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Genetic enhancement of inbred lines through pre-breeding in maize

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Abstract

The enhancement of genetic yield potential in maize has resulted in the development of a vast number of cultivars globally. However, this progress has gradually narrowed the genetic diversity among modern maize cultivars. To broaden the genetic base, pre-breeding through wide hybridization between elite inbred lines and wild species is essential. In this study, 69 elite inbred lines were crossed with two wild relatives of maize — *Zea mays* ssp. *parviglumis* (WS-5) and *Zea luxurians* (WS-1). The resulting interspecific hybrids exhibited typical wild species traits such as tillering, prolificacy on both main and side tillers, and distinctive ear characteristics, indicating successful introgression. Combining ability analysis revealed significant variability among parents and crosses for most studied traits. *Zea luxurians* was a good general combiner for all traits except tassel length, anthesis-silking interval, and days to 75% dry husk based on General Combining Ability (GCA) effects. Inbred lines IC0621049 and IC0621565 were also identified as good general combiners, except for days to 75% dry husk. Significant Specific Combining Ability (SCA) effects in the desirable direction were observed in crosses such as PFSR-3 × WS-5, EC618215 × WS-1, and IC213122 × WS -1 for traits like plant height, ear diameter, kernel rows per ear, kernels per row, ear weight, and grain yield per ear. SSR marker-based molecular analysis confirmed hybridity. Cluster analysis grouped all parental lines into two clusters, with most inbred lines clustering with WS-5, suggesting closer genetic relatedness to WS-5 than to WS-1, which could explain the higher success rate of crosses with WS-5.

Abbreviations

ANOVA – Analysis of Variance
ASI – Anthesis-Silking Interval
bp – Base Pair
CIMMYT – International Maize and Wheat Improvement Center
CTAB – Cetyltrimethylammonium Bromide
CW – Cob Weight
DARwin – Diversity Analysis and Representation for Windows
DFS – Days to 50% Silking
DFT – Days to 50% Tasseling
DNA – Deoxyribonucleic Acid
dNTPs – Deoxyribonucleotide Triphosphates
DT75DH – Days to 75% Dry Husk
DUS – Distinctness, Uniformity and Stability
ED – Ear Diameter
EL – Ear Length
F_1 – First Filial Generation
GCA – General Combining Ability
GYPC – Grain Yield per Cob
NKPR – Number of Kernels per Row
NKRPE – Number of Kernel Rows per Ear
PCR – Polymerase Chain Reaction
PH – Plant Height
PPV&FRA – Protection of Plant Varieties and Farmers' Rights Authority
RCBD – Randomized Complete Block Design
RNA – Ribonucleic Acid
RNase – Ribonuclease
SCA – Specific Combining Ability
SEm – Standard Error of Mean
SSR – Simple Sequence Repeat
TAE – Tris-Acetate-EDTA
Taq – *Thermus aquaticus*
TE – Tris-EDTA
TL – Tassel Length
UPGMA – Unweighted Pair Group Method with Arithmetic Mean
UV – Ultraviolet
WS – Wild Species
WS-1 – *Zea luxurians*
WS-5 – *Zea mays* ssp. *parviglumis*

Introduction

Maize (*Zea mays* L.), a member of the tribe Maydeae in the family Poaceae, is the third most significant cereal crop globally, following wheat and rice. As a C4 plant with a chromosome number of 2n = 20, maize exhibits high photosynthetic efficiency and is inherently cross-pollinated, necessitating careful measures to maintain genetic purity. It is a monoecious, protandrous, short-day plant with a determinate growth habit, first domesticated nearly 8000 years ago and introduced to India by the Portuguese in the 16th century (Suto and Yoshida, 1956). Globally, maize is cultivated on more than 200 million hectares, with total production exceeding 1.2 billion tons annually (FAOSTAT, 2023). In India, maize ranks third in importance after rice and wheat, covering approximately 10.4 million hectares and yielding around 33.7 million tons during the 2022–23 season (Ministry of Agriculture & Farmers Welfare, 2024). Future projections indicate that maize demand in developing countries is expected to double by 2050, driven by population growth and industrial applications. However, achieving this target is hindered by both abiotic and biotic stresses, which drastically affect productivity (Rosegrant *et al.*, 2009). Given the constraints on land expansion and the environmental repercussions of unsustainable cultivation, there is a pressing need to develop climate-resilient cultivars (Cairns *et al.*, 2012). Climate projections forecast substantial challenges to food security, emphasizing the urgency for yield enhancement through genetic improvement. Harnessing the full spectrum of genetic diversity is imperative to meet these challenges. While landraces and heirloom cultivars retain valuable diversity, their low yields present a major limitation. Pre-breeding programs play a critical role in bridging this gap by transferring useful traits from wild relatives to elite inbred lines (Dempewolf *et al.*, 2014). Wild relatives of maize have historically contributed to crop improvement, offering beneficial alleles for kernel traits and stress tolerance through sexual hybridization (William *et al.*, 2007; Wang *et al.*, 2008; Karn *et al.*, 2017).

Effective crop improvement depends on the extent of genetic variability in a population. This variability can stem from genetic makeup or environmental conditions and is quantified through parameters like genotypic and phenotypic coefficients of variation, heritability, and genetic advance (Johnson *et al.*, 1955). High heritability combined with substantial genetic advance indicates strong potential for selection-based improvement. Hybrid breeding relies heavily on the identification of superior inbred lines. Combining ability analysis, particularly through line × tester mating designs, helps estimate general combining ability (GCA) and specific combining ability (SCA), which are crucial for selecting parents and optimal hybrid combinations (Kempthorne, 1957; Abdel-Moneam *et al.*, 2014).

While morphological traits are informative, they are often influenced by the environment. Molecular markers, particularly simple sequence repeats (SSRs), offer an environment-independent and robust approach for genotyping. Due to their high polymorphism, reproducibility, and automation compatibility, SSRs are extensively used in maize for fingerprinting and diversity analysis (Smith *et al.*, 1997). Molecular grouping of hybrids based on SSR data helps assess genetic diversity, validate parentage, and guide breeding decisions (Fan *et al.*, 2018). This approach is particularly valuable when introgressing wild genomes into the cultivated gene pool, as it clarifies genetic relationships among hybrids and their wild progenitors, ultimately contributing to the broadening of genetic architecture. Considering these factors, the present study was undertaken to characterize the morphology of wild species and hybrids, determine the combining ability (GCA and SCA) of inbred lines with wild species, and group the developed hybrids using molecular markers.

Materials and Methods

Plant Material and Hybrid Development

Sixty-nine elite maize inbred lines and two wild relatives - *Zea luxurians* (WS-1) and *Zea mays* subsp. *parviglumis* (WS-5) - were selected as parental material. These were sown at the Winter Nursery Centre, Rajendranagar 17.322168°N,78.400616°E, during *Kharif* 2022, using an Alpha Lattice Design with two replications for selfing and evaluation. Standard crop management practices were followed, and traits of the parental lines such as plant height, tassel length, ear length, ear diameter, number of kernel rows per ear, number of kernels per row, cob weight, and grain yield per plant were recorded as per guidelines. In case of wild species some distinctive traits were recorded: days to flowering, no. of tillers per plant, no. of tassels per plant, no. of silks per plant, no. of nodes, internodal length, leaf length and leaf width. Crosses were made using a line × tester mating design, resulting in 63 successful interspecific F_1 hybrids. Specifically, 40 lines were crossed with WS-5, seven lines with WS-1, and eight lines were common to both. In total, 48 inbred lines participated in crosses with WS-5 and 15 with WS-1. Due to this imbalance, Henderson's method was employed for statistical correction, using the Line × Tester analysis package from CIMMYT, Mexico, to obtain valid estimates of general and specific combining ability.

Hybrid Evaluation and Phenotypic Trait Measurement.

The 63 interspecific hybrids were evaluated during Rabi 2022–23 at the same location using a Randomized Complete Block Design (RCBD) with two replications. Of the 63 hybrids, only 58 hybrids were germinated. These 58 hybrids were developed from 50 inbred lines and two wild species. The germinated hybrids were used for further study. Each hybrid was planted in a single row of 3 meters length with a row-to-row spacing of 60 cm and plant-to-plant spacing of 20 cm. Recommended agronomic practices were uniformly applied. Observations were recorded on three randomly selected plants per plot for a set of 12 agronomic and yield-related traits, including days to 50% tasseling, days to 50% silking, anthesis-silking interval, days to 75% dry husk, plant height, tassel length, ear length, ear diameter, number of kernel rows per ear, number of kernels per row, cob weight, and grain yield per plant. Data collection adhered to the PPV&FRA DUS test guidelines, and genetic advance as percent of mean was interpreted following Johnson *et al.* (1955).

Genomic DNA Isolation and Quality Assessment

Leaf tissue samples were collected from the 69 inbred lines, two wild species, and 63 hybrids. Parental tissues were collected during *Kharif* 2022, and hybrids during *Rabi* 2022–23 and stored at –80°C until use. Genomic DNA was extracted using the CTAB method described by Doyle and Doyle (1990), with minor modifications. The process of DNA extraction involved tissue grinding using a tissue lyser, lysis in CTAB buffer, purification with chloroform:isoamyl alcohol (24:1), and precipitation with cold isopropanol, followed by washing with 70% ethanol. The dried DNA pellet was rehydrated in 1X TE buffer. To eliminate RNA contamination, samples were treated with RNase A (10 mg/ml), incubated at 37.5°C for 1 hour, followed by deactivation at 60°C. DNA purity and concentration were quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific). Samples showed concentrations between 200–3000 ng/µl, and were diluted to 100 ng/µl for downstream use.

SSR Marker Amplification and Electrophoresis

A set of 12 maize specific polymorphic SSR primers were employed to assess the genetic diversity and cluster the interspecific hybrids. PCR was carried out in 10 µl reaction volumes, containing 2.5 mM SSR primers, 2.5 mM dNTPs (Takara), *Taq* DNA polymerase (1 U/µl; Himedia), and template DNA (2.0 µl). Amplifications were performed in an Applied Biosystems™ Veriti™ 96-well thermal cycler, following standard cycling parameters. PCR products were resolved on 3% agarose gels prepared in 1X TAE buffer, stained with ethidium bromide (10 mg/ml), and electrophoresed at 120 V for ~90 minutes. A 100 bp DNA ladder (Takara) was used to estimate band sizes. DNA bands were visualized under a UV transilluminator and documented using a BIO-RAD gel documentation system.

Marker based grouping of developed hybrids

SSR Scoring

SSR scoring was performed based on the presence ('1') or absence ('0') of bands in inbred lines, wild species, and hybrids, using gel images captured by the BIO-RAD documentation system (BioRad Laboratories, India). Band sizes were analyzed with Alpha Imager software, using a 100 bp DNA ladder (Thermo Fisher Scientific) as the size standard.

Grouping of hybrids

The binary data matrix (score '1' and '0') of the alleles of SSR marker obtained from parents {lines, testers (wild species)} and hybrids were subjected to grouping using DARwin 6.0.021 software (Perrier and Jacquemoud, 2006) through which a radial tree diagram was generated. Based on the radial tree diagram, hybrids were assigned to specific groups based on similarity.

Statistical Analysis

Phenotypic data were subjected to Analysis of Variance (ANOVA), and genetic variability parameters such as heritability, genetic advance, and genotypic and phenotypic coefficients of variation were estimated. Combining ability analysis was conducted using Henderson's method, accounting for the unbalanced mating design, and implemented through CIMMYT's Line × Tester analysis tool (Henderson, C.R. 1952).

Results and discussion

Morphological characterisation of wild species

Wild species exhibited several characteristic traits that were absent in the maize inbred lines. Data on various morphological characteristics were recorded, and the mean values are presented in Table 1. The data presented in Table 1 are descriptive in nature and were used to characterize the distinguishing morphological features of wild relatives and cultivated maize; therefore, inferential statistical analyses were not performed. The observations revealed that the wild species exhibited protogyny, whereas cultivated maize is predominantly protandrous. In addition, the wild species displayed tillering, prolificacy on both main and side tillers, and

Table 1 - Comparative morphological characteristics of wild relatives and cultivated maize

Traits recorded	*Zea mays ssp. parviglumis*	*Zea luxurians*	*Zea mays*
Days to anthesis	92.0	107.0	62.0
Days to silking	89.0	104.0	65.0
No. of tillers per plant	8.8	2.2	0.0
No. of tassels per plant	21.0	12.0	1.0
No. of silks per plant	80.3	14.8	1-2
Ear length (cm)	3.2	3.8	15.8
No. of kernel rows per ear	2.0	1.7	14.7
No. of nodes per plant	98.5	20.3	13.5
Internodal length (cm)	14.8	18.5	9.5
Leaf length (cm)	53.4	63.3	78.3
Leaf width (cm)	5.3	6.4	7.2

the production of multiple tassels per plant, traits that were absent in the inbred lines. In the wild species, multiple tassels emerged from side branches, while silks and ears developed from the nodes. On average, each node produced a silk-bearing ear. Compared with cultivated maize, the wild species possessed a greater number of nodes and longer internodes. They also exhibited markedly shorter ears and fewer kernel rows per ear, highlighting the effects of domestication and artificial selection during maize improvement. Among the wild relatives, *Zea mays ssp. parviglumis* recorded a higher number of tillers (8.8), tassels (21.0), silks (80.3), and nodes per plant (98.5) than *Zea luxurians*. A similar trend in the expression of wild-species characteristics has been reported by Adhikari *et al*. (2019).

Morphological characterization of hybrids

In the hybrids, characteristic wild-species traits such as tillering and prolificacy on both main and side tillers were observed and are presented in Table 2. The number of tillers ranged from 1.0 to 8.3, with a mean of 3.81, while the number of silks ranged from 1.0 to 8.0, with a mean of 3.05. Among the F_1 hybrids, IC0612770 × WS-5 recorded the highest number of tillers (8.3), followed by IC0621166 × WS-5 (6.6), IC0621634 × WS-5 (6.5), IC212875 × WS-5 (6.3), IC420922 × WS-1 (6.2) and IC522300 × WS-1 (6.2). For prolificacy, as measured by the number of silks, IC522300 × WS-1 exhibited the highest value (8.0), followed by IC212875 × WS-1 (7.0), IC0612832 × WS-5 (6.4), MIL6-2 × WS-5 (5.5) and IC0621166 × WS-5 (5.5). The presence of these traits in the F_1 hybrids suggests successful introgression and expression of wild-species characteristics in the cultivated background. Figures 1 and 2 illustrate prolificacy on the main and side tillers and the tillering habit observed in the hybrids. The relatively low CV values for number of tillers (12.11%) and number of silks (11.43%) indicate good experimental precision and reliability of the recorded observations

Combining ability in maize

Analysis of variance (Table 3) revealed significant differences in replications for traits such as plant height, tassel length, days to 50% tasseling and silking, anthesis-silking interval, and days to 75% dry husk. All treatments showed highly significant variation, indicating sufficient genetic variability among genotypes. While inbred lines differed significantly for several traits, testers did not, yet hybrids showed significant variation across all traits. This suggests the influence of non-additive gene action, including dominance and overdominance, likely due to complementary alleles at different loci (Ahmed *et al.*, 2020; Kumar *et al.*, 2022). Such interactions can lead to enhanced hybrid performance through additive × dominant or dominance × dominance effects (Tulu *et al.*, 2021; Singh *et al.*, 2023). These findings emphasize the role of hybrid vigor and underline the importance of identifying suitable parental combinations for trait improvement (Reddy *et al.*, 2024).

The trait-wise estimates of General Combining Ability (GCA) and Specific Combining Ability (SCA) are presented in Tables 4 and 5, respectively. The GCA effects revealed that none of the lines or testers were good general combiners for all 12 quantitative characters studied. However, among the testers, WS-1 was a good general combiner for all the traits except tassel length, anthesis silking interval, and days to 75% dry husk. Among the lines EC618215, IC0621049, IC0621061, IC0621565, IC522300, and IC563961 were good general combiners for plant height, tassel length, days

Table 2 - Mean values for Number of tillers and silks observed in hybrids

Hybrid. No	Hybrid	No. of tillers	No. of Silks	Hybrid. No	Hybrid	No. of tillers	No. of Silks
H1	BJIM-08-27× WS-5	4.6	3.3	H33	IC212876× WS-5	4.8	3.0
H2	BML-7× WS-5	2.5	3.0	H34	IC213004× WS-5	5.0	4.0
H3	EC0758189× WS-5	6.0	2.0	H35	IC213007× WS-5	1.2	1.0
H4	EC618215× WS-1	5.3	4.5	H36	IC213035× WS-5	2.0	5.2
H5	EC618215× WS-5	1.0	1.0	H37	IC213053× WS-5	2.5	4.0
H6	HKI-163× WS-1	4.3	4.0	H38	IC213068× WS-1	2.0	1.0
H7	HKI-163× WS-5	3.0	1.0	H39	IC213090× WS-5	5.0	2.5
H8	IC0597385× WS-5	1.0	2.0	H40	IC213120× WS-5	3.4	1.5
H9	IC0598546× WS-5	3.7	4.0	H41	IC213122× WS-1	2.0	1.0
H10	IC0612729× WS-5	3.0	4.0	H42	IC213122× WS-5	4.6	3.4
H11	IC0612770× WS-1	4.6	2.0	H43	IC213132× WS-1	2.2	3.3
H12	IC0612770× WS-5	8.3	1.0	H44	IC213132× WS-5	3.8	1.8
H13	IC0612821× WS-1	4.6	3.0	H45	IC254061× WS-5	5.0	4.2
H14	IC0612832× WS-5	5.4	6.4	H46	IC254062× WS-5	5.2	3.8
H15	IC0612864× WS-5	4.2	5.0	H47	IC420922× WS-1	6.2	4.5
H16	IC0616502× WS-5	5.0	2.0	H48	IC522300× WS-1	6.2	8.0
H17	IC0620929× WS-5	2.3	2.3	H49	IC522300× WS-5	1.5	1.0
H18	IC0620969× WS-5	4.2	3.3	H50	IC563961× WS-5	2.0	1.0
H19	IC0620978× WS-5	5.2	5.0	H51	MIL6-1× WS-5	1.2	1.0
H20	IC0621049× WS-1	1.5	1.0	H52	MIL6-19× WS-5	1.0	1.0
H21	IC0621061× WS-5	4.5	2.7	H53	MIL6-2× WS-5	3.0	5.5
H22	IC0621159× WS-5	4.3	4.6	H54	MIL6-26× WS-5	6.1	5.0
H23	IC0621166× WS-5	6.6	5.5	H55	MIL6-P6× WS-5	2.5	3.4
H24	IC0621168× WS-5	5.5	4.0	H56	MIL6-P6M× WS-5	1.5	1.0
H25	IC0621430× WS-5	5.9	1.0	H57	PFSR-3× WS-1	4.2	5.0
H26	IC0621565× WS-5	1.8	1.0	H58	PFSR-3× WS-5	3.0	1.0
H27	IC0621578× WS-5	3.5	2.0		**Mean**	3.81	3.05
H28	IC0621634× WS-5	6.5	4.0		**Min**	1.00	1.00
H29	IC212838× WS-5	3.0	3.0		**Max**	8.30	8.00
H30	IC212871× WS-5	2.0	4.0		**CV (%)**	12.11	11.43
H31	IC212875× WS-1	4.0	7.0		**SEm**	0.23	0.23
H32	IC212875× WS-5	6.3	1.0				

to 50% tasseling, days to 50% silking, and ASI. The lines MIL6-P6M, MIL6-26, MIL6-19, MIL6-1, IC213007, IC0621565, and IC0621049 were good general combiners for ear length, ear diameter, no. of kernel rows per ear, no. of kernels per row, ear weight, and grain yield per ear.

Among the 58 crosses, significant high SCA effects in desirable direction were recorded by the crosses PFSR-3 × WS-5, EC618215 × WS-1 and IC213122 × WS-1 for plant height, ear diameter, no. of kernel rows per ear, no. of kernels per row, ear weight and grain yield per ear; IC212875 × WS-1 and EC618215× WS-5 for tassel length; IC213132 × WS-1 for days to 50% tasseling and days to 50% silking; IC212875 × WS-1 and IC522300 × WS-5 for ASI and ear length and none of the crosses showed significant SCA effects in desired direction for days to 75% dry husk. These results are in accordance with Akshaya *et al*. (2022), Italia *et al*. (2022).

Best cross combinations with desirable SCA effects for 12 quantitative traits, along with GCA effects of their parents, revealed that the best specific combiners were resultant of good × good, good × poor, and poor ×

Table 3 - Analysis of variances for combining ability for various traits under investigation

Source of variations	d.f.	PH	TL	DFT	DFS	ASI	DT75DH	EL	ED	NKRPE	NKPR	CW	GYPC
Replications	1	290.96*	68.42**	82.84**	155.56**	11.36*	295.57**	0.50	0.02	0.54	5.09	88.21	9.85
Treatments	109	1543.53**	25.41**	79.98**	70.42**	6.23**	61.04**	28.25**	2.24**	37.22**	124.16**	3620.08**	2255.35**
Parents	51	1984.13**	39.40**	104.93**	81.43**	7.39**	59.52**	13.40**	0.82**	12.22**	71.99**	1981.89**	1315.79**
Parents vs. Crosses	1	41648.54**	100.02**	255.56**	297.85**	1.62	206.03**	585.70**	109.95**	1466.99**	4128.9**	46770.69**	24195.19**
Crosses	57	445.71**	11.58**	54.57**	56.57**	5.28**	59.86**	31.76**	1.63**	34.5**	100.58**	4328.83**	2711.10**
Lines	49	425.87	11.17	58.44**	60.12*	5.51	65.46**	29.78	1.47	33.33	95.37	4375.27	2742.33
Testers	1	1050.30	0.12	42.78	28.13	1.53	36.12	34.94	4.15	10.15	39.72	3590.86	2308.94
Lines vs. Testers	7	498.3**	16.08**	29.21**	35.12**	4.17	24.05	45.09**	2.41**	46.15**	145.77**	4109.16**	2549.94**
Error	109	58.64	4.9	5.12	5.97	2.01	14.59	1.75	0.05	1.34	4.84	51.68	35.08
Total	219	798.00	15.40	42.73	38.73	4.15	39.00	14.94	1.14	19.19	64.23	1827.5	1140.02

**, * Significant at 1% and 5% levels, respectively Where, PH-plant height, TL-tassel length, DFT- days to 50% tasseling, DFS-days to 50% silking, ASI-anthesis silking interval, DT75DH-days to 75% dry husk, EL-ear length, ED- ear diameter, NKRPE-no. of kernel rows per ear, NKPR-no. of kernels per row, CW-cob weight, GYPC-grain yield per cob

poor general combiners. The interaction between good × good combiners in a cross in plant height, ear diameter, no. of kernel rows per ear, no. kernels per row, ear weight, and grain yield per ear can be fixed in subsequent generations, as it was due to additive × additive interaction. The superiority of crosses involving good × poor combiners could be due to additive × dominant interaction. They may produce good segregants only if general combiners were governed by additive effects and crosses were governed by epistatic effects. Whereas superior crosses involving poor × poor general combiners may be due to overdominance. The high yield of crosses involving non-additive gene action would not be fixable (Arulselvi *et al*. 2024)

It is also observed that females (lines) made a greater contribution to the total variance for all the studied traits compared to males (testers), and the proportion of line × tester interaction to the total variance was hi-

Fig. 1 - Prolificacy on the main tiller and side tillers habitat observed in hybrids

Fig. 2 - Tillering habitat observed in hybrids

gher than that of testers for all the traits. Proportional contribution of parents and hybrids were represented in Figure 3. For all the traits under study, the ratio between GCA to SCA was less than unity, indicating the prevalence of non-additive gene action in the inheritance of these traits.

Molecular analysis

Molecular grouping refers to clustering hybrids - related or unrelated -based on genetic similarity, which offers insights into their genetic relationships (Fan *et al.*, 2018). Such grouping plays a crucial role in assessing hybrid authenticity, genetic diversity, and introgression

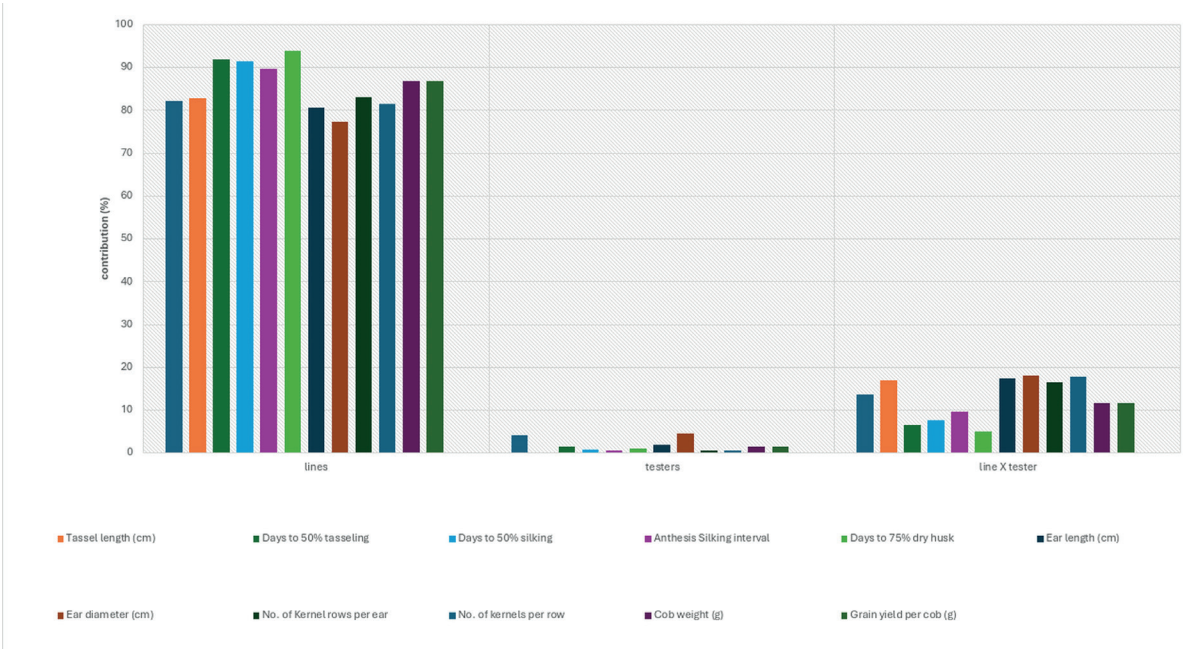

Fig. 3 - Proportional contribution of lines, testers and line × tester interactions to total

Table 4 - Estimates of General Combining Ability (GCA) effects of lines and testers for various traits under investigation

Parents	PH	TL	DFT	DFS	ASI	DT75DH	EL	ED	NKRPE	NKPR	CW	GYPC
Testers												
WS-5	1.44	-0.13	0.53	0.42	-0.11	0.27	-0.43	-0.12*	-0.30	-0.55	-3.43*	-2.61*
WS-1	-5.50**	0.5	-2.04**	-1.61*	0.43	-1.05	1.66**	0.45**	1.15**	2.12**	13.18**	10.01**
SE (tester)	0.76	0.22	0.22	0.24	0.14	0.38	0.13	0.02	0.11	0.21	0.71	0.58
Lines												
BJIM-08-27	-10.01**	0.70	-2.79**	0.55	3.35**	3.78**	-1.64**	-0.35**	-1.90**	-3.49**	-21.68**	-17.17**
BML-7	35.82**	4.04**	12.70**	11.05**	-1.65**	11.78**	-3.15**	-0.60**	-2.90**	-5.16**	-25.68**	-20.11**
EC0758189	-0.02	0.70	-2.79**	-1.94**	0.85	-6.22**	-1.64**	-0.52**	-2.24**	-3.49**	-23.34**	-18.45**
EC618215	-10.85**	-0.13	-2.04**	-2.94**	-0.91*	-2.45*	1.73**	0.52**	1.76**	4.43**	5.74	3.13
HKI-163	20.40**	3.20**	10.45**	10.30**	-0.16	7.53**	-2.85**	-0.56**	-2.90**	-4.99**	-24.04**	-18.99**
IC0597385	22.49**	-2.63**	9.71**	9.55**	-0.16	8.28**	-3.31**	-0.52**	-2.40**	-4.99**	-23.68**	-18.62**
IC0598546	-15.01**	-0.96	-3.29**	-2.45**	0.85	1.28	-0.81*	-0.43**	-1.90**	-3.16**	-21.51**	-16.95**
IC0612729	10.82**	-4.29**	5.21**	5.05**	-0.16	1.28	-1.65**	-0.35**	-1.24**	-2.99**	-24.01**	-19.28**
IC0612770	-5.43**	0.70	-3.04**	-0.95	2.09**	3.03**	2.10**	0.82**	2.76**	5.26**	40.98**	31.88**
IC0612821	-9.18**	-1.79**	-1.79**	-0.95	0.85	1.78	-0.98**	-0.43**	-2.24**	-1.49*	-23.68**	-18.61**
IC0612832	-9.18**	-4.29	-1.29	-0.95	0.35	-2.71**	-2.39**	-0.35**	0.43	-2.32**	-21.68**	-17.17**
IC0612864	3.32	1.54*	1.20	1.05	-0.16	1.28	-2.73**	-0.52**	-1.90**	-3.82**	-24.34**	-19.28**
IC0616502	7.45**	1.20	-1.29	-6.95**	-5.66**	-6.71**	-2.23**	-0.52**	-2.57**	-3.16**	-22.01**	-17.11**
IC0620929	4.99*	-0.96	-0.79	-6.45**	-5.66**	-3.71**	-0.73	-0.43**	-2.24**	-0.49	-22.68**	-18.11**
IC0620969	9.15**	4.04**	4.2**	5.55**	1.35**	-1.22	-1.23**	-0.43**	-3.57**	-2.66**	-23.85**	-18.78**
IC0620978	9.15**	-2.63**	3.20**	3.05**	-0.16	3.78**	-1.98**	-0.35**	-1.24**	-3.66**	-22.68**	-17.20**
IC0621049	-19.18**	1.53*	-9.29**	-9.95**	-0.66	-9.22**	4.19**	1.15**	8.76**	11.34**	104.15**	75.05**
IC0621061	-22.51**	-3.46**	-2.29**	-2.45**	-0.16	-4.22**	-1.89**	-0.52**	-2.90**	-2.88**	-25.18**	-19.45**
IC0621159	-0.85	-2.63**	-2.29**	-2.95**	-0.66	-4.72**	-3.31**	-0.52**	-2.24**	-4.49**	-24.68**	-19.78**
IC0621166	-9.18**	-2.63**	-3.29**	-3.95**	-0.66	-1.72	-2.15**	-0.35**	-2.57**	-2.66**	-22.34**	-17.45**
IC0621168	8.32**	1.54*	10.70**	10.55**	-0.16	10.28**	-2.64**	-0.52**	-1.56**	-4.69**	-19.84**	-16.11**
IC0621430	18.32**	0.70	-2.79**	-2.95**	-0.16	-5.22**	-2.23**	-0.35**	-1.24**	-4.66**	-22.34**	-18.11**
IC0621565	-21.68**	-1.79**	-7.29**	-7.95**	-0.66	-9.22**	5.35**	1.73**	5.76**	9.68**	112.48**	85.38**
IC0621578	-12.51**	-0.96	6.20**	7.05**	0.85	5.28**	-2.89**	-0.60**	-2.24**	-5.33**	-24.51**	-19.28**
IC0621634	3.32	-0.46	8.21**	7.05**	-1.16**	8.78**	-2.73**	-0.19**	-1.90**	-3.99**	-21.51**	-17.12**
IC212838	6.65**	0.70	-7.29**	-7.45**	-0.16	-8.22**	-1.98**	-0.60**	-1.24**	-3.16**	-22.52**	-17.95**
IC212871	-7.52**	-0.96	0.20	-0.45	-0.66	2.28	-3.15**	-0.52**	-1.90**	-4.67**	-23.68**	-18.45**
IC212875	-10.43**	1.04	-2.54**	-1.19	1.35**	0.03	-0.56	-0.52**	-2.07**	-1.91**	-22.93**	-18.28**
IC212876	2.49	-2.63**	3.21**	2.55**	-0.66	1.78	-1.39**	-0.52**	-1.57**	-2.49**	-24.02**	-18.61**
IC213004	24.15**	2.37**	-2.79**	-2.45**	0.35	1.78	-1.39**	-0.52**	-2.24**	-6.66**	-22.85**	-18.11**
IC213007	-14.18**	-1.79**	-2.79**	-2.95**	-0.16	-5.22**	7.19**	1.48**	6.76**	11.67**	80.15**	60.71**
IC213035	-6.68**	-0.97	6.70**	7.55**	0.85	9.28**	-1.56**	-0.52**	-1.90**	-3.32**	-23.68**	-18.78**
IC213053	-6.68**	-0.13	9.21**	8.55**	-0.66	6.28**	-1.56**	-0.52**	-1.90**	0.84	-22.68**	-18.11**
IC213068	16.65**	3.2**	-1.79**	-3.45**	-1.66**	-2.22	9.85**	1.65**	8.09**	12.18**	36.65**	30.21**
IC213090	-3.35	-0.13	-0.29	-0.45	-0.16	2.28	-1.14**	-0.43**	-2.24**	-5.32**	-23.18**	-18.45**
IC213120	-5.85**	0.70	-1.29	-0.45	0.85	1.28	-1.81**	-0.43**	-1.57**	-4.49**	-24.01**	-18.78**
IC213122	2.49	0.28	-2.79**	-2.95**	-0.16	-1.97	3.35**	0.86**	1.43**	2.5**	8.73*	8.3**
IC213132	8.32**	-0.55	3.70**	3.8**	0.09	6.03**	-2.89**	-0.52**	-2.41**	-5.07**	-24.26**	-18.78**

IC254061	-5.85**	-0.96	-3.29**	-2.45**	0.86*	-6.72**	-3.31**	-0.52**	-2.90**	-3.65**	-25.01**	-19.78**
IC254062	14.15**	0.70	-1.29	-0.95	0.35	-3.72**	0.02	-0.52**	-1.24**	-0.99	-22.35**	-17.61**
IC420922	9.15**	0.70	0.21	1.05	0.85	-2.72**	-2.48**	-0.52**	-3.57**	-6.65**	-21.85**	-16.95**
IC522300	-26.68**	-2.21**	-2.54**	-0.69	1.85**	-0.22	-2.02**	-0.39**	-3.24**	-4.99**	-23.43**	-18.28**
IC563961	-13.35**	4.04**	-7.29**	-7.95**	-0.66	-3.22**	9.69**	1.65**	7.43**	9.07**	45.15**	49.38**
MIL6-1	15.82**	3.20**	-2.29**	-3.45**	-1.16**	-4.22**	4.02**	1.73**	8.76**	12.50**	94.98**	70.88**
MIL6-19	-0.02	1.53*	-3.29**	0.55	3.85**	-0.72	6.85**	1.48**	7.76**	14.68**	126.99**	99.21**
MIL6-2	-8.35**	-5.13**	-3.29**	-1.95**	1.35**	0.28	-2.22**	-0.27**	-1.91**	-3.66**	-23.51**	-18.61**
MIL6-26	24.98**	0.70	-2.79**	-3.95**	-1.16**	-12.22**	7.52**	1.40**	8.09**	15.17**	83.65**	64.54**
MIL6-P6	-3.35	-0.13	6.21**	6.55**	0.35	6.78**	-1.23**	-0.68**	-2.24**	-2.49**	-23.68**	-18.78**
MIL6-P6M	-7.51**	4.87**	-0.79	-1.95**	-1.16**	-3.22**	9.02**	1.90**	9.09**	17.84**	123.82**	112.71**
PFSR-3	4.57	-0.55	-3.79**	-3.95**	-0.16	-3.72**	2.06**	0.44**	2.93**	5.93**	17.31**	13.55**
SE (line)	3.13	0.91	0.92	0.99	0.58	1.56	0.54	0.09	0.47	0.89	5.08	2.41

Where, PH-plant height, TL-tassel length, DFT- days to 50% tasseling, DFS-days to 50% silking, ASI-anthesis silking interval, DT75DH-days to 75% dry husk, EL-ear length, ED- ear diameter, NKRPE-no. of kernel rows per ear, NKPR-no. of kernels per row, CW-cob weight, GYPC-grain yield per cob

efficiency, especially when wild relatives are used as donors. In maize, incorporating wild germplasm is a promising approach to broaden the genetic base of elite lines. Molecular classification of hybrids developed from wild relatives confirms successful hybridization and facilitates assessment of genetic relationships among wild accessions, elite inbred lines and their derived hybrids. This, in turn, supports long-term breeding goals such as

Table 5 - Estimates of Specific Combining Ability (SCA) effects for crosses of various traits under investigation

Crosses	PH	TL	DFT	DFS	ASI	DT75DH	EL	ED	NKRPE	NKPR	CW	GYPC
BJIM-08-27× WS5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
BML-7× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
EC0758189× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
EC618215× WS-1	-8.67*	2.84**	1.79	1.16	-0.68	-2.20	2.80**	0.59**	2.51**	5.79**	17.06**	12.57**
EC618215× WS-5	12.73**	-3.20**	-0.28	0.08	0.36	2.98	-4.03**	-0.93**	-3.36**	-7.36**	-26.81**	-19.9**
HKI-163× WS-1	-0.75	-1.33	1.79	1.87	0.07	2.78	-1.84**	-0.41**	-1.48**	-3.45**	-12.51**	-9.22**
HKI-163× WS-5	4.86	0.96	-0.28	-0.67	-0.39	-2.02	0.61	0.07	0.63	1.89	2.77	1.82
IC0597385× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	-13.78**	-2.61
IC0598546× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0612729× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0612770× WS-1	0.92	0.33	2.79**	3.12**	0.32	-2.20	2.26**	0.80**	3.18**	6.29**	51.82**	41.51**
IC0612770× WS-5	3.15	-0.70	-1.28	-1.92	-0.64	2.98	-3.48**	-1.13**	-4.03**	-7.86**	-61.56**	-48.55**
IC0612821× WS-1	5.5	-0.49	2.04	1.61	-0.43	1.05	-1.66**	-0.44**	-1.15	-2.12	-13.17**	-10.01**
IC0612832× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0612864× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0616502× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0620929× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0620969× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0620978× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC0621049× WS-1	5.5	-0.49	2.04	1.61	-0.43	1.05	-1.66**	-0.45**	-1.15	-2.12	-13.18**	-10.01**
IC0621061× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61

IC0621159× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC0621166× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC0621168× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC0621430× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC0621565× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC0621578× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC0621634× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC212838× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC212871× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.11	0.30	0.55	3.44	2.61
IC212875× WS-1	-0.75	-3.33**	1.29	-1.14	-2.43**	-0.20	-1.57**	-0.36**	-1.65**	-3.20**	-13.93**	-10.34**
IC212875× WS-5	4.82	2.95**	0.21	2.33	2.11**	0.97	0.35	0.03	0.80	1.63	4.18	2.95
IC212876× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC213004× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC213007× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC213035× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC213053× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC213068× WS-1	5.5	-0.49	2.04	1.66	-0.43	-1.05	-1.66**	-0.45**	-1.15	-2.12	13.18**	-10.14**
IC213090× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.43	2.92
IC213120× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC213122× WS-1	-7.83*	-0.08	-1.96	-1.89	0.07	-1.70	4.34	0.84**	2.85**	4.88**	20.57**	18.23**
IC213122× WS-5	11.9**	-0.29	3.47**	3.08**	-0.39	2.47	-5.57**	-1.18**	-3.7**	-6.45**	-30.31**	-25.63**
IC213132× WS-1	-3.67	-1.75	-4.46**	-4.64**	-0.18	-2.20	-2.24**	-0.45**	-1.98**	-3.70**	-14.76**	-11.34**
IC213132× WS-5	7.73	1.38	5.96**	5.83**	-0.14	2.97	1.01	0.12	1.13	2.13	5.02	3.95
IC254061× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC254062× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
IC420922× WS-1	5.50	-0.49	2.04	1.62	-0.43	1.05	-1.66**	-0.45**	-1.15	-2.12	-13.17**	-10.01**
IC522300× WS-1	-6.99	-1.74	2.29	3.37**	1.07	2.05	-3.37**	-0.32**	-1.15	-3.96**	-13.26**	-9.85**
IC522300× WS-5	11.06**	1.38	-0.78	-2.17	-1.39**	-1.27	2.13**	-0.01	0.30	2.39*	3.52	2.45
IC563961× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
MIL6-1× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
MIL6-19× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
MIL6-2× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
MIL6-26× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
MIL6-P6× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
MIL6-P6M× WS-5	-1.44	0.13	-0.53	-0.42	0.11	-0.27	0.43	0.12	0.30	0.55	3.44	2.61
PFSR-3× WS-1	25.91**	1.59	3.54**	3.61**	0.07	3.55	-5.28**	-1.41**	-6.98**	-10.71**	-55.67**	-43.34**
PFSR-3× WS-5	-21.85**	-1.96	-2.03	-2.42	-0.39	-2.77	4.05**	1.08**	6.13**	9.14**	45.94**	35.95**
SE (crosses)	5.42	1.57	1.60	1.72	0.58	2.70	0.93	0.17	0.82	1.56	5.08	4.19

Where, PH-plant height, TL-tassel length, DFT- days to 50% tasseling, DFS-days to 50% silking, ASI-anthesis silking interval, DT75DH-days to 75% dry husk, EL-ear length, ED- ear diameter, NKRPE-number of kernel rows per ear, NKPR of kernels per row, CW-cob weight, GYPC-grain yield per cob.

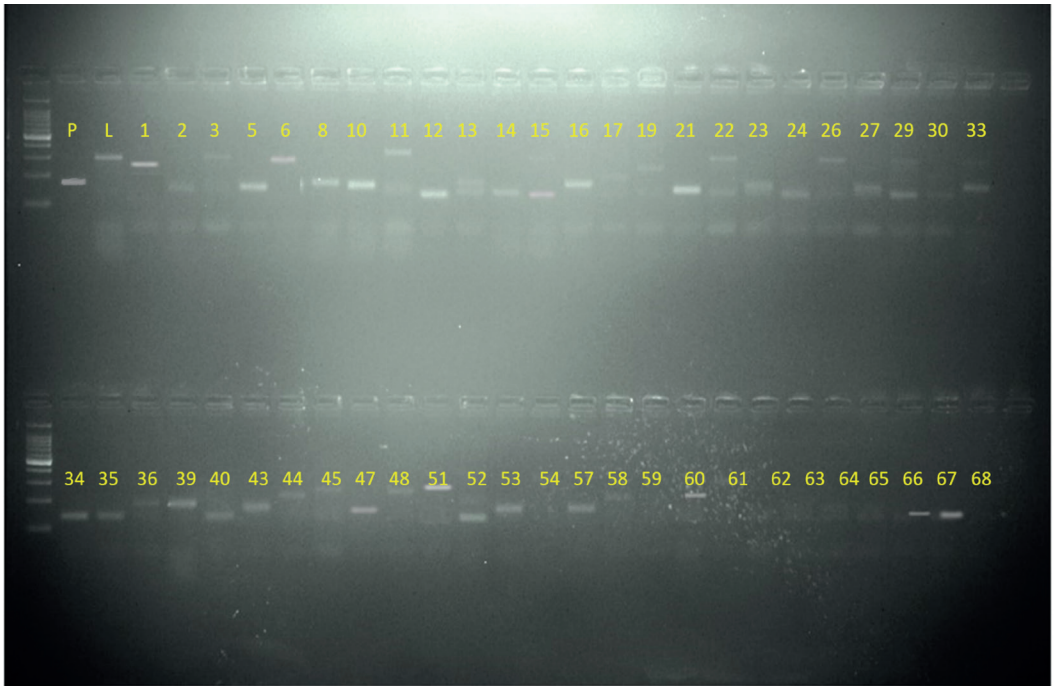

Fig. 4 - Molecular profile of parents with bnlg 1185 primer

enhancing heterotic pools and generating novel inbred lines with wider genetic backgrounds.

In the present study, 63 interspecific maize hybrids were developed using 69 inbred lines crossed with two wild species- *Zea luxurians* (WS-1) and *Z. mays* ssp. *parviglumis* (WS-5) - following a line × tester design. Out of these, 58 hybrids germinated and were subsequently used for molecular characterization. Specifically, 12 hybrids were derived from WS-1 and 46 from WS-5. Eight inbred lines were common to both crossing programs. Thus, 50 inbred lines which were successfully crossed with the two wild testers, and the 58 hybrids were considered for molecular analysis.

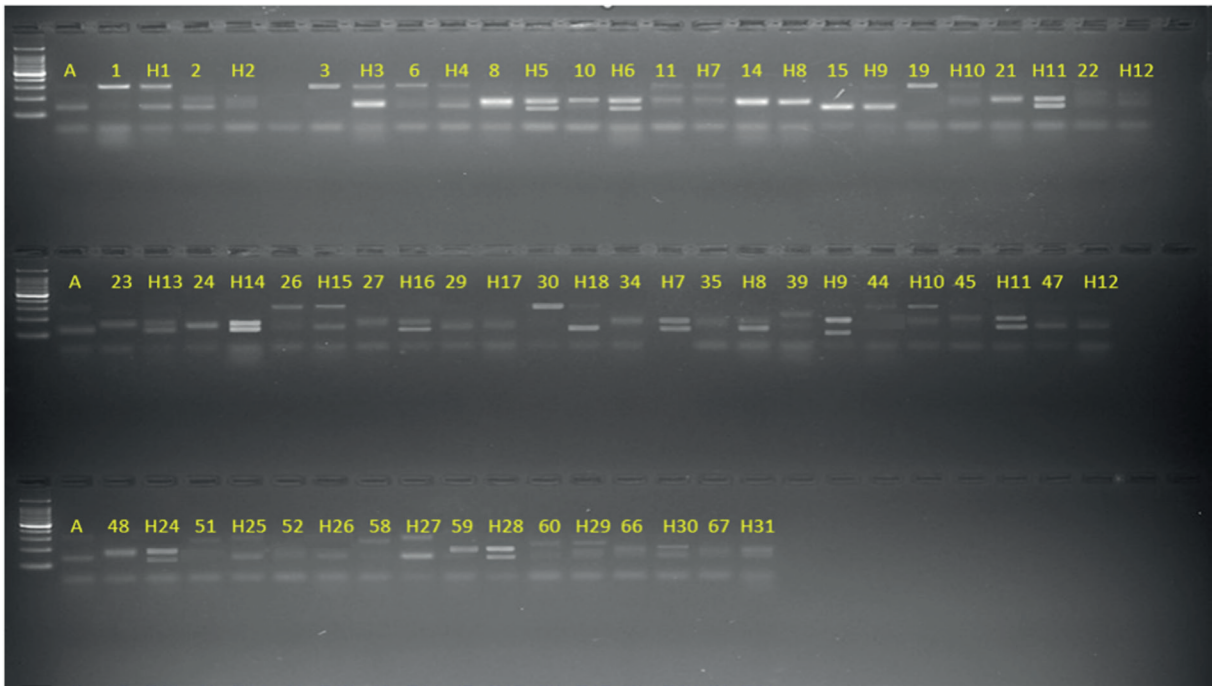

Fig. 5 - Molecular profile of parents along with their respective hybrids developed with WS-5

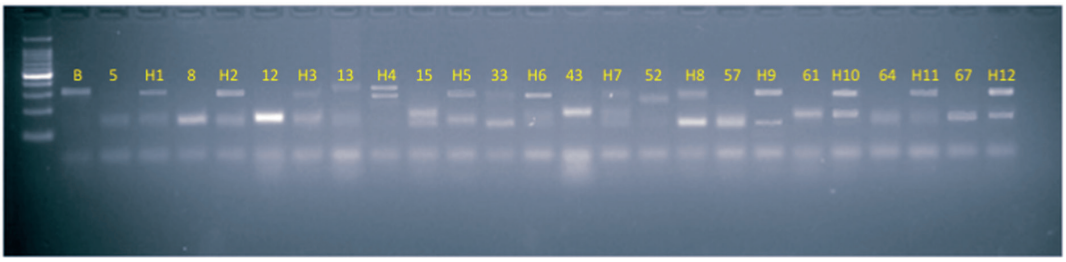

Fig. 6 - Molecular profile of parents along with their respective hybrids for hybrids developed from WS-1

Parental Polymorphism

Before grouping hybrids, a parental polymorphism survey was conducted to identify SSR markers capable of distinguishing the wild species from the inbred lines. This is essential to confirm hybrid identity and to trace the introgression of wild alleles. Among the 12 **Simple Sequence Repeat (SSR)** markers screened, only *bnlg1185* showed distinct polymorphism between the inbred lines and wild testers and was thus selected for further analysis. This marker produced five different alleles, effectively differentiating the wild parents and inbred lines. Gel images showing the amplification patterns of the parents are here reported (Figure 4).

Out of the 50 inbred lines, 40 showed polymorphisms with at least one of the two wild testers. Among these, 32 lines were involved in hybrids with WS-5, and 12 lines were involved in crosses with WS-1; four inbred lines were common to both. Consequently, 40 inbred lines and two wild species were used to group 44 successfully derived hybrids for molecular analysis.

Molecular Grouping of Hybrids and Parents

Although 46 hybrids were initially developed using WS-5, only 32 hybrids were retained for molecular grouping based on confirmed parental polymorphism. All 12 WS-1-derived hybrids were included in the analysis. The grouping was performed using the marker *bnlg1185*, which amplified alleles at 160 bp for WS-5, 340 bp for WS-1, and at 210, 270, and 290 bp for the inbred lines, as detailed in Table 6. All the hybrids showed the presence of alleles from both female (inbred) and male (wild species) parents, confirming interspecific hybridization and heterozygous banding patterns. These observations were supported by the amplification profiles and gel documentation as shown in Figures 5a and 5b. The scored banding data were analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm implemented in DARwin v6.0.021 software (Perrier and Jacquemoud-Collet, 2006). The resulting radial tree diagram illustrating the grouping pattern is shown in Figure 6. Cluster analysis grouped

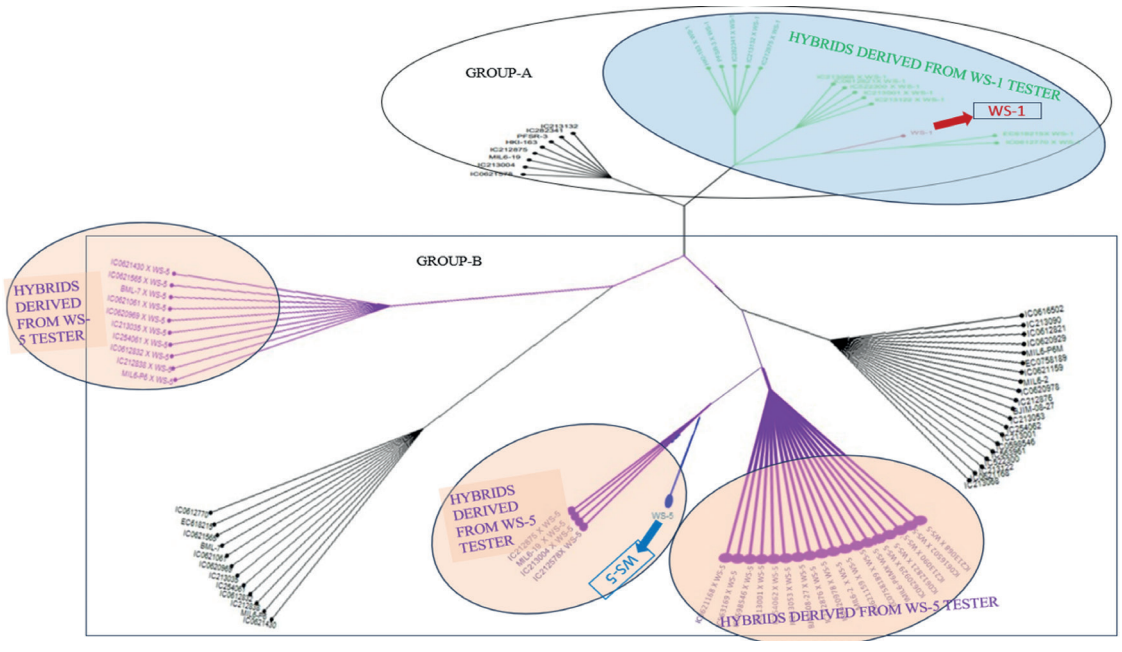

Fig. 7 -Clustering pattern of parents along with hybrids constructed by using Darwin software version 6.0.021

the 44 hybrids and 42 parents into two major clusters: Group A: contained WS-1, its 12 derived hybrids, and eight inbred lines, all of which amplified at 290 bp. Group B: contained WS-5, 32 hybrids, and 32 inbred lines, further subdivided into two subgroups based on amplification at 210 bp and 270 bp.

The banding profiles of hybrids consistently showed alleles from both the parents, verifying true hybrid status. Importantly, the clustering of hybrids along with their respective wild male parents indicates successful introgression of wild alleles. The greater number of hybrids successfully developed with WS-5 and their close clustering with inbred lines suggests a higher degree of genetic similarity between WS-5 and the elite germplasm, compared to WS-1. This may explain the relatively better cross-compatibility and hybrid establishment seen with WS-5. Morphological observations, such as tillering and multiple silking, further supported these genetic findings. These molecular results align with previous reports by Ahmed *et al.* (2019), Kumar *et al.* (2019), and Reif *et al.* (2003), who emphasized the role of wild relatives in expanding the genetic base and enhancing variability in maize.

Conclusion

This study demonstrated the effective use of wild relatives in maize pre-breeding to expand the genetic base and introduce desirable traits not commonly found in cultivated varieties. Interspecific crosses between elite inbred lines and two wild species *Zea luxurians* (WS-1) and *Zea mays* ssp. *parviglumis* (WS-5) resulted in hybrids expressing distinct wild traits such as prolific tillering and multiple silks per plant. These features, absent in modern cultivars, present potential advantages for developing dual-purpose maize suited for both grain and fodder. A key outcome was the identification of promising parental combinations through combining ability analysis. WS-1 emerged as a strong general combiner for several traits, while inbreds such as MIL6-P6M, MIL6-26, and IC0621565 showed potential for enhancing ear and yield traits. Specific cross combinations like PFSR-3 × WS-5 and EC618215 × WS-1 recorded high SCA effects, indicating the possibility of heterosis even among genetically contrasting parents. Molecular validation using SSR markers confirmed the hybrid nature and successful introgression of wild alleles, with clustering patterns revealing closer genetic relationships between WS-5-derived hybrids and elite inbreds. This suggests better compatibility of WS-5 with cultivated lines, whereas WS-1 appeared more distant, possibly limiting hybrid success. Overall, the research highlights the utility of wild germplasm in introducing novel variability, improving adaptive traits, and supporting the development of resilient, high-yielding maize hybrids. These findings provide a valuable foundation for future breeding strategies aimed at enhancing productivity and sustainability under increasingly variable climatic conditions.

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Author contributions

Study material: Chikkappa G Karjagi and Sunil Neelam; Conceptualization : Sunil Neelam, Chikkappa G Karjagi; Conduct of experiment, Writing: Srikanth O and Jyothi B; Review: Sunil N, Nagesh Kumar MV Jyothi B; Editing: Sunil N, Jyothi B and Srikanth O; Validation: Sunil N, Jyothi B, Sri kanth O, Chikkappa G Karjagi, Vanishree S.

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References

Abdel-Moneam MA, Sultan MS, Sadek SE, Shalof MS (2014) Estimation of heterosis and genetic parameters for yield and yield components in maize using the diallel cross method. Asian Journal of Crop Science 6:101-111.

Adhikari S, Joshi A, Singh NK (2019) Phenotypic characterization and microsatellite marker analysis of elite maize inbred and teosinte (*Zea mays* ssp. parviglumis) accession. Pantnagar Journal of Research 17:123-128.

Ahmed HGMD, Rizwan M, Naeem M, Khan MA, Baloch FS, Sun S, Chung G (2022) Molecular characterization and validation of sunflower (Helianthus annuus L.) hybrids through SSR markers. PLoS ONE 17:e0268561.

Ahmed R, Hussain M, Ali M (2020) Genetic analysis for yield and associated traits in maize using line × tester mating design. Plant Breeding and Biotechnology 8:205-214.

Akshaya M, Shantakumar G, Sridevi O, Harlapur S (2022) Combining ability and heterosis study for grain yield and yield attributing traits in maize (*Zea mays* L.). Journal of Farm Sciences 35:28-32.

Arulselvi S, Umadevi M, Tamilselvi C, Karunakaran V, Selvamurugan M, Dhanushkodi V, Anuratha A, Shibi S (2024) Combining ability for grain yield and its contributing traits over environments in maize (*Zea mays* L.). Applied Ecology and Environmental Research 22:3217-3232.

Cairns JE, Sonder K, Zaidi PH, Verhulst N, Mahuku G, Babu R, Nair SK, Das B, Govaerts B, Vinayan MT, Rashid Z (2012) Maize production in a changing climate: impacts, adaptation and mitigation strategies. Advances in Agronomy 114:1-58.

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Muller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve and use crop wild relatives. Agroecology and Sustainable Food Systems 38:369-377.

Dowswell CD, Paliwal RL, Cantrell RP (1996) Maize in the Third World. Westview Press, Boulder, Colorado, USA.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15.

Fan X, Bi Y, Zhang Y, Jeffers D, Yin X, Kang MS (2018) Improving breeding efficiency of a hybrid maize breeding program using a three heterotic-group classification. Agronomy Journal 110:1209-1216.

FAOSTAT (2023) Food and Agriculture Organization of the United Nations. Available at: https://www.fao.org/faostat/ (accessed 2 August 2025)

Findley WR, Nault LR, Styer WE, Gordon DT (1982) Inheritance of maize chlorotic dwarf virus resistance in maize × *Zea diploperennis* backcrosses. Maize Genetics Cooperation Newsletter 56:165-166.

Henderson CR (1952) Specific and general combining ability. In: Gowen JW (ed) Heterosis. Iowa State College Press, Ames, Iowa, pp 375–530

Italia PB, Izge AU, Sabo MU, Buba UM, Fagam AS (2022) Genetic analysis among elite Nigerian open-pollinated varieties and inbred lines of maize (*Zea mays* L.) for grain yield and other yield components. Direct Research Journal of Agriculture and Food Science 10:11-21.

Johnson HW, Robinson HF, Comstock RE (1955) Estimates of genetic and environmental variability in soybeans. Agronomy Journal 47:314-318.

Karn A, Gillman JD, Flint-Garcia SA (2017) Genetic analysis of teosinte alleles for kernel composition traits in maize. G3: Genes, Genomes, Genetics 7:1157-1164.

Kempthorne O (1957) An Introduction to Genetic Statistics. John Wiley and Sons, New York, pp 458-471.

Kumar A, Singh NK, Adhikari S, Joshi A (2019) Morphological and molecular characterization of teosinte derived maize population. Indian Journal of Genetics and Plant Breeding 79:670-677.

Kumar A, Verma R, Meena R (2022) Combining ability analysis in maize (*Zea mays* L.) for grain yield and yield contributing traits. International Journal of Agriculture Sciences 14:35-40.

Ministry of Agriculture and Farmers Welfare (2024) Agricultural Statistics at a Glance 2024. Department of Agriculture and Farmers Welfare, Government of India, New Delhi.

Perrier X, Jacquemoud-Collet JP (2006) DARwin software. Available at: http://darwin.cirad.fr/darwin (accessed 28 July 2025)

Ramirez DA (1997) Gene introgression in maize (*Zea mays* ssp. *mays* L.). Philippine Journal of Crop Science 22:51-63.

Ray JD (1999) Introgressing root aerenchyma into maize. Maydica 44:113-117.

Reddy KM, Swathi G, Rao MS (2024) Evaluation of combining ability and gene action in maize (*Zea mays* L.) using diverse inbred lines. Indian Journal of Genetics and Plant Breeding 84:88-95.

Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK, Beck D, Bohn M, Frisch M (2003) Use of SSRs for establishing heterotic groups in subtropical maize. Theoretical and Applied Genetics 107:947-957.

Rosegrant MR, Ringler C, Sulser TB, Ewing M, Palazzo A, Zhu T, Nelson GC, Koo J, Robertson R, Msangi S, Batka M (2009) Agriculture and food security under global change: prospects for 2025/2050. International Food Policy Research Institute, Washington, DC.

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S, Ziegle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with

data from RFLPs and pedigree. Theoretical and Applied Genetics 95:163-173.

Singh P, Sharma HK, Sharma RC (2023) Genetic analysis and heterotic grouping in maize for hybrid development. Journal of Cereal Research 15:45-52.

Suto T, Yoshida Y (1956) Characteristics of the oriental maize. In: Land and Crops of Nepal Himalaya, Vol. 2, pp 375-530.

Tulu L, Mekonen D, Feyisa H (2021) Genetic variability and combining ability analysis in maize inbred lines using line × tester mating design. Agriculture and Food Security 10:74.

Wang Q, Jiang Y, Liao Z, Xie W, Zhang X, Lan H, Hu E, Xu J, Feng X, Wu F, Liu Y (2020) Evaluation of the contribution of teosinte to the improvement of agronomic, grain quality and yield traits in maize (*Zea mays* L.). Plant Breeding 139:589-599.

Briggs WH, McMullen MD, Gaut BS, Doebley J (2007) Linkage mapping of domestication loci in a large maize–teosinte backcross resource. Genetics 177:1915–1928.

Yadav OP, Karjagi CG, Dhillon BS (2014) Overview of maize improvement. Indian Farming 64:5-11.

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