversity

Some morphological traits represented a higher contribution towards genetic variation compared to others. In this study, the contribution of 11 morphological traits towards the genetic divergence is presented in Figure 2. The maximum contribution towards total diversity in interspecific derivatives was by harvest index (18.7%) followed by number of pods plant^{-1} (15.7%), primary branches plant^{-1} (14.9%), seed yield plant^{-1} (12.4%), days to 50 % flowering (9.0%), secondary branches plant^{-1} (7.9%), 100-seed weight (6.6%), days to maturity (5.1%), seeds pod^{-1} (4.0%), biological yield plant^{-1} (3.7%) and plant height (2%).

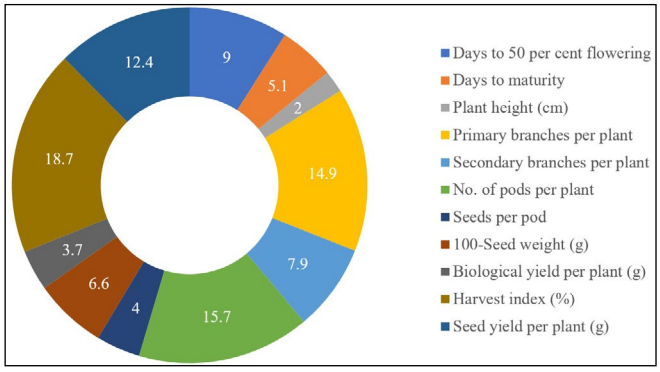

Figure 2: % contribution of various traits to diversity of pigeonpea derivatives

The findings indicated that the pigeonpea derivatives analysed in this study exhibited significant divergence primarily for harvest index, number of pods plant^{-1}, primary branches plant^{-1} and seed yield plant^{-1}, which collectively contribute to 61.70% of the total diversity. Hence, these traits should be prioritized when selecting at least one parent for hybridization programs.

3.5. Multi-trait genotype-ideotype distance index (MGIDI)

The Multi-Trait Genotype-Ideotype Distance Index (MGIDI) was utilized as a selection method to evaluate genotypes based on multiple traits. MGIDI identified genotypes with performance closest to an ideal ideotype, defined by desired characteristic values and ranked them according to their "distance" from the ideal. This index proved valuable for breeders, enabling efficient selection of high-performing genotypes by integrating multiple trait evaluations into a single score. As such, MGIDI served as a powerful tool for optimizing breeding programs focused on complex traits.

Considering four factors from the initial set of 11 traits, which collectively accounted for 76.77% of the total variation among traits, analysis revealed distinct trait groupings. These factors provided a more streamlined view of interrelationship of traits. Factor 1 (FA1) encompassed days to 50% flowering, days to maturity and plant height. Factor 2 (FA2) was associated with traits like primary branches plant^{-1}, no. of pods plant^{-1}, harvest index and seed yield plant^{-1}. Factor 3 (FA3) was linked to traits secondary branches plant^{-1}and biological yield plant^{-1}. Lastly factor 4 (FA4) was associated with traits like seeds pod^{-1} and 100-Seed weight (Table 3).

Using the MGIDI index, only biological yield plant^{-1} resulted in an undesirable selection gain (-2.51%). The MGIDI index achieved the highest total gains, 59.4% for traits that wanted to be enhanced and -8.0% for traits that wanted to be reduced. Notable parameters such as no. of pods plant^{-1}, primary branches plant^{-1}, seed yield plant^{-1} and harvest index showed significant percent selection gains of 8.68, 9.78, 11.7 and 14.4, respectively (Table 3). This highlighted the MGIDI index's efficiency in supporting targeted and advantageous trait selection for enhanced crop improvement tactics.

3.6. Selection of genotypes using MGIDI

In this study, 11 traits were analysed to evaluate differences among 40 pigeonpea interspecific derivatives. The MGIDI index identified six accessions-AL 2581, AL 2611, AL 2577, AL 2613, AL 2606 and AL 2597-as high-performing for multiple traits, demonstrating significant potential for simultaneously improving the 11 measured traits in pigeonpea breeding programs (Figure 3). These genotypes were particularly notable for traits such as early flowering, short stature and early maturity. Among them, AL 2597, positioned near the cut-off point indicated by the red line, displayed intriguing characteristics warranting further investigation, as suggested by Olivoto and Nardino (2021).

To our knowledge, no existing literature documented the use of the MGIDI selection index in pigeonpea. This study was the first to employ the MGIDI index for selecting derivatives in pigeonpea. Successful applications of this selection index had been demonstrated in evaluating ideal

Table 3: Original value (X_o), selected value (X_s), selection differential (SD%), heritability (h^2) and selection gain (SG%) for the MGIDI in 40 pigeonpea derivatives

Trait	Factor	X_o	X_s	SD (%)	h^2	SG (%)	Sense
DFF	FA1	96.40	92.60	-3.98	0.913	-3.63	decrease
DTM	FA1	143.00	140.00	-1.59	0.868	-1.38	decrease
PH	FA1	220.00	218.00	-0.79	0.668	-0.528	decrease
PBPP	FA2	8.78	9.70	10.50	0.933	9.78	increase
PPP	FA2	223.00	244.00	9.37	0.926	8.68	increase
HI	FA2	23.00	27.00	17.20	0.836	14.4	increase
SYPP	FA2	49.30	55.40	12.50	0.933	11.70	increase
SBPP	FA3	12.70	13.50	6.45	0.900	5.80	increase
BYPP	FA3	216.00	209.00	-3.30	0.763	-2.51	increase
SPP	FA4	4.33	4.65	7.32	0.771	5.65	increase
HSW	FA4	7.02	7.31	4.10	0.820	3.36	increase

DFF: Days to 50% flowering; DTM: Days to maturity; PH: Plant height (cm); PBPP: Primary branches plant^{-1}; SBPP: Secondary branches plant^{-1}; PPP: No. of pods plant^{-1}; SPP: Seeds pod^{-1}; HSW: 100: Seed weight (g); BYPP: Biological yield plant^{-1} (g); HI: Harvest index (%); SYPP: Seed yield plant^{-1} (g)

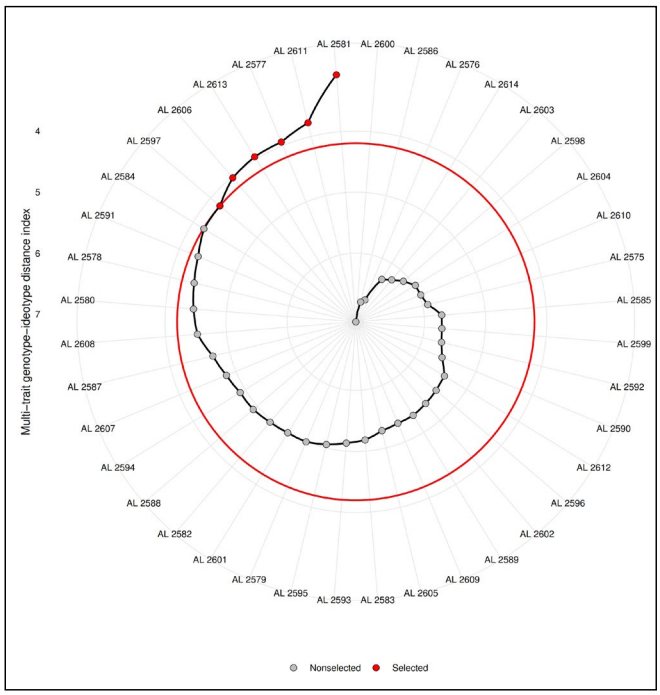

Figure 3: Genotype ranking in ascending order based on the MGIDI index, with selected genotypes highlighted in red. The red circle indicates the cut point defined by 15% selection pressure

yield and yield-related traits across various crops, including maize (Palaniyappan et al., 2023), wheat (Meier et al., 2021), brinjal (Uddin et al., 2021), guar (Benakanahalli et al., 2021) and soybean (Woyann et al., 2020; Volpato et al., 2020). These different studies demonstrated the effectiveness of multivariate selection indices for simultaneous trait selection. The MGIDI is the most efficient index for choosing genotypes with desirable features, demonstrating its relevance and usefulness in crop development (Olivoto and Nardino, 2021). These selected derivatives serve as the foundation for establishing recombinant populations through judicious crossings, ensuring maximum genetic diversity for the breeding of novel pigeonpea lines.

3.7. Strengths and weaknesses of genotypes based on MGIDI factors

The strengths and weaknesses of genotypes were accounted for by the proportion of each factor to the MGIDI index of the genotypes (Figure 4). Derivatives associated with FA1, such as AL 2577 and AL 2597, had distinct strengths in traits such as days to 50% flowering, days to maturity and plant height. On the other side, AL 2577 and AL 2597, which were also related to FA2, displayed strength in traits such as primary branches plant^{-1}, no. of pods plant^{-1}, harvest index and seed yield plant^{-1}. Furthermore, AL 2613 and AL 2581, which were related with FA3, exhibited strength in traits such as secondary branches plant^{-1} and biological yield plant^{-1}. Subsequently FA4 with derivatives like AL 2611 and AL 2613 showed strength in traits like seeds pod^{-1} and 100-Seed weight.

In the study, FA1, FA2, FA3, and FA4 contributed the most to the MGIDI of AL 2613, AL 2581, AL 2597 and AL 2577, respectively, indicating that these genotypes were poor for traits associated with their respective factors. These insights into the strengths and weaknesses of genotypes provided valuable guidance for selecting parents in breeding programs. The contributions were utilized to

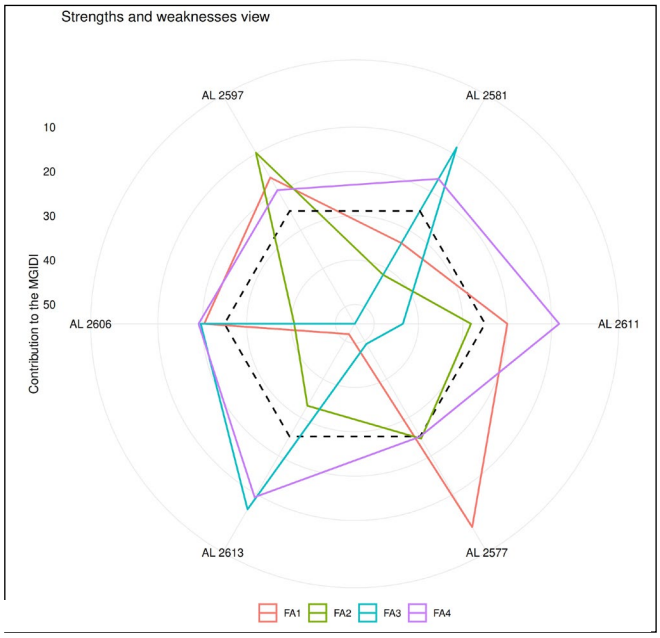

Figure 4: The strengths and weaknesses of the selected genotypes are represented by the proportion of each factor in the computed multi-trait genotype-ideotype distance index (MGIDI). A smaller proportion attributed to a factor (FA) (nearer to the outer edge) indicates that the traits within that factor are closer to the ideotype. The dashed line represents the theoretical value assuming equal contribution from all factors

identify potential parents for future crosses. Specifically, AL 2577, AL 2597 and AL 2611 were identified as promising candidates for inclusion in a crossing block aimed at developing plants with improved traits. AL 2611 was noted for its potential to contribute higher seed weight and an increased number of seeds, while AL 2577 and AL 2597 were valued for traits such as a shorter vegetative period, low stature, and early maturity. This graphical technique effectively highlighted the strengths and weaknesses of genotypes based on multiple attributes, aiding in the strategic selection of parental lines for breeding programs.

The detailed examination of strengths and weaknesses yielded useful insights, emphasising the importance of selecting the best pigeonpea genotype with superior quantitative traits. These selected genotypes stood out as promising candidates for future breeding projects, establishing MGIDI as a revolutionary technique for improving pigeonpea varieties with early maturity and yield attributes. The use of MGIDI represented a promising and creative approach for enhancing pigeonpea breeding tactics, helping to generate resilient and high-performing pigeonpea varieties suitable for sustainable farming practices.

4. CONCLUSION

The genotypes identified through MGIDI-AL-2581, AL-2611, AL-2577, AL-2613, AL-2606 and AL-2597 demonstrated strong potential for commercial release or use as key breeding materials in pigeonpea improvement programs.

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