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Research Article

Integration of canonical correlation, cluster analysis, and MGISI for identifying stable fertility restorers in *Maldandi* cytoplasmic background of sorghum

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Abstract
Sorghum, often referred to as the "camel crop," is one of the most important nutri-cereals for the global population. Though *rabi* sorghum is better in quality as compared to *kharif* crop, the yield level of *rabi* hybrids is lesser, which necessitates development of high yielding *rabi* hybrids. Additionally, to overcome cytoplasmic uniformity, it is essential to develop hybrids based on alternate cytoplasmic sources other than *milo* (A_1 cytoplasm). In this context, a study was conducted using an F_4 population derived from the cross PKV Kranti × DSMR 8, where DSMR 8 is a reported restorer for the A_4 cytoplasm, while PKV Kranti is known for its quality and is a ruling *rabi* variety. The population was subjected to Canonical Correlation Analysis (CCA) to identify the relationships between fertility restoration traits and yield-related traits. A mutualistic association was observed between the two groups of variables, indicating that improvement in one could positively influence the other. Among the ten F_4 families evaluated, the families 75R, 69R, and 54R exhibited strong restoration ability along with desirable yield traits, as confirmed through integrated multivariate analyses. These promising families can be further advanced in cascade breeding programs to develop superior restorers with enhanced agronomic performance for the *maldandi* cytoplasmic background in sorghum.

Keywords: Sorghum, A4 cytoplasm, *Maldandi*, CCA-multivariate analysis, Restorer.

INTRODUCTION

Sorghum is called as camel crop, and is one of the important nutri-cereal for the world population. In India, sorghum is grown in two seasons namely, *kharif* and *rabi.* The productivity of *kharif* sorghum has increased over decades but similar breakthrough has not been achieved in *rabi* sorghum (Reddy *et al.,* 2008). This is mainly due to the non-availability of hybrids that are suitable for *rabi* season. Till date, the available sorghum hybrids are based on the *milo* cytoplasm which is coming under the classification of A_1 cytoplasm. When the *milo* cytoplasmic background comes in interaction with the *rabi* conditions, the resulting hybrids often fail to perform as expected (Arunkumar *et al*., 2001; Verma *et al.,* 2022a). This is because of the cold stress during the flowering stage in *rabi* season that leads to pollen sterility and a low seed set percentage **(**Brooking *et al.,* 1976). This ultimately causes a reduction in single plant yield of the current hybrids of *rabi* sorghum.

So, there is an urgent need to create *rabi* hybrids based on cytoplasm adapted for *rabi* season as the productivity of *rabi* hybrids is less. One among such cytoplasm is A_4 indigenous cytoplasm of sorghum, in particular the *maldandi* male sterile cytoplasm (Reddy *et al.,* 2011), which could be an alternative. The line DSMR 8 was identified as a restorer for *maldandi* cytoplasm by Verma *et al.* (2024). Once a suitable restorer is identified the next objective of the breeder is to make the restorer agronomically superior, with good restoration ability. To achieve the same, it is important to understand the association among characters and identification of the superior genotypes that satisfies both the yield and restoration traits. Hence, this study focuses on assessment of association between traits influencing yield and fertility restoration, along with the identification of superior restorer in *maldandi* cytoplasm.

The association study was further advanced using Canonical Correlation Analysis (CCA), a powerful multivariate technique (Hotelling *et al.,* 1992) that enables simultaneous evaluation of relationships among groups of interrelated traits, providing deeper insights into their combined influence and mutual associations between restoration and agronomic traits. This multivariate analysis is also a method for indirect selection (Sedaghati *et al.,* 2023 and Martin *et al.*, 2005). As of now only few reports are available for estimating the relationship of yield traits and restoration trait, as two dependent groups in sorghum. Thus, the primary objective of this study was to elucidate the nature of association between yield related traits and restoration traits, viewed as two interdependent groups, having a relationship that holds significant importance for cytoplasmic diversification and for the development of *rabi* specific hybrids in sorghum.

MATERIALS AND METHODS

The present study was carried out with 10 F_4 families of the combination PKV Kranti x DSMR8. PKV Kranti is a ruling *rabi* variety and DSMR 8 is a reported restorer of *maldandi* cytoplasm, having a restoration percentage greater than ninety (Verma *et al.,* 2022b). This cross was forwarded from F_1 to F_3 generations during *rabi* 2018-2021. Thus, the 10 F_4 families that were used in the study, were selected for agronomic traits in F_2 generation and for restoration behaviour in the F_3 generation by studying the test cross hybrids obtained from crossing the male sterile line. In line with this, the yield traits were evaluated during the F_4 generation during *rabi* 2022 and the restoration trait was evaluated in the testcross hybrid developed from the cross between selected individuals with in each family and male sterile line M31-2A. With in each family, 10 selections were made and totally 100 F_4 individuals were selected based on the earliness and vigour and they were simultaneously crossed with M31-2A a *maldandi* cytoplasm based male sterile line and 100 such test cross hybrids were produced. This resulted in 100 testcross hybrids, which were evaluated in the next *rabi* season (*rabi* 2023) for pollen viability and seed set percentage as a measure of restoration.

Canonical correlation analysis (CCA) is a multivariate statistical technique used to identify the linear relationship between two or more sets of variables by forming groups based on the similarity of traits under study (Hotelling *et al.,* 1992), such as yield and restoration traits. Unlike Pearson's correlation, which measures the association between two individual traits, CCA evaluates the interrelationship between two groups of traits simultaneously. As a type of association analysis, its primary objective is to determine the degree of dependence among combined variables, thereby providing insight into their collective behavior. CCA can also serve as a preliminary step in multi-trait selection. However, while multi-trait selection assigns weights to each trait based on the breeder's preference or the economic importance of individual traits, in CCA, the weights are derived from the underlying Pearson's correlation coefficients between the variables.

Thus in CCA three types of variables are obtained namely, canonical weight, canonical loading and canonical cross loading which are statistically driven by using the linear combination of the characters within each group. In the present investigation, the two traits that were used for CCA analysis were agronomic traits and restoration traits.

Set 1: $X_{PH}, X_{NL}, X_{DF}, X_{DM}, X_{PL}, X_{PW}, X_{PWE}, X_{HGW}, X_{SPY}, X_{PVF4}$
Set 2**:** Y_{SS}, Y_{PVF41}

For the same, a collective canonical variate will be calculated by assigning the weight to individual character that in turn will depend in the pearson's correlation co-efficient. Thus, the canonical variates are calculated as

$$U = a_1 X_{PH} + a_2 X_{NL} + a_3 X_{DF} + a_4 X_{DM} + a_5 X_{PL} + a_6 X_{PW} + a_7 X_{PWE} + a_8 X_{HGW} + a_9 X_{SPY} + a_{10} X_{PVF4}$$

$$V = b_1 Y_{SS} + b_2 Y_{PVF41}$$

Here $a_{1\ldots}a_{10}$ are the canonical weights of X variable and b_1 and b_2 are canonical weights of Y variable. The canonical loadings which represents the correlation between the original variables and the canonical variates can be calculated as,

Canonical loading = corr (X_i,U)
Canonical loading = corr (Y_j,V)

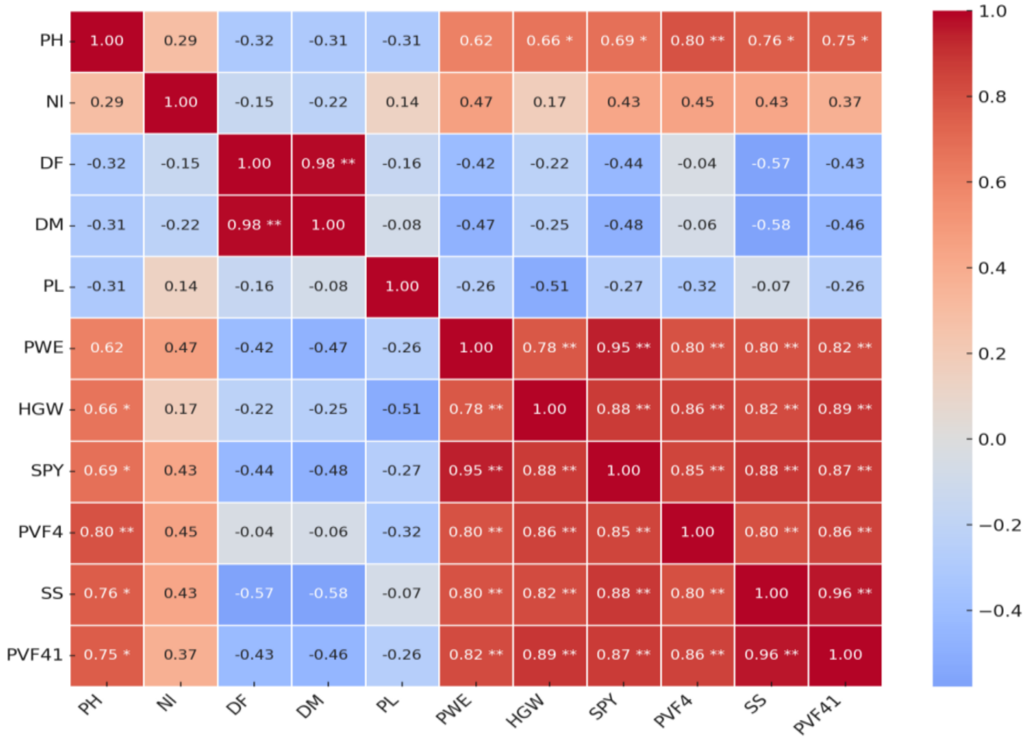
Similarly, canonical cross-loadings are estimated by multiplying the canonical loadings by the canonical correlations, which could be mentioned as,

Cross loading = corr(X_i,V)
Cross loading = corr(Y_j,U)

Then the dataset was subjected MGISI-Multitrait Genotype Ideotype Selection Index (Olivoto and Nardino, 2021) and cluster analysis to find out the superior families that will perform well in both the restoration as well as the yield traits for their utilization in the breeding programme.

RESULTS AND DISCUSSION

A significant positive correlation was found among most of the yield traits. But the trait, panicle length showed a negative association with yield (**Table 1**). Similar trend was noticed by Swamy *et al.* (2018) and Sathya *et al.* (2021). But the same had shown a negative correlation with days to fifty percent flowering and days to maturity. A pairwise character association was shown by Pearson's correlation co-efficient. It is also essential to identify how a group of traits are affecting the restoration behaviour (Arunkumar *et al.*, 2013). So the population was further evaluated by canonical correlation analysis.



	PH	NI	DF	DM	PL	PWE	HGW	SPY	PVF4	SS	PVF41
PH	1.00	0.29	-0.32	-0.31	-0.31	0.62	0.66 *	0.69 *	0.80 **	0.76 *	0.75 *
NI	0.29	1.00	-0.15	-0.22	0.14	0.47	0.17	0.43	0.45	0.43	0.37
DF	-0.32	-0.15	1.00	0.98 **	-0.16	-0.42	-0.22	-0.44	-0.04	-0.57	-0.43
DM	-0.31	-0.22	0.98 **	1.00	-0.08	-0.47	-0.25	-0.48	-0.06	-0.58	-0.46
PL	-0.31	0.14	-0.16	-0.08	1.00	-0.26	-0.51	-0.27	-0.32	-0.07	-0.26
PWE	0.62	0.47	-0.42	-0.47	-0.26	1.00	0.78 **	0.95 **	0.80 **	0.80 **	0.82 **
HGW	0.66 *	0.17	-0.22	-0.25	-0.51	0.78 **	1.00	0.88 **	0.86 **	0.82 **	0.89 **
SPY	0.69 *	0.43	-0.44	-0.48	-0.27	0.95 **	0.88 **	1.00	0.85 **	0.88 **	0.87 **
PVF4	0.80 **	0.45	-0.04	-0.06	-0.32	0.80 **	0.86 **	0.85 **	1.00	0.80 **	0.86 **
SS	0.76 *	0.43	-0.57	-0.58	-0.07	0.80 **	0.82 **	0.88 **	0.80 **	1.00	0.96 **
PVF41	0.75 *	0.37	-0.43	-0.46	-0.26	0.82 **	0.89 **	0.87 **	0.86 **	0.96 **	1.00

Table 1. Pearson's correlation coefficient between the individual traits

PH-Plant height, NL-Number of leaves, DF-Days to fifty percent flowering, DM-Days to maturity, PL-Panicle length,PWE-Panicle weight,HGW-Hundred grain weight,SPY-Single plant yield,PVF4- Pollen viability in F4 generation, SS- Seedset, PVF41-Pollen viability in F4:1 generation

To investigate the relationship between yield performance and fertility restoration efficiency as two groups, canonical correlation analysis (CCA) was performed using two restoration traits which includes seed set percentage along with the pollen viability in $F_{4:1}$ as the dependent variable set (Y) and eight agronomic yield traits, namely, plant height, number of leaves, days to fifty percent flowering, days to maturity, panicle length, panicle width, single plant yield and pollen viability in F_4 generation as independent traits (X). The traits hundred grain weight and panicle weight were excluded to eliminate multicollinearity. The multicollinearity diagnostics presented in **Table 2**, had showed a very high condition number (575.88) with an extremely high variance inflation factor (97.95), thus confirming the severe multicollinearity among the shown agronomic traits. A strong correlation was observed between the panicle weight and single plant yield, which suggest that these characters were linearly dependent and there is a requirement of dimensional reduction before carrying out the CCA analysis. So, within the two traits, single plant yield was the major determinant of the final agronomic potential and thus panicle weight was eliminated. The test of multi collinearity was carried out and the CN was 13.81, which had showed a moderate collinearity, though with a VIF value of zero. Though this is statistically valid, to improve the efficiency, hundred grain weight was also removed from the agronomic traits and the downstream analysis was carried out. A comparable procedure for eliminating highly correlated traits for CCA analysis was reported by Silva *et al.* (2016) in their study on multicollinearity among agronomic characters. As observed in Keskin and Yasar, (2007) and Martin *et al.* (2005), the first canonical variate had captured the strongest linear association between the two sets of traits. Similarly, in the present study, out of the two canonical variates obtained, the first variate had showed the strongest relationship between agronomic traits and restoration traits (**Table 3**).

Table 2. Test of multi-collinearity with in the variables

Condition number	Largest correlation	Between traits	Variation inflation factor
575.89	0.93	Panicle weight, Single plant yield	97.96
13.81	0.76	Hundred grain weight, Single plant yield	0

Table 3. Significance for canonical variate

Function	Canonical correlation	Squared canonical correlation	df	Chi-Square	p-value
1	0.93	0.88	22	36.07	0.000038
2	0.33	0.47	9	27.77	0.18

The three metrics obtained by canonical correlation analysis is furnished in the **Table 4** and the results were discussed here by. The standardized canonical coefficients revealed that pollen viability at F_4 generation had contributed most positively to the first canonical variate U_1 (coefficient = +0.757), followed by days to fifty percent flowering with a negative contribution (–0.612). This implies how each variable had contributed in the formation of canonical variate. Among the restoration traits, seed set percentage had the dominant contribution to V1 (coefficient = +0.990), indicating that this trait strongly governs the variation explained by the canonical relationship.

Canonical loadings which are also called as the structure correlations confirmed that single plant yield (+0.812) and pollen viability at F_4 generation (+0.803) had the strongest positive correlations with U1, followed by days to fifty percent flowering (–0.565) and days to maturity (–0.294) which had showed a moderate negative loading. While dealing with restoration traits, seed set percentage (loading = +0.998) and pollen viability at $F_{4:1}$ generation (+0.904) were highly associated with V1.

Cross-loadings shows how well each trait correlates with the opposite canonical variate. The yield traits, namely, single plant yield (0.776) and pollen viability at F_4 generation (0.768) showed strong positive cross-loadings with V1, while days to fifty percent flowering had a negative cross-loading (–0.540), which suggests that these traits are responsive to the restoration performance that was captured in the canonical function. Similarly, seed set percentage and pollen viability at F_4 generation (0.768) had high cross-loadings with U1 (0.955 and 0.864, respectively), which is a great positive sign, which tend to imply that the population that were utilized for studying the yield and restoration traits were aligning in the same direction thus increase in the one group will simultaneously improve the other. Thus, both the yield trait and the restoration trait could be combined in an effective manner as both are having a mutualistic effect in concern with the population pertaining to PKV Kranti and DSMR-8. With respect to the pollen viability percentage, irrespective of the seed set, majority of the genotypes had shown a higher percentage of pollen fertility. Similar pattern of pollen fertility was obtained by Secrist *et al.* (1989) where they studied the restoration behaviour of various cytoplasmic restorers including *maldandi* restorers. They noticed that the A_4 cytoplasmic restorers had fallen majorly under high fertility restoration class, while dealing with pollen fertility percentage but when the final seed set percentage was analyzed it was not satisfactory and the reason behind this is the higher accumulation of starch in the pollen grain resulting in higher percentage of dark stained pollen. Since both the variables within this linear combination have same sign, it suggests that both are having direct effect among each other.

Table 4. Canonical weight, canonical loadings and cross loadings of the variables

Variables		Canonical weight		Canonical loadings		Canonical cross loadings	
		1	2	1	2	1	2
Agronomic traits	Plant height	0.06	0.02	0.29	-0.48	0.27	-0.30
	Number of leaves	0.17	-0.34	0.19	-0.39	0.19	-0.24
	Days to fifty percent flowering	-0.61	0.51	-0.56	0.51	-0.54	0.31
	Days to maturity	0.13	-0.45	-0.29	0.09	-0.28	0.05
	Panicle length	0.00	-0.08	-0.13	-0.34	-0.12	-0.21
	Panicle width	0.02	0.00	0.27	-0.18	0.25	-0.11
	Single plant yield	-0.06	-0.36	0.81	-0.07	0.78	-0.04
	Pollen viability percentage in F4 generation	0.76	0.53	0.80	0.52	0.77	0.32
Restoration traits	SS% F4:1	0.99	-0.14	1.00	-0.06	0.95	-0.04
	PVF4:1	0.14	0.99	0.90	0.43	0.86	0.26

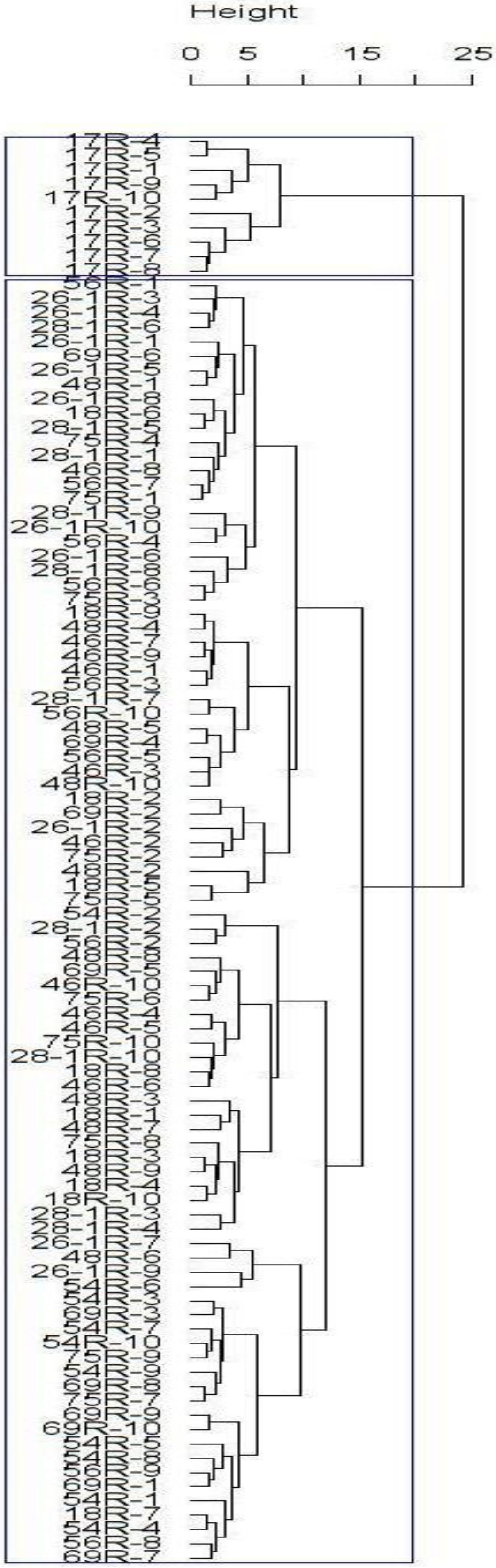


Fig. 1. Cluster dendrogram for F_4 seggregants

The canonical coefficients of plant height and number of leaves imply their independent nature and their contribution towards the variability is also negligible. As the days to fifty percent flowering showed negative trend of canonical loading and cross loading but the magnitude of seed set percentage was found to be positive they are tending to show an inverse association. Similar patterns have been noted in other crops, where phenological traits (such as days to flowering) show negative canonical cross-loadings with reproductive output traits (seed set or yield), while traits more directly related to yield show positive associations. For example, in soybean, Pereira *et al.* (2017) demonstrated that the first canonical variate coupled a reduction in flowering and maturity duration with improved seed germination and emergence traits. Similar trend was also observed by Veena *et al.* (2013). Thus, earliness might be one of the desired characters for restoration. This could be a cause of delayed days to fifty percent flowering, which can make the population to survive in the cold stress effectively, since the *maldandi* cytoplasm is cultivated mainly under the *rabi* season where the cold stress mediated pollen sterility hinders seed set s a major problem in the expected seed set percentage (Kariyannanavar *et al.,* 2024 and Murty, 2024).

The box plot (**Fig. 2**) shows that most of the families under study were on the higher scale for CC1. Further to pinpoint the family that is performing better for both yield and restoration trait, the individual families were subjected to cluster analysis and Multi trait genotype ideotype selection index.

To investigate the genetic divergence among the F_4 recombinants derived from 10 different families, a hierarchical cluster analysis was conducted using the Ward-D² method. The resulting dendrogram distinctly grouped the 100 selections of F_4 families into two major clusters, which showed a clear differences in performance across the population. The first cluster consisted of genotypes only from the family (17R), which consistently exhibited poor performance for both fertility restoration and yield traits. This cluster represented a group of inferior recombinants that could be removed for further genetic improvement. In contrast, the second cluster comprised of genotypes from the remaining nine families, and showed a wider distribution with greater phenotypic variation thus indicating the segregating nature of the population. The clear partitioning of genotypes into two clusters suggested that significant genetic divergence had existed among families, particularly with the 17R family forming a distinct underperforming group.

The MGISI score provided a multivariate selection index that ranked family based on their overall similarity to a defined ideotype. An ideotype was constructed using weighted trait contributions, with the highest weight given for single plant yield, followed by seed set percentage

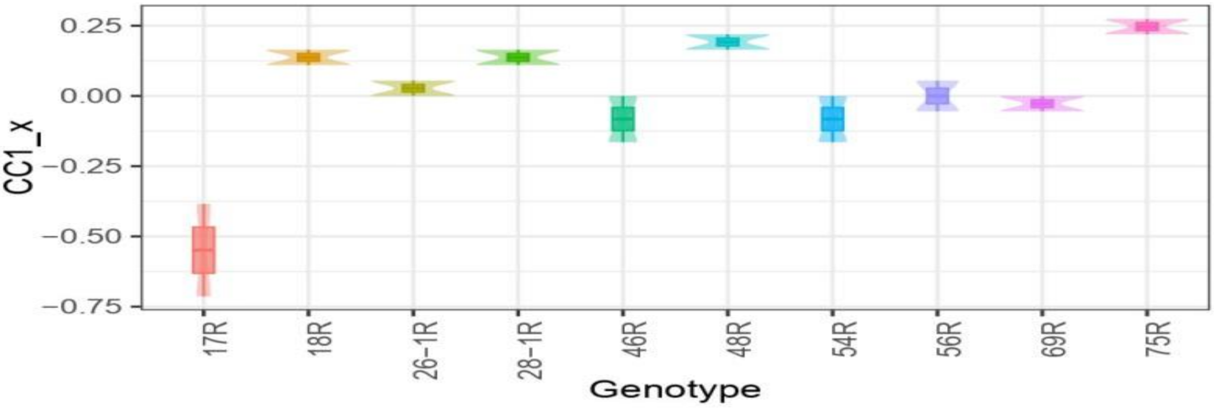


Fig.2. Box plot of 10 F_4 families of the cross PKV Kranti and DSMR-8

which is a measure of restoration and desired agronomic ability, and minimal contribution was provided for days to fifty percent flowering to improve the earliness.

Genotypes with higher MGISI scores were closer to this ideotype and thus exhibited favorable multi-trait performance, particularly for key traits like single plant yield, restoration, and days to fifty percent flowering. In contrast, genotypes with lower or negative MGISI scores were characterized by reduced expression in high weighted traits leading to their ranking farther from the ideotype (Karthik and Hanamaratti, 2025), thus enabling the selection of the F_4 families that have performed well for both restoration and agronomic traits. The range of standardized score for calculating the MGISI value are furnished in the **Table 5.**

Based on this, 28-1R outperformed all others families, for most traits (MGISI score -1.80). The highest score was attained by panicle weight (9.0), single plant yield (8.7), and strong scores in pollen viability traits in both parental as well in the test cross generation were observed [PVF4 (8.5) and PVF41 (8.7)]. Similarly, this family had the maximum scoring for seed-set percentage reflecting its potential use as a restorer. Thus, in this family both the yield and restoration were found to be maximum. The family 75R also showed higher values for panicle weight (8.0), single plant yield (8.2), with a total MGISI score of 0.58 , thus becoming the second improved family related to yield and fertility restoration. The family 54R recorded MGISI of 0.23, with balanced trait expression, making the family suitable for stable yield selection as it showed good weight for yield traits. The families 48R, 46R, 26-1R, and 56R exhibited intermediate performances.18R, and 17R ranked lowest across multiple traits, which indicates the inferior overall yield and restoration performance. Thus, the obtained result was in line with the cluster and box plot analysis.

The superior performance of family 28-1R across multiple traits (1.80) is consistent with the results of Swamy *et al.* (2018), validating the efficiency of selection indices in distinguishing high-yielding and stable restorer families. The observed superiority in panicle weight, single-plant yield, and pollen viability concurs with the findings of Kasanaboina *et al.* (2024), highlighting that fertility restoration potential is intrinsically linked to major yield component traits, with environmental and climatic factors further modulating this association.

Thus, the families 28-1R, 75R and 54R could be given importance in breeding programmes as they could be transferred for the upcoming generation in order to achieve the goal of creating a restorer for *maldandi* cytoplasm combining both the yield traits and restoration traits. On the other hand, the least performing genotype for these characters was 17R suggesting the poor performance for both the target traits. Families like 18R, 26-1R and 28-1R were having average performance for the traits of interest. In concern with the variation among the within the genotype, though they are F_4 seggregants, theoretically speaking the level of homozygosity is expected to be 93.75%. But the present investigation showed a greater variability with in the famliy, for the characters that were evaluated. The presence of considerable within-family variation among certain F_4 segregants in the present study agrees with earlier findings that advanced selfing generations can still exhibit measurable segregation (Longkho *et al*., 2020; Eglington and Moore, 1973). Similar reports of residual heterogeneity despite high theoretical homozygosity have also been documented in cereal crops (Cornish, 1990). One such family was 46R. Contrastingly other the two families namely 18R and 26-1R had narrow boxplot width implicating the presence of lower variability. Thus, the CCA and MGISI analyses had provided an inter connected idea about the association between the yield trait and restoration trait as a whole along with the identification of superior genotypes.

The combined CCA and MGISI analyses provided a comprehensive understanding of the interrelationship between yield components and fertility restoration traits, leading to the identification of superior F_4 families. The CCA results revealed that the studied population possessed promising yield potential and strong restoration ability, while the MGISI analysis identified 28-1R, 75R,

Table 5. MGISI scores for F_4 families of PKV Kranti and DSMR-8

Genotype	Panicle weight	Seedset	Hundred grain weight	Single plant yield	Days to fifty percent flowering	Plant height	Number of leaves	Panicle length	Panicle width	Days to maturity	Pollen viability in F4 generation	Pollen viability in F4:1 generation	Multitrait genotype ideotype selection index
28-1R	9	8.4	8.1	8.7	7.8	8.2	8.3	8.5	8.2	8.8	8.5	8.7	1.80
75R	8	7.8	7.7	8.2	7.6	8	7.7	8	7.9	8.4	8.1	8.3	0.58
54R	8.2	7.6	7.5	8	7.4	7.8	7.5	7.8	7.7	8.2	7.9	8.1	0.23
48R	7.6	7.1	7.3	7.7	7.9	8.3	7.1	7.4	7.3	7.8	7.5	7.8	0.04
46R	7.5	7	7.2	7.6	8	8.5	7.2	7.5	7.4	7.9	7.6	7.9	0.02
26-1R	7.8	7.2	7	7.3	8	8.2	7	7.3	7.2	7.8	7.3	7.6	-0.09
56R	7.9	7.3	7.1	7.5	7.7	8	7.3	7.6	7.5	8	7.7	7.9	-0.11
69R	7.7	7.1	7	7.4	7.6	8.1	7	7.3	7.2	7.7	7.4	7.6	-0.44
18R	7.5	7	6.7	7.4	7.6	8	6.8	7	6.9	7.5	7.1	7.4	-0.73
17R	7.2	6.8	6.5	7	7.5	7.8	6.5	6.8	6.7	7.3	6.9	7.2	-1.30

and 54R as superior families. These families exhibited high single-plant yield, favorable flowering behavior, and enhanced restoration potential. The findings emphasize the importance of single-plant yield and reduced days to 50% flowering in determining the restoration efficiency of genotypes. Consequently, families such as 28-1R, 75R, and 54R can be effectively utilized in future breeding programs as reliable restorers combining both yield and fertility restoration traits. Overall, this study identified novel F_4 families with dual advantages in agronomic performance and fertility restoration, thereby contributing to the diversification of cytoplasmic sources and reducing the dependency on single-cytoplasm-based hybrids in sorghum.

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