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Utilizing morphological variation and genetic divergence for pre-breeding in finger millet (*Eleusine coracana* L.) under Bastar plateau conditions

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Abstract

A pre-breeding study on 100 finger millet (*Eleusine coracana* L.) accessions was undertaken on Kharif 2024 at S.G. CARS of college Agriculture and Research Station, Kumhrawand, Jagdalpur, Bastar (C.G.) to identify promising donors for yield enhancement. Pigmentation traits were useful for parental classification such as leaf juncture pigmentation was observed in GEC-46, while ICO-477132 lacked it. Similarly, pollen pigmentation was present in ICO-476636 and absent in GEC-93, providing distinct donor groups. Trait-based classification highlighted ICO-476838 × GEC-93 for finger length and ICO-476846 × GEC-135 for test weight as potential cross combinations. Flowering synchronisation further enabled compatible crosses such as GEC-181 × ICO-587981 and GEC-122 × ICO-476958. Genetic divergence analysis revealed the widest inter-cluster distance between clusters VIII and IX (11.68), with promising heterotic combinations like GEC-127 × ICO-476958 and GEC-122 × ICO-477385. High-yielding accessions including GEC-53 (24.4 g/plant) and ICO-476711 (20 g/plant) were identified as donors. The integration of pigmentation markers, trait grouping, synchronisation, and divergence analysis strengthens pre-breeding strategies in finger millet.

Keywords: Pre-breeding, cross combination, pigmentation marker, finger length, test weight, flowering synchronisation, genetic divergence

Introduction

Finger millet (*Eleusine coracana* L.) is a self-pollinated allotetraploid cereal crop belonging to the family Poaceae and subfamily Chloridoideae. Cultivated widely in Asia and Africa, it is also referred to as bird's foot millet, ragi, African millet, coracana, and kurukkan. This annual cereal grows between 30-150 cm in height and matures within 75-160 days. Plants are herbaceous with slender leaves and profuse tillering. The panicle is characteristically digitate, comprising 4-19 finger-like spikes radiating from a common point, resembling a clenched fist—hence the name “finger millet.” Its growth cycle is divided into four stages: booting (S1), flowering/anthesis (S2), grain filling (S3), and ripening (S4). Inflorescences bear 2-11 curved or straight fingers with the basal spike often referred to as the thumb. Owing to its adaptability across diverse agro-ecological regions, it is largely cultivated under rainfed conditions in India (Singamsetti, 2018) [14].

Globally, finger millet accounts for ~3.7 million tonnes annually, harvested from about 6.1 million ha with an average productivity of 600 kg/ha. It represents ~12% of the world's millet area, making it one of the most important small millets in tropical regions. In India, it dominates minor millet cultivation, contributing ~81% of total production. The crop occupies ~1.0 million ha with a yield of 1.76 million tonnes and an average productivity of 1747 kg/ha. While cultivation area and production have declined in recent years, productivity has improved through the release of superior varieties. Major producing states include Karnataka, Tamil Nadu, Uttarakhand, Maharashtra, Andhra Pradesh, Odisha, Jharkhand, Gujarat, West Bengal, Bihar, and Chhattisgarh (Thakur *et al.*, 2023) [16]. Morphological characterization plays a pivotal role in identifying desirable genotypes that can serve directly as cultivars or act as donors in breeding programs (Upadhyaya *et al.*, 2006). Finger millet is primarily autogamous (Goron *et al.*, 2015), with anther dehiscence coinciding with style and filament elongation, ensuring the stigma is enveloped by pollen at anthesis.

This arrangement greatly reduces cross-pollination (Dodake *et al.*, 1998; Gupta *et al.*, 2012) ^[2, 5]. Since stigmas are often inconspicuous and anther dehiscence occurs within the palea under high temperatures, the natural outcrossing rate seldom exceeds 1% (Dodake *et al.*, 1998; Seetharam, 1998) ^[2, 13]. Although cross-pollination is widely used to create genetic variability in crop plants (Krishna *et al.*, 2017) ^[7], systematic breeding strategies in finger millet remain limited. Early evidence of outcrossing in improved lines was documented in 1934 (Ayyangar), but subsequent breeding progress has been relatively modest (Reddy *et al.*, 2013) ^[11]. Nevertheless, diverse germplasm collections offer substantial variability, forming the basis for genetic improvement. To broaden the crop’s genetic base, the use of efficient crossing techniques is critical (Rizal *et al.*, 2015) ^[12]. Traditionally, emasculation in finger millet has been achieved through manual and hot-water methods (Sood *et al.*, 2016) ^[15]. The hot-water technique has proven superior due to its efficiency, reduced labor demand, and higher success rate compared to hand emasculation (Sood *et al.*, 2016; Rao *et al.*, 1962) ^[15, 10]. Moreover, hybrids produced by hot-water emasculation often display greater genetic purity (Krishna *et al.*, 2019) ^[8]. Pollination is done extremely early in the morning, that is, before 6 a.m., and hand emasculation is done in the evening. The emasculation of florets using hot water is also effective. Based on the percentage of hybrid seed-set, hot water treatment at 52 °C for two minutes was the most effective (Ganapathy, 2021) ^[3]. Purple pigmentation at the seedling stage has also been established as a reliable marker for hybrid identification (Krishnappa *et al.*, 2009) ^[9].

Materials and Methods

The present study was conducted at the Instructional-cum-Research Farm, S.G. College of Agriculture and Research Station, Kumhrawand, Jagdalpur, Bastar (Chhattisgarh), situated at 19°4’0” N latitude, 82°2’0” E longitude, and an altitude of 552 m above mean sea level. The experiment aimed to evaluate the performance of genotypes and undertake pre-breeding studies for yield and related traits in finger millet (*Eleusine coracana* L.). A set of 100 genotypes was planted using the Augmented Complete Block Design (ACBD). The crop was sown on 31st August 2024, maintaining 23 cm row-to-row and 10 cm plant-to-plant spacing. Standard agronomic practices were followed to ensure healthy crop establishment and growth. Data were recorded on 15 traits, comprising 12 quantitative and 3 qualitative characters. The quantitative traits included days

to 50% flowering, days to maturity, plant height (cm), flag leaf length (cm), flag leaf width (cm), finger length (cm), peduncle length (cm), number of fingers per plant, number of tillers per plant, number of grains per plant, test weight (g), and grain yield per plant (g). The qualitative traits assessed were plant pigmentation, pollen colour, and ear shape.

Results and Discussion

In the present experiment, possible cross combinations among finger millet genotypes were inferred using dominant morphological markers, genetic divergence, test weight, finger length, and flowering synchronization as key criteria.

Dominant morphological markers

In finger millet genotypes, the presence of purple pigmentation at the leaf juncture or nodal region and the occurrence of pink-colored pollen were considered as dominant morphological markers.

Pigmentation at leaf Juncture

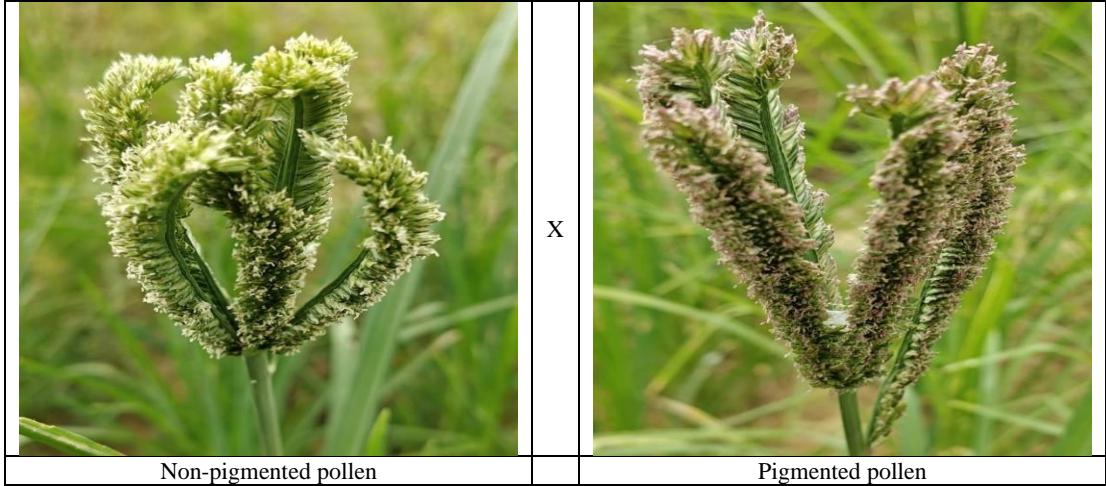
Purple pigmentation expressed at the seedling stage is widely recognized as a reliable marker for confirming hybridization success in finger millet (Krishnappa *et al.*, 2009) ^[9]. Crosses between pigmented male and non-pigmented female genotypes can be validated by the emergence of purple pigmentation at the leaf base in the resulting progeny, which confirms effective pollen transfer. In support of this, Veligatla *et al.* (2023) ^[18] demonstrated that pigmentation appeared in otherwise non-pigmented female genotypes (GPU 28 and GPU 67) when pollinated by pigmented male parents (IR and CG Ragi 02). In current study, the pigmented leaf juncture or nodal regions were observed in genotypes GEC-398, ICO-476789, IR-1, ICO-476636, ICO-477385, ICO-476711, ICO-476511, ICO-476520, ICO-587964, CG RAGI-2, GEC-46, ICO-477838, GEC-421, GEC-437, ICO-477426, ICO-587989, ICO-476687, ICO-476378, ICO-477323, GEC-109, ICO-477601, GEC-440, ICO-477314, ICO-476913 while those genotypes with absent pigmentation were ICO 476838, GEC-93, GEC-181, GEC-122, ICO-476958, ICO-477132, GEC-452, ICO-477831, ICO-476409, ICO-587981, ICO-476921, GEC-147, GEC-310, ICO-477659, GEC-5, ICO-476707, ICO-477017, GEC-161, ICO-476539, GEC-58, GEC-446, ICO-476216, GEC-92, GPU-28, ICO-477340 X, etc. The potential genotype combinations identified for crossability assessment are presented in Table 1.



Pollen pigmentation

Pollen pigmentation can be used as a crucial morphological marker to evaluate successful transfer of pollen grain from male parent. Further, difference in pollen pigmentation depicted presence of genetic variability among the genotypes (Gupta *et al.*, 2024) ^[6]. Crosses involving pigmented male and non-pigmented female parents can be

confirmed by the occurrence of pink pollen in the progeny. In the present study, the pigmented genotypes were GEC-398, ICO-476789, IR-1, ICO-476636, ICO-477385, ICO-476711, ICO-476511, ICO-476520, ICO-587964, CG RAGI-2, etc. The potential genotype combinations identified for crossability assessment are presented in Table 2.



Mahalanobis D² Genetic divergence

Mahalanobis D² statistics serve as an effective tool for evaluating genetic divergence and identifying potential genotype combinations for pre-breeding in finger millet. By selecting donor parents occupying distinct positions in the dendrogram, breeders can exploit heterosis and develop superior recombinants. Large inter-cluster distances highlight the most promising pairs for hybridization, while high intra-cluster distances indicate substantial genetic variability within a cluster. In the present study, the maximum inter-cluster distance was recorded between Cluster VIII and Cluster IX (11.683), followed by Cluster VI and VIII (11.024), Cluster IV and VIII (11.023), Cluster I and VIII (10.730), and Cluster V and VIII (10.574). These

results suggest that crosses involving genotypes from Cluster VIII and IX are most promising for generating heterotic hybrids, followed by combinations between Cluster VIII and VI. The present findings align with the results of Gupta (2024) ^[6], who reported the highest inter-cluster divergence between Cluster I and V (4.686), followed by Cluster V and IV (4.560), Cluster III and IV (4.203), and Cluster I and IV (4.115). Consequently, crosses between genotypes of Cluster V and I, followed by Cluster V and IV, were recommended for producing heterotic individuals. Based on these observations, the selected genotype combinations suitable for crossability studies are summarized below.

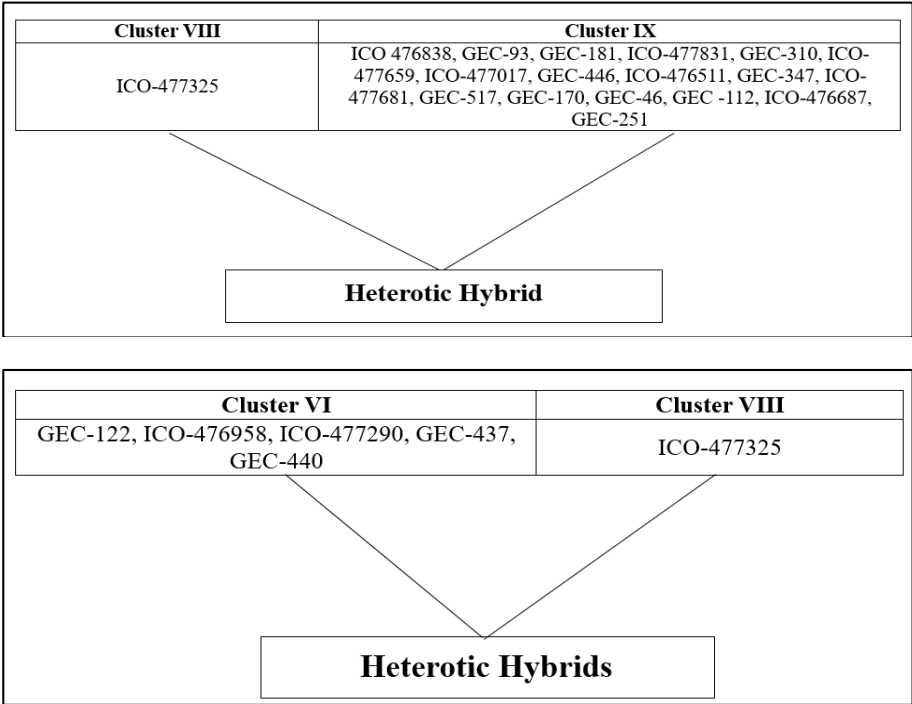


Table 1: Depiction of pigmented and non-pigmented genotypes

Pigmented genotypes at leaf juncture	Non-Pigmented genotypes at leaf juncture
GEC-398, ICO-476789, IR-1, ICO-476636, ICO-477385, ICO-476711, ICO-476511, ICO-476520, ICO-587964, CG RAGI-2, GEC-46, ICO-477838, GEC-421, GEC-437, ICO-477426, ICO-587989, ICO-476687, ICO-476378, ICO-477323, GEC-109, ICO-477601, GEC-440, ICO-477314, ICO-476913	ICO 476838, GEC-93, GEC-181, GEC-122, ICO-476958, ICO-477132, GEC-452, ICO-477831, ICO-476409, ICO-587981, ICO-476921, GEC-147, GEC-310, ICO-477659, GEC-5, ICO-476707, ICO-477017, GEC-161, ICO-476539, GEC-58, GEC-446, ICO-476216, GEC-92, GPU-28, ICO-477340 X, GEC-314, ICO-477561, ICO-587961, ICO-475933, GEC-53, GEC-347, ICO-587985, ICO-477681, ICO-587982, GEC-517, GEC-341, GEC-170, GEC-127, ICO-477290, GEC-112, GEC-134, ICO-476495, GEC-400, GEC-331, GEC-496, ICO-77264, GEC-254, GEC-488, GPU-67, GEC-259, GEC-105, GEC-313, ICO-477556-X, ICO-476299, ICO-476846, GEC-485, ICO-477890, ICO-476558-X, ICO-476882, GEC-473, ICO-477304, ICO-477166, GEC-135, GEC-260, ICO-477274, GEC-222, ICO-477325, GEC-415, GEC-362, GEC-331, ICO-477591, GEC-69, GEC-144, ICO-477429

Table 2: Representation of genotypes with pigmented pollen and non-pigmented pollen

Pigmented pollen	Non-pigmented pollen
GEC-398, ICO-476789, IR-1, ICO-476636, ICO-477385, ICO-476711, ICO-476511, ICO-476520, ICO-587964, CG RAGI-2, GEC-46, ICO-477838, GEC-421, GEC-437, ICO-477426, ICO-587989, ICO-476687, ICO-476378, ICO-477323, GEC-109, ICO-477601, GEC-440, ICO-477314, ICO-476913	ICO 476838, GEC-93, GEC-181, GEC-122, ICO-476958, ICO-477132, GEC-452, ICO-477831, ICO-476409, ICO-587981, ICO-476921, GEC-147, GEC-310, ICO-477659, GEC-5, ICO-476707, ICO-477017, GEC-161, ICO-476539, GEC-58, GEC-446, ICO-476216, GEC-92, GPU-28, ICO-477340 X, GEC-314, ICO-477561, ICO-587961, ICO-475933, GEC-53, GEC-347, ICO-587985, ICO-477681, ICO-587982, GEC-517, GEC-341, GEC-170, GEC-127, ICO-477290, GEC-112, GEC-134, ICO-476495, GEC-400, GEC-331, GEC-496, ICO-77264, GEC-254, GEC-488, GPU-67, GEC-259, GEC-105, GEC-313, ICO-477556-X, ICO-476299, ICO-476846, GEC-485, ICO-477890, ICO-476558-X, ICO-476882, GEC-473, ICO-477304, ICO-477166, GEC-135, GEC-260, ICO-477274, GEC-222, ICO-477325, GEC-415, GEC-362, GEC-331, ICO-477591, GEC-69, GEC-144, ICO-477429

Flowering synchronisation

Finger millet is recognized as a predominantly self-fertilizing crop (Goron *et al.*, 2015) ^[4]. During anthesis, the simultaneous growth of the style with the filaments ensures that the stigma remains enclosed within a dense cloud of pollen grains, thereby restricting opportunities for natural outcrossing (Dodake *et al.*, 1998; Gupta *et al.*, 2012) ^[2, 5]. For effective hybridization, however, synchronization of flowering between parental lines is crucial. Aligning genotypes that reach 50% flowering at the same time improves the chances of successful pollination and seed development. In the present investigation, genotypes were classified according to their flowering periods, allowing identification of those with overlapping phases. These groupings serve as a practical basis for selecting potential parental combinations. Together with dominant morphological markers (leaf juncture pigmentation and pollen colour) and genetic divergence analysis, flowering synchronization formed an important criterion in determining the crossability prospects among finger millet genotypes. On the basis of flowering synchronization, possible crosses can be made among genotypes GEC-181, ICO-587981, ICO-476958, ICO-477659, ICO-476707, ICO-476539, ICO-476511, GEC-347, GEC-112, ICO-476378, ICO-477556-X, ICO-476299, GEC-109, ICO-476558-X, ICO-477325, GEC-251 as there days to 50% flowering were similar (50-55 DAS) and genotypes which took 56-60 days for 50% flowering were ICO 476838, GEC-93, ICO-476786, ICO-477831, ICO-476409, GEC-310, ICO-477017, GEC-161, ICO-476711, GEC-58, etc. The detailed grouping of genotypes for synchronized flowering and the corresponding cross combinations is presented in Table 3.

Crossability through test weight

Genotypes exhibiting wide variation in test weight generally reflect diverse genetic constitutions, making them promising candidates for hybridization to generate useful variability. The inclusion of parents with consistently high-test weight can further enhance seed development, grain filling, and seed viability, thereby contributing to improved yield

potential in subsequent segregating generations. In this study, test weight was considered as one of the important parameters for identifying potential cross combinations among finger millet genotypes and is categorized into three groups i.e., genotypes with test weight between 3.00-3.5 g (ICO-587981, ICO-476707, ICO-476711, GEC-92, ICO-476511, GEC-314, GEC-341, ICO-477838, GEC-331, GEC-400, GEC-496, ICO-77264, GEC-254), genotypes those with a test weight of 3.51-4 g (GEC-135, ICO-476558-X, ICO-476299, ICO-476378, GEC-488, GEC-112) and 4.1-4.5 g (ICO-476846, ICO-477325). The results are summarized in Table 4.

Crossability through Finger length

Finger length, representing the length of the spike-like digits on the panicle, is a critical yield-contributing trait in finger millet. Genotypes exhibiting contrasting finger lengths are likely to differ in their genetic makeup and growth patterns, making them valuable candidates for hybridization. Crossing such diverse parents can increase the probability of obtaining transgressive segregants with superior panicle architecture and enhanced grain yield in subsequent generations.

In the present investigation, finger length was employed as one of the important criteria for assessing crossability among genotypes (Table 5). The genotypes were divided into two groups i.e., genotypes with finger length between 7-10 cm (GEC-122, ICO-476958, ICO-477132, ICO-587981, ICO-476921, ICO-476216, GEC-314, ICO-477561, ICO-587961, ICO-587985, GEC-341, ICO-476495, etc.) and genotypes with finger length 10.1-13 cm (ICO-477385, ICO-476711, GEC-53, GEC-127, ICO-477325). By grouping genotypes based on this character, divergent parental lines were identified that may serve as promising sources in hybridization programs. This strategy not only facilitates the improvement of yield-related traits but also enhances the efficiency of selection and the potential for generating desirable recombinants in the breeding population.

Table 3: Possible crosses on the basis of days to 50% flowering

Days to 50% flowering	Selection of parents for making possible crosses
50-55 DAS	GEC-181, ICO-587981, ICO-476958, ICO-477659, ICO-476707, ICO-476539, ICO-476511, GEC-347, GEC-112, ICO-476378, ICO-477556-X, ICO-476299, GEC-109, ICO-476558-X, ICO-477325, GEC-251
56-60 DAS	ICO 476838, GEC-93, ICO-476786, ICO-477831, ICO-476409, GEC-310, ICO-477017, GEC-161, ICO-476711, GEC-58, GEC-446, ICO-476216, GEC-92, ICO-477340 X, GEC-314, ICO-475933, ICO-476520, ICO-477681, ICO-587982, ICO-587964, GEC-341, GEC-170, GEC-127, GEC-46, ICO-477838, GEC-400, GEC-496, ICO-77264, GEC-254, ICO-587989, GEC-488, GEC-259, ICO-476687, GEC-105, ICO-476846, GEC-485, ICO-477890, ICO-476882, ICO-477304, ICO-477166, ICO-477601, GEC-260, GEC-222, GEC-415, GEC-362, GEC-69, GEC-147, GPU-67
61-65 DAS	GPU-28, ICO-477274, GEC-473, GEC-313, GEC-421, ICO-476495, GEC-134, GEC-517, ICO-587985, ICO-587961, ICO-477561, ICO-477385, GEC-452, ICO-477132, GEC-398
66-70 DAS	ICO-476921, ICO-476636, GEC-5, ICO-4587989, ICO-477426, GEC-135, ICO-477314, GEC-331
71-75 DAS	CG RAGI-2, IR-01, ICO-477429, ICO-476913, ICO-477323, ICO-477290
76-80 DAS	GEC-122, ICO-476958

Table 4: Possible crosses on the basis of test weight (g)

Test weight (g)	Selection of parents for making possible crosses
3.00-3.5	ICO-587981, ICO-476707, ICO-476711, GEC-92, ICO-476511, GEC-314, GEC-341, ICO-477838, GEC-331, GEC-400, GEC-496, ICO-77264, GEC-254, GEC-437, ICO-477426, ICO-587989, GEC-259, GEC-105, ICO-477323, GEC-109, GEC-485, ICO-477890, GEC-473, ICO-477304, ICO-477601, GEC-440, ICO-477314, ICO-476913, GEC-251, GEC-69, ICO-477429, IR-01, GPU-67
3.51-4.0	GEC-135, ICO-476558-X, ICO-476299, ICO-476378, GEC-488, GEC-112
4.01-4.5	ICO-476846, ICO-477325

Table 5: Possible crosses on the basis of finger length

Finger length	Selection of parents for making possible crosses
7-10 (cm)	GEC-122, ICO-476958, ICO-477132, ICO-587981, ICO-476921, ICO-476216, GEC-314, ICO-477561, ICO-587961, ICO-587985, GEC-341, ICO-476495, GEC-421, GEC-400, ICO-77264, GEC-437, GEC-313, ICO-477323, GEC-473, GEC-222, ICO-476913, GEC-144, ICO-477429, IR-01
10.1-13 (cm)	ICO-477385, ICO-476711, GEC-53, GEC-127, ICO-477325

Conclusion

In the present pre-breeding cataloguing study, five major criteria were considered for evaluating the genotypes: presence of morphological markers, extent of genetic divergence, flowering synchrony, variation in test weight, and finger length. Pigmented lines such as GEC-398, CG Ragi-02, and ICO-476636 were identified as potential male parents, while non-pigmented types like GEC-93 and GEC-112 were better suited as female parents. These pigmentation traits, observed at the leaf node and in pollen, were particularly useful as reliable morphological markers for confirming true F₁ progeny. The assessment of genetic divergence through Mahalanobis D² statistics grouped the genotypes into ten distinct clusters. Among these, Clusters VIII and IX (11.683) were the most genetically divergent, indicating that crosses such as ICO-477325 × GEC-112 are promising for exploiting maximum heterosis. The next highest divergence was observed between Cluster VI and Cluster VIII (11.024), suggesting further opportunities for creating genetically diverse hybrids.

Genotypes were also classified based on flowering time to ensure crossing compatibility. Early to medium flowering types between 50-55 DAS (e.g., GEC-181, ICO-587981, ICO-476958) and 56-60 DAS (e.g., ICO-476838, GEC-93, ICO-476786, ICO-477831, ICO-476409) were identified as suitable groups for synchronized hybridization. Substantial variability was also recorded in test weight, ranging from 2.2 g to 4.04 g. Genotypes like ICO-477325 (4.04 g) and ICO-476846 (4.01 g) were recognized as promising donor lines for improving grain quality traits. Similarly, finger length exhibited considerable diversity, ranging between 10.1 cm to 13 cm, with genotypes such as ICO-477385, ICO-476711, GEC-53, GEC-127, and ICO-477325 showing

superior values. Since finger length is a vital yield component, this variation highlights their potential to generate transgressive segregants with improved panicle architecture and grain yield.

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