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Integrated morpho-molecular and genetic dissection reveals nitrogen-efficient parental lines and superior hybrids for sustainable improvement of Indian mustard (Brassica juncea L.)

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Abstract
Aims: To evaluate genetic variability for NUE and yield traits under controlled N-levels (N_0 and N_{40}) followed by morpho-molecular diversity in Indian mustard; selection of superior genotypes for hybridisation; to identify superior N-efficient parental lines and high-yielding hybrid combinations
Methods: experiment conducted at Assam Agricultural University, Jorhat, during Rabi seasons (2021-22 to 2023-24). Evaluated 44 genotypes in a pot trial under two N-doses (0 and 40 kg N ha^{-1}) using a completely randomized design (CRD). Mahalanobis D^2 and SSR study revealed genetic diversity among the genotypes. Line × Tester design involved eight superior lines and three testers; field Evaluation of 24 hybrids hybrids and parents under recommended N levels (N_{40}) using a randomized complete block design (RCBD).
Results: ANOVA revealed significant differences for most traits; high heritability and GAM for NUE and yield was recorded. PIC values ranged from 0.17 to 0.62, indicating moderate to high marker informativeness. ANOVA (LXT) revealed significant genotypic differences for most traits; Kranti, RRE 2012/14, PM25, and NRCHB101 emerged as superior combiners while superior hybrids with strong positive specific combining ability were Kranti × TM2, Kranti × PM25, Kranti × NRCHB-101, RRE2012/14 × PM25, RRE2012/14 × NRCHB-101, and JM13-4 × PM25. RRE2012/14 x PM25 and Kranti x NRCHB-101 revealed considerable positive specific combining ability and standard heterosis of up to 104% and 8.3%, respectively, over the check variety NRCHB-101 for seed production $hectare^{-1}$.
Conclusions: The study successfully identified genetically diverse, N-efficient parents and high-yielding hybrids. These genotypes provide valuable genetic resources for sustainable Indian mustard breeding programs.

Keywords: Brassica juncea, NUE, SSR diversity, Line × Tester, heterosis

1. Introduction

Indian mustard (Brassica juncea L. Czern & Coss.) is a commercially significant oilseed crop from the Rapeseed-Mustard group under family Brassicaceae. With a physical genome size of 922 Mb, it is an amphidiploid crop (AABB, 2n = 36) that developed thru natural hybridization between two primary diploids, Brassica rapa and Brassica nigra following chromosomal duplication (K. H. Singh et al., 2022). Its high monounsaturated and polyunsaturated fatty acid content, omega-3 content, and low saturated fat content make mustard oil healthy. Beside cooking oil, its oil is used for body massage, paints and varnishes, and biofuel. Its seedmeal is highly rich in proteins, making it an ideal feed for poultry animals (Thakur et al., 2020). Mustard is utilized as a spice due to its distinct sharp spicy pungent flavor. Mustard is also used medicinally, with the leaves acting as a diuretic, stimulant, and expectorant. Particularly, fermented mustard has been proven to have a number of health advantages and impacts on preventing disease (Tian & Deng, 2020). Rapeseed-mustard contributes greatly to the Indian economy by providing edible oils, vegetables, sauces, and livestock feed. In 2024, India produced 11.9 million metric tons (MMT) of rapeseed-mustard, harvested from 9.3 million hectares with an average yield of 2.05 metric tonnes per hectare (USDA, 2024); making it the -largest oilseed crop in India, surpassing soybean (DRMR, 2024). In Assam, rapeseed-mustard are cultivated on 0.32 million hectares, yielding 0.28 million tonnes, with an average yield of 775 kg/ha (DRMR, 2022). Here short-duration toria (Brassica rapa) stands as the most important oilseed crop, although the productivity of rapeseed-mustard in the region remains significantly below the national average. Recently, mustard has gained prominence as a strategy to enhance oilseed production. Identifying

genotypes with improved nitrogen use efficiency (NUE) is essential for developing high-yielding rapeseed-mustard varieties and for optimizing the use of nitrogen fertilizers. Adequate nutrient provision enhances seed and oil yields by improving yield-related traits. Understanding the genetic basis of NUE variation and associated traits is crucial for improving these characteristics in breeding programs. NUE in plants is a complex process, influenced by various internal and external factors, including soil nitrogen availability, uptake, and assimilation of carbon and nitrogen (Jat and Choudhury, 2019). It is an essential macronutrient crucial for plant growth and development. Under nitrogen deficit environments, plants display stunted growth and experience chlorosis and therefore, efficient nitrogen utilization is a critical factor in breeding high-yielding mustard varieties. Indian mustard is noted for its low nitrogen use efficiency (Bouchet et al., 2016). Investigations revealed a toria genotype TS65 produced the high seed yield (9.9 q/ha) coupled with the high NUE (17.5 kg/kg); the high yield was primarily due to higher NUE, indicating the significance of this trait for enhancing yield of oilseed brassicas (Barua et al., 2024). Gaining insight into the nitrogen use characteristics of oilseed brassicas can enhance NUE and reduce production costs. Identifying genotypes with high nitrogen utilization efficiency (NUtE) under variable nitrogen supply levels can improve the nitrogen economy of Indian mustard in different soil fertility conditions.

Indian mustard hybrids often exhibit higher yield potential and adaptability compared to pure lines. Line × tester analysis is an important method for identifying the best parental combinations to produce high-yielding hybrids. This technique allows breeders to evaluate general combining ability (GCA) of parental lines via reflecting additive genetic effects and the specific combining ability (SCA) of hybrids indicating non-additive genetic effects. Additionally, the line × tester analysis helps in effectively utilizing genetic diversity within the breeding material, enabling breeders to explore a wide range of genetic combinations and to increase the chances of finding superior hybrids (Singh et al.,2022). Although genetic variability for nitrogen use efficiency (NUE) has been reported in Indian mustard, most studies have focused either on physiological evaluation under different nitrogen levels or on genetic analyses independently. Comprehensive studies integrating phenotypic screening under contrasting nitrogen environments, molecular diversity, and combining ability to identify superior parents and hybrids for NUE improvement remain limited. Such an integrated approach is essential for understanding the genetic architecture of NUE and its association with yield-related traits, thereby facilitating the identification of genetically diverse, nitrogen-efficient parental lines and superior hybrid combinations for sustainable mustard improvement. Therefore, the present study aimed to characterize genetic variability for NUE and yield-related traits under contrasting nitrogen regimes, assess genetic diversity through morpho-molecular approaches, elucidate combining ability and heterosis, and identify superior parents and hybrids for developing nitrogen-efficient Indian mustard cultivars.

Materials And Methods

The experiments were carried out utilizing Indian mustard (Brassica juncea L. Czern & Coss.) genotypes over three successive Rabi seasons (2021-22 to 2023-24) at Instructional-Cum-Research (ICR) Farm, Assam Agricultural University, Jorhat, India (latitude: 26°47/ north; longitude: 94°12/ east; elevation: 86.6 m above the mean sea level). First experimental year (Rabi, 2021-22) followed pot trials for forty-four genotypes Fs1utilizing completely randomized design (CRD) with two replications under two nitrogen doses, N0 (0kg N/ha) and N40 (40kg N/ha) to determine the nitrogen use efficiency (NUE). Genotypes with high NUE and yielding ability were selected based on their nitrogen use efficiency indices as parental lines for hybridization. Pot culture was used to calculate NUE indexes as it provides for precise control over nutrient delivery and minimizes nutrient losses or transfer between treatments, resulting in accurate measurements of NUE components. Additionally, uniform root-zone conditions minimize soil variability, making it feasible to attribute observed variations in NUE only to genetic variation rather than environmental heterogeneity. Random soil samples (0–20 cm depth) were taken with the objective to document the soil fertility level of the plot area from which the soil was obtained for pot filling .The pot screening revealed eight nitrogen-efficient genotypes, which were used as lines and crossed with three testers (one nitrogen-efficient genotype and two released varieties) in a Line x Tester mating system in the second season (Rabi 2022-23). Twenty-four F1 hybrids were generated from the eight lines and three testers, which were further studied for combining ability and genetic parameters related to NUE and yield attributes following recommended nitrogen dose for Assam (N40) under randomized complete block design with two replications in the third year (Rabi, 2023-24). Random soil samples (0–20 cm depth) were collected from the experimental site to document the soil fertility level of the plot area . The experiment was conducted following cultural approaches and plant protection measures recommended for mustard cultivation in Assam. Weekly meteorological data for the cropping season 2021-2024 were obtained from the Assam Agricultural University's Meteorological Observatory, Jorhat (Table 1).

The meteorological conditions recorded during the three cropping seasons (Rabi 2021-22 to 2023-24) are summarised in Table 1.

Table 1: Meteorological data during the period of experimentation (Source: Department of Agrometeorology, AAU, Jorhat)

Period	Temperature (°C)		RH (%)		Total RF (mm)	Total BSSH (hr)
	Max.	Min.	Morn.	Even.		
2021-22						
Nov	28.37	14.36	96.63	55.8	0.77	8.1
Dec	26.01	10.79	97.9	54.87	0.02	8.1
Jan	23.3	9.6	99	60	18.9	4.7

Feb	23.6	10.2	98	53	49.7	5.7
March	30.8	16.1	96	49	42.6	5.7
2022-23						
Nov	28.9	14.1	98	56	0	8.6
Dec	25.8	11.4	99	58	0.9	6.4
Jan	24.1	8.7	99	55	0	6.1
Feb	25.3	13.1	98	59	40.5	3.6
March	26.9	15.3	96	61	86	4.1
2023-24						
Nov	28.9	15.2	97	62	19.4	7.2
Dec	25.4	11.8	98	64	37.4	6.2
Jan	58	8.4	98	58	0	6.1
Feb	60	11.1	97	60	28	3.5
March	55	15	94	55	54.1	4.1

The data were recorded for the traits viz. days to 50% flowering (DFF), days to leaf senescence (DLSC), days to 75% maturity (DM), plant height (PH), no. of primary branches/plant (PBP), no. of second. branches/ plant (SBP), foot length (FL), length of main raceme (LMR), no. of siliquae/plant (SQP), no. of seeds/siliqua (SQS), thousand seed weight (TSW), oil content (OC), leaf area index (LAI) , maximum root length (MRL), root volume (RV), root dry weight (RDW), shoot dry weight (SDW), root/shoot ratio (R-S), biological yield per plant (BYP), leaf area index (LAI), harvest index (HI), seed yield per plant (SYP), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and nitrogen use efficiency (NUE). In first season, the data was recorded for three sample plants under pot culture, while in third year the data was recorded for five sample plants under field conditions for both the nitrogen doses.

The data on various traits were subjected to analysis of variance (ANOVA) following fixed effect model based on statistical and biometric techniques (Gomez and Gomez, 1984; Dabholkar, 1999). Genotypic and phenotypic variance were estimated as suggested by Allard (1960) and Dabholkar (1999); coefficients of genotypic and phenotypic variations were estimated as suggested by Burton and Devane (1953); broad-sense heritability (h2) and genetic advance expected at 5% selection intensity were calculated as per Allard (1960); Pearson correlation analysis (Singh and Chaudhary, 1985) was performed using Microsoft Excel 2019 and classified as suggested by Dospekov (1984). The estimates of genetic parameters were categorized into low, moderate, and high as suggested by Sivasubramanian and Menon (1973) for GCV and PCV; Robinson et al. (1949) for heritability and Johnson et al. (1955) for genetic advance as percent of mean. Line × Tester analysis was carried out as explained by Kempthorne (1957), and Dabholkar (1999). Standard heterosis was estimated based on the released variety NRCHB-101. Seed oil content (%) was analyzed using FOSS Infratec NIR (FOSS Analytics, Hillerød, Denmark); nitrogen use efficiency was evaluated utilizing the standard procedures; nitrogen uptake efficiency, (NUpE) and nitrogen utilization efficiency (NutE) were calculated as per Moll et al. (1982); and plant nitrogen content was estimated separately from grains, roots and shoots through Kjeldahl Digestion Method (Kirk, 1950). Mahalanobis D^2 statistics (Mahalanobis 1936) was carried out in R statistical software utilizing package BIOTOOLS (da Silva 2021) to study the genetic diversity among the forty-four genotypes in first year. Molecular characterization was carried out to investigate the molecular diversity among the forty-four genotypes using eighty random SSR markers (Table 2). DNA isolation of young leaf tissues (3-4 leaves stages) were carried out according to the modified protocol of Dellaporta et al. (1983) using SDS method. A Nanodrop at 260 and 280 nm was used to determine the quality and content of genomic DNA samples; contamination-free DNA is indicated by an absorbance ratio (A260/A280) of 1.8 (Desjardins and Conklin, 2010). Simultaneously, 0.8% agarose gel electrophoresis was also used to verify the quality of DNA at 100 volt for 90 minutes using 1XTBE buffer. PCR amplification was conducted using a Gene AMP® 2400 thermal cycler (Applied Biosystems, USA); the reaction profile consisted of an initial denaturation at 95°C for 3 min, followedf by 36 cycles of denaturation at 94°C for 1 min, primer annealing at 50–60°C for 1 min, and extension at 72°C for 1 min. A final extension step was performed at 72°C for 7 min. PCR products were resolved via 3.0% agarose gel electrophoresis; amplicons (10 µL each) were loaded and run at 70-80 volts for 150 minutes and the scoring of prominent bands were recorded using a ladder of 100 bp. Bands were visualized and recorded using a VILBER Gel Documentation System and scoring of prominent bands were done based on presence (1) or absence (0). Percentage of polymorphism and polymorphism information content (PIC) were calculated as per Anderson et al. (1993). Genetic similarity between genotypes was calculated through Jaccard's Similarity Coefficient (Jaccard, 1908) and cluster analysis was done through SIMQUAL module in NTSYS-pc software.

The 80 SSR markers employed for the molecular characterisation, together with their primer sequences, annealing temperatures and original sources, are listed in Table 2.

Table 2: List of 80 SSR markers used in the study

SI No.	Marker code	Forward Primer	Reverse primer	Tm (0C)	Reference
1	BRMS-003	ACGAATTGAATTGGAC AGAG	CAGATGGGAGTCAAGT CAAC	55	Yadava et al., 2012
2	Ol10B11	AAAATGTGAGGCTGTT TGGG	TTTCGCAGCAGTAAAC ATGG	55	Lowe et al., 2004

3	E05	CTCGTCTCAGGGATTATGTCG	CAGACAGAGGATAGACCGAACC	56	Ramchiary et al. (2007)
4	E07	GAGCGAGTCGATTACTTTTGC	GAATGGATTTCCGATGATGG	55	Lowe, Moule, Trick & Edwards (2004)
5	BRMS-017	GGAAAGGGAAGCTTCATATC	CTGGAAAGCATACACTTTGG	55	Suwabe et al. (2002, 2006
6	SORF-73 (B-27)	CGTGGGCCAAGCTTAGATTA	CGTTCAAGAAGACACAGATCAAA	55	Raman et al. (2002)
7	CN -52 (B-16)	CCGGCTTGGTTCGATACTT	TTGCGAATCTTTAAGGGACG	55	Raman et al. (2002)
8	Ra2-E03	AGGTAGGCCCATCTCTCTCC	CCAAAACTTGCTCAAAACCC	57	Lowe et al., 2004
9	BRMS-093	TCCAAGTAGACCGAATCAAGAGT	ATAAATCGAACCTGAAACCATGT	57	Suwabe et al. (2006),
10	SR_94102	ATCCCCAAACTACCCTCACC	AGGATGAGCAAAGGAAAGCA	57	Wang et al. (2007)
11	SSR_Na10-D11	GAGACATAGATGAGTGAATCTGGC	CATTAGTTGTGGACGGTCGG	57	Wang et al. (2011)
12	BRMS-240	CAAGAGTATTTGTGTGGGTTGAC	AAATAACGAACGGAGAGAGAGG	57	Suwabe et al. (2002, 2006)
13	SSR_Na10D09	AAGAACGTCAAGATCCTCTGC	ACCACCACGGTAGTAGAGCG	57	Wang et al. (2011)
14	NI-F02a	TGCAACGAAAAAGGATCAGC	TGCTAATTGAGCAATAGTGATTCC	57	Lowe et al. (2004)
15	BRMS_324	AACTTAACCGAAACCGAGSTAGG	AATCTCGAAATTCATCGACTTCC	58	Suwabe et al. (2006)
16	SR_9222	CACCGAACAAAACTGAGGGT	CGTTTCACTGCGTTCTACCA	59	Wang et al. (2007)
17	sR7178	CTGATGAGGTGACGCAGTGT	GGCTTGAGTAAAGCGACCTG5	59	Wang et al. (2007)
18	Ol10B01	CCTCTTCAGTCGAGGTCTGG	AATTTGGAAACAGAGTCGCC	59	Lowe et al. (2004
19	Ni3-B07	GGAGAAGAGGAAGAAGAAGCC	CGACTTCTAGAGGAACCCCC	52	Lowe et al. (2004
20	sS1702	CAGATGAGACAACACAGGAAACA	ACTCAATACGTTTTTCGCGG	60	Wang et al. (2007)
21	Ol10-H02	AACAGGAAGAAACGACGAGG	AGAGAGCCATGAGAAGCACC	60	Lowe et al. (2004)
22	BRMS-040	TCGGATTTGCATGTTCCTGACT	CCGATACACAACCAGCCAACTC	60	Suwabe et al. (2002)
23	Ni2B03	ACTTCTTGCCCTCCTCACC	AAATACTCACTGCAATACCCAGG	54	Lowe et al. (2004
24	sORA43	GCGCGTGTGGGATCAGAA	CTTCTCCACCGTCGATCG	54	Raman et al. (2002)
25	BRMS-011	GAACGCGCAACAACAAATAGTG	CGCGTCACAATCGTAGAGAATC	52	Suwabe et al. (2002)
26	Ni2D10	GATGCCCCAAATCTGTTACG	CAATTCGTGAAAAATAGCCG	57	Lowe et al. (2004)
27	PBCESSRJU13	TTGCTTTGACTGAGCCTG	CCATTCATGGAGCCTGTAG	57	Parida et al. (2009)
28	NI02-D08a	TTTAGGGAAAGCGAATCTGG	ACAACAACCATGTCTTCCG	57	Lowe et al. (2004)
29	EJU1	GGTGAAAGAGGAAGATTGGT	AGGAGATACAGTTGAAGGGTC	57	Luy et al. (2008)
30	NI03-H07a	GCTGTGATTTTAGTGCACCG	AGCCGTTGATGGAATTTTTG	58	Lowe et al. (2004)
31	PBCESSRJU1	GGTGAAAGAGGAAGATTGGT	AGGAGATACAGTTGAAGGGTC	55	Parida et al. (2009)
32	PBCESSRJU2	TTCACATCTTCTTCATCTTCC	TTGCTATTCGTTCTCAGTCTC	55	Parida et al. (2009)
33	PBCESSRJU3	CCTCTTTTAATTCAAACAAGAAATA	TTCGGACAATGGCAGTGATA	55	Parida et al. (2009)
34	PBCESSRJU4	GCGAAGCAGTCTGAAA	GCGAATCCGGTGAGAA	55	Parida et al. (2009)

		CC	AC		
35	Ra2-E11	GGAGCCAGGAGAGAAGAAGG	CCCAAAACTTCCAAGAAAAGC	58	Lowe et al. (2004)
36	Ra2-E12	TGTCAGTGTGTCCACTTCGC	AAGAGAAACCCAATAAAGTAGAACC	58	Lowe et al. (2004)
37	G02	TTGGTGTCAGAAACAACG	ACACACGACGGATCTCTGC	58	Lowe et al. (2004)
38	Ra2-D04	TGGATTCTCTTTACACACGCC	CAAACCAAAATGTGTGAAGCC	56	Lowe et al. (2004)
39	OL10-A03a	CTGGTTTTCTCCTTCATCAG	CTGTGTAGCTTTTAGTCTTT	57	Lowe et al. (2004)
40	OL10-F11a	TTTGGAACGTCCGTAGAAGG	CAGCTGACTTCGAAAGGTCC	57	Lowe et al. (2004)
41	RA02-A04a	AAAAACTCCTCTTCAACG	CCCAAAGTTAGGTTTTAATGTAATCTC	55	Lowe et al. (2004)
42	RA02-E01a	TCTATATTAACGCGCGACGG	GCACACACACACTCAAACCC	55	Lowe et al. (2004)
43	SSR_Na10-D11	GAGACATAGATGAGTGAATCTGGC	CATTAGTTGTGGACGGTCGG	56	Wang et al. (2011)
44	BrgMS710	ATCATCATCGTCTTCCTCTTCC	CTGCCCAAAATAAACACAGTCA	57	Iniguez-Luy et al. (2008)
45	SR_7223	AGGACCCGACTTTCCTTGTT	ACCAAACTCGGCGTACAAAT	57	Wang et al. (2007/2011)
46	BrgMS339	CTACCTGAAGATGACCCAGACG	GCATACAAACCTCGTCCTAAGC	59	Iniguez-Luy et al. (2008)
47	BRMS-019	CCCAAACGCTTTTGACACAT	GGCACAATCCACTCAGCTTT	59	Suwabe et al. (2002)
48	cnu_m60a	CTCCTTCATTTGATCCCCAA	CTTCTTCAGGGTTTCCAACG	56	Lowe et al. (2004)
49	MB5	AACATCTTTTTGCGTGATAT	AATAGCATTGAAGCCTTAC	56	Mayerhofer et al. (2005)
50	Na10-C01c	TTTTGTCCCACTGGGTTTTC	GGAAACTAGGGTTTTCCCTTC	57	Wang et al. (2011)
51	Ni3C05	TTTCGTGCTTTGGTGTGAAG	TCCCCAAATCGAACCATAAG	57	Lowe et al. (2004)
52	Ni3-G07	CACTCTCTCCGCCATTTTTC	CTTGAAGCGTTAAAGCCGAC	55	Lowe et al. (2004)
53	BN6A3	GCTACCCACTCATGTCCTCTG	CCAAGCTTATCGAATCTCAGCTA	55	Piquemal et al. (2005)
54	BRMS-033	GCGGAAACGAACACTCCTCCCATGT	CCTCCTTGTGCTTTCCCTGGAGACG	55	Suwabe et al. (2002)
55	CB-10065	CGGCAATAATGGACCACT	CGGCTTTCACGCAGACTTCG	55	Cheng et al (2016)
56	ENA17	CAGTTATTTCGCCTCGTCT	TATTTGTGGTCTGTTATTGGA	55	Lowe et al. (2004)
57	cnu_m62a	GCAGAAGCCTGAGAGTCTGG	AACAAGGCTGAATGCTACCG	55	Lowe et al. (2004)
58	NA10-C01a	TTTTGTCCCACTGGGTTTTC	GGAAACTAGGGTTTTCCCTTC	55	Wang et al. (2011)
59	Na14-D07	GCATAACGTCAGCGTCAAAC	CTGCGGGACACATAACTTTG	56	Wang et al. (2011)
60	E05	CTCGTCTCAGGGATTATGTCG	CAGACAGAGGATAGACCGAACC	54	Lowe et al. (2004)
61	sS1702	CAGATGAGACAACACAGGAAACA	ACTCAATACGTTTTTCGCGG	54	Wang et al. (2011)
62	Ra2-H10	GCGCGTGTAGGCTACGTC	CGGCCGCGGCAACTG	55	Lowe et al. (2004)
63	Ra3E05	TTCTCATGCTCCAACCACAG	GTTTCTTCCAAGCCAAGCTG	59	Lowe et al. (2004)
64	Ra2-F11	TGAAACTAGGGTTTCCAGCC	CTTCACCATGGTTTTGTCCC	60	Lowe et al. (2004)
65	Ra2-H06	GAATTCAGAGGTATCTACACGGC	TAACAAAGACCCTGCGTTCC	58	Lowe et al. (2004)

66	nia_m026a	AATGAGTAATGTCCCACACGA	TGAAATTGCGGATTCTTTAGC	58	Lowe et al. (2004)
67	nia_m043a	CCATTCGAGGTGGTCGTAAA	AGAAAACGGACCTCGATTCA	55	Lowe et al. (2004)
68	BN25A	CACGTGGTATGTTGGTATTGGG	TGATTCTCCTCCGACGCATGC	57	Piquemal et al. (2005)
69	BrgMS1237	ATCAAAAGATGCAGGGAGAGAG	GTCCTCAATGGATTACACATGC	55	Iniguez-Luy et al. (2008)
70	BrgMS329	TCATCATCATAGCTTTCGCTTC	AAAACCTCCTCCTCCTCCTC	54	Iniguez-Luy et al. (2008)
71	BrgMS787	CCATCTCAGCTCTATCTACCAAAA	TCAAAACACCGAGTAAACTGGA	56	Iniguez-Luy et al. (2008)
72	BrgMS802	TCCCACCCTCAAAATATACAGC	TGCTCGTTGGAAGAGGACAT	56	Iniguez-Luy et al. (2008)
73	Ol10B11	AAAATGTGAGGCTGTTTGGG	TTTCGCAGCAGTAAACATGG	54	Lowe et al. (2004)
74	Ol10-A05	TGTAATAACCCGACCCATCC	CTCTCTCGCTCTCTCGATCC	54	Lowe et al. (2004)
75	Ra3E05	TTCTCATGCTCCAACCACAG	GTTTCTTCCAAGCCAAGCTG	59	Lowe et al. (2004)
76	Ni2A07	GGAACCCAACAAGTGAGTCC	AGAGCTTGAGACACATAACACC	54	Lowe et al. (2004)
77	Ra2-H07	ATCATCAATCCTGACGAGGC	CGCGCACACACACACAC	57	Lowe et al. (2004)
78	Ra2-H10	GCGCGTGTAGGCTACGTC	CGGCCGCGGCAACTG	57	Lowe et al. (2004)
79	E07	GAGCGAGTCGATTACTTTTGC	GAATGGATTTCCGATGATGG	53	Lowe et al. (2004)
80	BrgMS70	TACAATGAAGATGTGATCCCGA	CGTGCGTGAGCTTATCAATACA	52	Matsumoto et al. (2012)

Results

Morpho-molecular characterisation of Indian mustard (Pot experiment)

Analysis of variance

ANOVA for the forty-four genotypes revealed significant genotypic variations among all the traits except leaf area index and thousand grain weights under both N0 and N40 doses, suggesting the presence of high genetic variation among the genotypes. Pooled ANOVA for two levels of nitrogen (combined N_0 and N_{40}) doses revealed significant environmental (nitrogen dose level) differences for the traits studied except for root-shoot ratio and nitrogen uptake efficiency (NUpE), and significant genotypic variations among all the traits were observed. Significant GXE interactions were recorded for days to leaf senescence, primary branches, siliquae per plant, seeds per siliqua, root traits, nitrogen content traits, biological yield, harvest index, seed yield, NUE, NUpE and NUtE (Table 3).

The mean squares from the analysis of variance for the individual nitrogen doses (N_{40} and N_0) and from the pooled analysis over the two nitrogen levels are presented in Table 3 (parts a and b, respectively).

Table 3: ANOVA for the traits (Rabi, 2021-22)

a. Analysis of variance (mean squares) for the traits studied during first year in pot experiment (Rabi, 2021-22) in both N0 and N40 doses

Source	df	DFF		DLSC		DM		PH (cm)		NPB		NSB		FL (cm)		LMR (cm)	
		N40	N0	N40	N0	N40	N0	N40	N0	N40	N0	N40	N0	N40	N0	N40	N0
Genotypes	43	2.96**	4.17**	29.49**	19.77**	5.03**	7.75**	243.46**	221.09**	0.73**	0.25**	6.52**	5.48**	7.79**	5.39**	37.33**	34.00**
Error	44	0.86	0.72	1.31	1.55	1.19	1.7	15.57	20.89	0.16	0.08	1.18	2	0.96	1.09	10.18	13.17
CV%		1.89	1.8	1.27	1.43	1.06	1.29	2.78	3.4	10.25	8.19	9.36	14.46	11.31	15.54	6.6	8.22

** Significant at P=0.05; ** Significant at P=0.01*

Table 3 (continued): part a, panel 2 of 3

Source	df	OC (%)		TGW		SQP		SSQ		MRL (cm)		RV (cc)		RDW(g)		SDW(g)	
		N40	N0	N40	N0	N40	N0	N40	N0	N40	N0	N40	N0	N40	N0	N40	N0
Genotypes	43	8.94**	24.27**	0.53	0.42	323.25**	383.13**	4.10**	2.86**	55.39**	47.72**	9.86**	8.80**	4.26**	1.75**	10.52**	8.31**
Error	44	0.1	11.61	0.31	0.02	6.94	9.85	0.84	0.63	2.76	1.87	2.71	1.92	0.76	0.3	0.83	0.61
CV%		0.1	10.05	11.79	3.47	1.71	2.17	7.72	8.54	3.35	6.72	13.51	14.06	22.99	20.01	10.66	12.92

** Significant at P=0.05; ** Significant at P=0.01*

Table 3 (continued): part a, panel 3 of 3

Source	df	R-S ratio		BYP (g)		LAI		HI (%)		Plant N		SYP (g)		NUpE (%)		NUtE (g/g)		NUE (g/g)	
		N40	N0	N40	N0	N40	N0	N40	N0	N40	N0	N0	N0	N40	N0	N40	N0	N40	N0
Genotypes	43	0.03**	0.02**	166.98**	155.96**	0.13	0.21	79.62**	5.09**	1.79**	0.88**	4.27**	4.27**	2.00**	2.18**	10.50**	15.24**	10.21**	12.49**
Error	44	0.01	0.01	12.42	6.4	0.31	0.36	7.93	0.54	0.05	0.01	0.37	0.37	0.15	0.04	0.85	0.83	1.09	1.08

CV%		13.29	9.78	12.89	12.57	19.12	20.83	10.09	10.13	9.01	5.98	11.3	11.3	10.38	6.41	9.14	9.31	10.13	11.3

** Significant at P=0.05; ** Significant at P=0.01*

Table 3 (continued): part b
b. Pooled ANOVA of pot culture experiments over two levels of N fertilizer (N40 and N0) during first year (Rabi,2021-22)

Source	df	DFF	DLSC	DM	PH (cm)	NPB	NSB	FL (cm)	LMR (cm)	OC (%)	TGW	SQP	SSQ	MRL (cm)
N levels	1	229.55**	416.57**	220.51**	2492.43**	14.15**	146.24**	164.82**	768.66**	26.01**	5.77*	4180.31**	294.28**	557.14**
Genotypes	43	5.95**	46.49**	11.39**	454.12**	0.70**	11.39**	12.14**	66.40**	27.70**	0.55	689.57**	4.07**	98.14**
G x N	43	1.19	2.77**	1.39	10.43	0.28**	0.62	1.04	4.93	5.51**	0.39	16.80**	2.89**	4.97**
Pooled error	88	0.79	1.43	1.45	18.23	0.12	2.56	2.01	11.68	5.81	0.16	8.39	0.73	2.32
CV%		1.85	1.35	1.18	3.09	9.47	15	18.44	7.39	7.03	8.98	1.94	8.1	6.88

** Significant at P=0.05; ** Significant at P=0.01*

Table 3 (continued): part b, panel 2 of 2

Source	df	RV (cc)	RDW (g)	SDW (g)	R-S ratio	Root N	Shoot N	Grain N	Plant N	SYP (g)	BYP (g)	HI (%)	NUE (g/g)	NUpE (%)	NUtE (g/g)
N levels	1	241.82**	48.89**	274.90**	0.0003	2.342**	1.89**	1.31**	17.02**	155.51**	2293.43**	64.59**	51.52**	0.01	4.23*
Genotypes	43	14.38**	4.42**	14.37**	0.02**	0.150**	0.11**	1.41**	1.87**	8.67**	302.68**	180.98**	21.00**	3.67**	21.57**
G x N	43	4.29**	1.59**	4.47**	0.03**	0.131**	0.04*	0.11**	0.30**	0.70**	20.27**	52.26**	1.70*	0.40**	4.17**
Pooled error	88	2.32	0.53	0.72	0.003	0.001	0.02	0.03	0.03	0.46	9.41	12.75	1.08	0.08	0.84
CV%		13.81	22.3	11.63	11.66	9.36	24.91	11.1	7.38	10.68	12.92	12.52	10.68	8.61	9.22

** Significant at P=0.05; ** Significant at P=0.01*

Mean performance of genotypes

Mean performances of the genotypes (Table 4) revealed that the earliest days to fifty percent flowering was attained for the genotype JMM-TM2-4 in both nitrogen doses; TM-204 recorded the earliest days to 75% maturity in both N0 and N40 dose followed by RRE 2012/14 in N0 levels. Maximum days to leaf senescence for high dose was recorded for NPJ-194 (96.3 days) followed by NPJ-193 (95.8 days); for low doses, NPJ-194 (93.4 days) recorded the maximum days followed by MM-TM2-160 (93.3 days). Genotype JMM-TM2-38 (122.3 cm at N40; 118.3 cm at N0) recorded the lowest plant height for both environments; the maximum primary branches were obtained in JMM-TM2-28 (6.7) followed by JMM-TM2-10 (5) for N40, and JMM-TM2-38 (4.3) had the maximum primary branches for N0 levels. KMH-405 (15.5) recorded the maximum secondary branches in N40, while JMM-TM2-34 (14.1) recorded the maximum secondary branches in N0. The longest main raceme was observed in the genotype JM16-1 (60.8 cm) for N40, in N0 Mustard No.2 (52.9 cm) recorded the longest main raceme. Foot length was the minimum for JMM-TM2-1 (4.9 cm) in high doses; conversely, JMM-TM2-1 (3.7 cm) had the minimum foot length for low dose environment. The maximum root length was exhibited under high fertilizer condition by PM26 (33.37 cm) followed by Pusa Bold (31.9 cm), under low doses it was maximum for PM26 (36.8 cm) followed by Pusa Bold (35.6 cm) and KMH405 (32.4 cm). Under N40 conditions, high root volume was exhibited by the genotypes JM16-2 (16.5 cc), Kranti (16.5 cc), and Pusa Bold (16.5 cc), while in N0 condition, it was recorded for the genotypes JMM-TM2-4 (14.5 cc), Kranti (14.4 cc), and JM16-2 (14.2 cc). The siliquae per plant was recorded in the genotype JM16-1 (177.1 no.) followed by JMM-TM2-34 (174.2) under N40 levels, in N0 levels, it was maximum for JMM-TM2-57 (166.3 no.) followed by JMM-TM2-34. Under high N condition, the highest number of seeds per siliqua was shown by the genotype Kranti (14.4 no.) followed by JM-16-2 (13.9), and in N0 condition, the highest number of seeds per siliqua was shown by the genotype JM16-3 (12.1) followed by JMM-TM2-4 (11.5). Plant N content was maximum for Pusa Bold (4.46%) followed by JM13-4 (4.42%) under high N fertilizer condition; simultaneously under N0 condition, it was highest for Kranti (3.83%) followed Pusa Bold (3.76%). At both N40 and N0 level, high root dry weight was obtained for Pusa Bold (10.2 g and 5.27 g, respectively); maximum shoot dry weight was recorded for Pusa Mahak (15.2 g) at N40 level and JMM-PM27-1 (10.74 g) at N0 levels. The maximum root by shoot ratio was obtained for Pusa Bold (0.73) followed by JMM-TM2-47 (0.70) under N40 conditions; under N0 conditions it was maximum for JM16-3 (0.70) followed by PM27 (0.68). The maximum biomass per plant was exhibited by Mustard No. 1 in both N levels; the maximum harvest index was recorded for JM16-2 in both fertilizer doses. Seed yield per plant was the maximum for Kranti followed by Pusa Bold in both N0 and N40 doses. High nitrogen use efficiency was observed for the genotypes Kranti and Pusa Bold in both environmental conditions; maximum nitrogen uptake efficiency was recorded for Pusa Bold and JM13-4 in both nitrogen dose levels. Nitrogen utilization efficiency was recorded maximum for Mustard No. 1 followed by PM25 in high fertilizer environments; while the genotypes Mustard No.1 and JMM-TM2-14 had the maximum nitrogen utilization efficiency under low fertilizer environments.

The mean performance of the forty-four genotypes under N40 and N0, together with the pooled means, is presented in Table 4 for reference.

Table 4: Mean performance for the traits in pot experiment at N_{40} and N_0 (Rabi 2021-22)

Genotype	Days to 50% flowering			Days to leaf senescence			Days to 75% maturity			Primary branches/ plant (No.)			Secondary branches/ plant (No.)			Plant height (cm)			Length of main raceme (cm)		
	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled
JM13-2	48.5	44.5	46.5	84	82.5	83.3	102	98	100	3.2	2.1	2.7	9.8	8.7	9.3	140.3	130.3	135.3	51	44	47.5
JM13-3	50.5	47.5	49	88.5	88.7	87.8	103.5	100.5	102	3.4	2.4	2.9	8.8	6.7	7.8	154.5	142.7	148.6	54.2	46.1	50.1
JM13-4	47.5	44.5	46	85.5	83.4	84.5	103	101	102	3.7	3.3	3.5	9.6	8.3	9	142.7	128.7	135.7	45	40.3	42.7
JM13-6	50.5	49.5	50	91.5	89	90.3	104.5	101.5	103	3.7	3.2	3.5	12.6	10.8	11.7	159.6	152.5	156	47.6	42.6	45.1
JM15-1	48.5	46.5	47.5	87.5	85.3	86.4	105.5	104.5	105	3.4	3.2	3.3	9.9	8.7	9.3	154.4	136.5	145.5	51.3	40.3	45.8
JM16-1	49.5	48.5	49	88.3	87	87.7	103.5	101	102.3	3.7	3.1	3.4	11.1	9.7	10.4	127.4	119.2	123.3	60.8	49.7	55.3
JM-16-2	50.5	48.5	49.5	89.5	87.9	88.7	103.5	99	101.3	3.8	3.5	3.7	10.3	8.7	9.5	136.7	130.5	133.6	49	44.1	46.5
JM16-3	47.5	45.5	46.5	93.4	91.3	92.4	107	105	106	3.5	3.5	3.5	11.7	10.9	11.3	154.2	145.7	149.9	41.7	39.4	40.5
JM16-4	50.5	49.5	50	88.8	87	87.9	104.5	103	103.8	3.4	3.1	3.3	9.6	8.8	9.2	144	138.1	141.1	45.4	42.1	43.7

JMM-13-57	49.5	48	48.8	86.7	85.9	86.3	106	104.5	105.3	3.3	3.3	3.3	12.9	10.7	11.8	144.8	134.5	139.6	47.3	45	46.2
JMM-PM27-1	47	45	46	85.4	83.3	84.4	103	99	101	4	3.4	3.7	10.6	9.5	10.1	142.3	136.3	139.3	49.3	46.3	47.8
JMM-PM27-11	50.5	48	49.3	90.5	87.5	89	104.5	100.5	102.5	3.7	3.3	3.5	10.4	9.8	10.1	150.4	141.8	146.1	48.2	42.6	45.4
JMM-TM2-1	48.5	46.5	47.5	91.4	88.8	90.1	100.5	98	99.3	4.3	3.1	3.7	11.5	9.8	10.7	138.5	130	134.3	50.3	46.2	48.2
JMM-TM2-10	49.5	48.5	49	88.8	85.8	87.3	105	102	103.5	5	4.1	4.5	14.5	12.4	13.5	154.2	146.8	150.5	46.1	44.4	45.3
JMM-TM2-14	47.5	45.5	46.5	85.8	83.8	84.8	102.5	102	102.3	4	3.6	3.8	11.1	9.1	10.1	137.1	130.5	133.8	41.5	37.6	39.6
JMM-TM2-160	48.5	46	47.3	94.8	93.3	94.1	105	102.5	103.8	4	3.6	3.8	13.7	10.9	12.3	135.1	126.9	131	48.3	45.5	46.9
JMM-TM2-17	47.5	45	46.3	91.4	87	89.2	105	103.5	104.3	3.7	3.3	3.5	10.4	8.7	9.6	129.1	122.4	125.8	48.4	45.6	47
JMM-TM2-2	50.5	48	49.3	85.8	84	84.9	101.5	99.5	100.5	3.6	3.7	3.6	11.2	9.6	10.4	136.3	132.5	134.4	47.8	45.2	46.5
JMM-TM2-20	48.5	46.5	47.5	85	83.8	84.4	102.5	100	101.3	3.7	3.3	3.5	11.1	8.9	10	136.5	129.5	132.9	46.3	42.8	44.5
JMM-TM2-208	49	48	48.5	89.8	86.8	88.3	101.5	98.5	100	5	3.8	4.4	13.7	10.8	12.3	138	132.4	135.2	39.5	34.3	36.9
JMM-TM2-23	50.5	47.5	49	86.8	84.5	85.7	103.5	102.5	103	4	3.6	3.8	12.1	9.8	11	137.9	129.7	133.8	37.8	34	35.9
JMM-TM2-28	50.5	45	47.8	84.9	83.5	84.2	103.5	101.5	102.5	6.7	3.4	5.1	12.5	11.1	11.8	133.9	134	134	40.3	37.9	39.1
JMM-TM2-34	49	47.5	48.3	85.8	82.8	84.3	101.5	100.5	101	3.8	3.6	3.7	14.3	14.1	14.2	136.4	122.8	129.6	50.2	42.6	46.4
JMM-TM2-38	49	47.5	48.3	88.9	86.3	87.6	103	102	102.5	4.7	4.3	4.5	7.3	6.3	6.8	122.3	118.2	120.2	52.3	49.4	50.8
JMM-TM2-4	46.5	44	45.3	84.8	81.7	83.3	101.5	100.5	101	4	3.4	3.7	12.1	9.2	10.7	129.5	119.5	124.5	50.3	48.2	49.2
JMM-TM2-47	47.5	45.5	46.5	86.4	83.6	85	103.5	101	102.3	3.7	3.4	3.6	13.5	11.8	12.7	139.2	132.4	135.8	53.3	48.9	51.1
JMM-TM2-73	48.5	46.5	47.5	90.9	86.6	88.8	106	102	104	5	3.5	4.3	14.7	13.1	13.9	136.2	128.2	132.2	51.5	47.8	49.6
JMM-TM2-15	50.5	48	49.3	88.8	84.2	86.5	102	99.5	100.8	4.4	3.9	4.2	11.2	8.3	9.8	137.4	128.9	133.2	47.2	46.8	47
KMH-405	49.5	47.5	48.5	88.3	85.2	86.8	103	101	102	3.9	3.5	3.7	15.5	11.5	13.5	139.2	135.3	137.2	50.9	46.7	48.8
KMR(E)16-1	49.5	47	48.3	83.8	83.3	83.6	104.5	103.5	104	3.7	3.3	3.5	13.7	10.1	11.9	140.7	133.6	137.1	51.9	50	51
Kranti	50.5	48	49.3	93.3	85.3	89.3	104	103	103.5	4.3	3.7	4	13.5	11.2	12.4	130.1	120.6	125.3	48.8	45.5	47.1
Mustard No-1	49.5	49	49.3	95.2	91.4	93.3	104.5	103.5	104	4	3.6	3.8	9.6	7.8	8.7	137.6	135.1	136.3	51	46.8	48.9
Mustard No-2	49	48.5	48.8	93.8	88.5	91.2	104.5	103	103.8	3.7	3.3	3.5	12	9.8	10.9	129.5	127.6	128.5	56.3	52.9	54.6
NPJ-193	50.5	48	49.3	95.8	89.8	92.8	104	100.5	102.3	4	3.4	3.7	11.5	9.3	10.4	157	145.8	151.4	45.8	42.7	44.3
NPJ-194	47.5	47	47.3	96.3	93.4	94.9	104	101	102.5	3.6	3.3	3.5	11.5	10.3	10.9	146.5	139.5	143	49.5	46.5	48
NRCHB-101	49.5	48	48.8	94.4	91.5	93	105.5	104.5	105	4	3.6	3.8	13.2	11.2	12.2	171.9	166.2	169	51.2	48.5	49.8
PM25	49	46	47.5	93.3	90	91.7	102.5	99	100.8	3.7	3.3	3.5	10.3	8.5	9.4	159.4	150.6	155	49.8	47.5	48.7
PM26	50.5	49	49.8	94	91.7	92.9	102.5	101	101.8	3.7	3.1	3.4	10.8	8.5	9.7	159.7	150.6	155.1	47.7	40.9	44.3
PM-27	48.5	45	46.8	95.2	91.5	93.4	101.5	102.5	102	4	3.3	3.7	9.2	8	8.6	166.4	159	162.7	46	43.2	44.6
Pusa Bold	51	48	49.5	94.9	88.3	91.6	101.5	99	100.3	3.7	3.3	3.5	11.1	9.8	10.5	129.1	123.9	126.5	44.2	40.3	42.2
Pusa Mahak	50.5	46.5	48.5	95.8	91.5	93.7	103.5	100	101.8	4	3.4	3.7	11.4	8.3	9.9	133.7	126	129.8	51.6	48.1	49.8
RRE-2012/14	50.5	46.5	48.5	94.9	88.9	91.9	101.5	97.5	99.5	3.5	3.2	3.4	14.4	13.3	13.9	136.8	133.1	134.9	46.6	40.2	43.4
TM-2	49.5	45.5	47.5	91.5	85.3	88.4	103.5	100.5	102	3.3	3.1	3.2	9.7	7.8	8.8	141.1	135.9	138.5	48.2	43.6	45.9
TM-204	51	47	49	92.9	88.5	90.7	99.5	97.5	98.5	3.7	3.1	3.4	10.4	9.2	9.8	143.1	135	139.1	44.7	39.1	41.9
Mean	49.3	47	48.1	90.1	87	88.5	103.4	101.1	102.3	3.9	3.4	3.7	11.6	9.8	10.7	142.1	134.5	138.3	48.3	44.1	46.2
CD5%	1.9	1.7	1.2	2.1	2.4	1.7	2.2	2.6	1.7	0.8	0.6	0.5	3.6	2.9	2.3	8	9.2	1.3	6.4	7.3	1

Table 4 (continued): panel 2 of 3

Genotype	Foot length (cm)			Main root length (cm)			Root volume (cc)			Siliquae/plant			Seeds/siliqua			Plant N %			Root dry wt. (g)		
	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled
JM13-2	9.6	9.7	9.6	30.4	21.9	26.1	14	10.4	12.2	103.5	88.3	95.9	12.5	7.8	10.1	4.4	3.4	3.9	1.6	1.5	1.6
JM13-3	8.8	6.7	7.7	20.2	15.4	17.8	12.4	9.7	11.1	144.3	129.6	137	13.3	9.3	11.3	1.8	1.5	1.6	3.8	3.9	3.9
JM13-4	10.1	6.1	8.1	26.5	21.5	24	11.4	8.9	10.1	137.3	122.9	130.1	8.7	7.8	8.3	4.4	3.5	4	4.8	3.8	4.3
JM13-6	9.1	7.7	8.4	24.6	20.9	22.7	13.7	8.1	10.9	146.7	134.9	140.8	12.5	8.8	10.7	3.8	2.1	3	3.6	3	2.4
JM15-1	6.6	4.7	5.7	27.9	17.8	22.8	13.4	9.5	11.5	134.2	123.6	128.9	10.2	7.3	8.8	3.1	1.7	2.4	4	3.3	3.7
JM16-1	5.8	4.3	5	23.8	18.8	21.3	12.7	9.2	10.9	177.1	160.1	168.6	11.1	9.1	10.1	2.3	2	2.2	3.9	2.8	3.3
JM-16-2	6.5	5	5.8	28.4	20.5	24.4	16.5	14.2	15.4	156.8	140.9	148.9	13.9	10.8	12.4	4.3	3.5	3.9	3.4	2.5	3
JM16-3	12.4	8.9	10.7	15.8	11.4	13.6	15	10.1	12.5	164.1	154.4	159.3	11.1	12.1	11.6	1.7	1.6	1.6	1.2	1.1	1.1
JM16-4	9.5	7.9	8.7	24.9	21.8	23.4	13.7	9	11.3	155.8	147	151.4	11.9	9.4	10.7	3.1	2.1	2.6	2.7	1.9	2.3
JMM-13-57	8.8	6.9	7.9	20.4	14.2	17.3	9.7	8	8.8	174.1	166.3	170.2	12.3	8.2	10.3	1.9	1.7	1.8	3.7	2.4	3.1
JMM-PM27-1	5.5	5.2	5.3	20.4	16.9	18.6	10.7	9.5	10.1	166	154.2	160.1	12.8	8.5	10.7	1.7	1.4	1.5	2.3	4.6	3.5
JMM-PM27-11	7.7	7.1	7.4	18.9	16.5	17.7	12.1	10.2	11.2	139.8	136	137.9	12.1	8.8	10.5	2.3	1.8	2	2.6	2	2.3
JMM-TM2-1	4.9	3.7	4.3	23.1	18	20.5	11.9	8.7	10.3	145.7	134.4	140.1	11.7	10.3	11	2.2	1.6	1.9	3.4	2.4	2.9
JMM-TM2-10	6.3	5.2	5.7	16.3	14.5	15.4	10.4	7.7	9.1	156.3	143.6	150	13.7	10	11.9	2.3	2.1	2.2	2.6	2.2	2.4
JMM-TM2-14	8.3	6.3	7.3	25	19.7	22.3	14.2	5.7	10	167.5	154.6	161.1	11.8	10.2	11	2.3	1.1	1.7	3.8	2.6	3.2
JMM-TM2-160	7.3	5.8	6.5	32.4	28.9	30.6	10.9	9.4	10.2	161.8	150.1	156	13.4	7.3	10.4	2.3	1.6	1.9	3.2	1.8	2.5
JMM-TM2-17	9.3	8.1	8.7	21.1	18.9	20	9.3	6.8	8.1	153.8	143.6	148.7	13	10.4	11.7	2.2	1.3	1.7	3.9	3.8	3.8
JMM-TM2-2	7.5	4.9	6.2	17.8	16	16.9	8	6.9	7.5	149.7	140.8	145.3	10.1	9	9.6	2	1.4	1.7	3.6	1.6	2.6
JMM-TM2-20	7.4	6.2	6.8	25.9	23.4	24.6	11.2	9.5	10.4	141.2	128.6	134.9	12.6	11	11.8	2.8	2.1	2.4	5.8	3.2	4.5
JMM-TM2-208	11.5	9.4	10.5	24.5	21.6	23.1	12.1	8.8	10.5	142.5	131.3	136.9	10.5	9	9.8	4	2.1	3	4.1	3.3	3.7
JMM-TM2-23	9.6	8.1	8.8	21	19	20	13.9	11.4	12.7	156.6	150.4	153.5	12.1	8.8	10.5	2.8	2.1	2.5	4.1	1.7	2.9
JMM-TM2-28	8.4	7.6	8	19.8	18.4	19.1	12.1	5.8	9	154.6	145.1	149.9	13.7	10.1	11.9	2.6	3	2.8	3.9	3.1	3.5
JMM-TM2-34	6.3	5	5.7	23.3	20.3	21.8	13	9.5	11.3	174.2	163.6	168.9	13.7	9.5	11.6	4.3	2	3.1	5.7	3.8	4.8
JMM-TM2-38	7.7	5.8	6.8	28.6	25.8	27.2	11.2	9.3	10.3	169.5	161.6	165.6	11.8	8.8	10.3	2.9	1.7	2.3	3.7	3.8	3.7
JMM-TM2-4	9.7	6.9	8.3	25.5	19	22.3	11.8	14.5	13.2	163.3	153.3	158.3	12.2	11.5	11.9	3	2.9	2.9	2.6	3.1	2.8
JMM-TM2-47	11.5	8.7	10.1	25.5	22.9	24.2	14.5	11.9	13.2	153	143.7	148.4	13.1	9.6	11.4	3	2	2.5	3.7	2.9	3.3
JMM-TM2-73	10.5	7.2	8.8	18.9	16.9	17.9	11.8	10.9	11.4	149.5	138.6	144.1	13.8	11.3	12.6	2.2	2	2.1	4.7	3.5	4.1
JMM-TM2-15	6.8	4.7	5.7	20.8	16.4	18.6	11.7	10.2	11	153.5	142.2	147.9	11.5	9.3	10.4	2.1	2	2.1	3.9	3.3	3.6
KMH-405	9.1	8.4	8.7	32.4	28.4	30.4	11.5	9.9	10.7	164.5	150.8	157.7	12	8.9	10.5	2	1.9	1.9	5.1	3.1	4.1
KMR(E)16-1	7.1	5.4	6.2	13.8	11.3	12.6	13.5	12.8	13.2	144.5	135.3	139.9	8.1	9	8.6	2.6	2	2.3	1.5	2.2	1.8
Kranti	8	8.2	8.1	24	26.9	25.5	16.5	14.4	15.5	142.6	126.6	134.6	14.4	8.5	11.5	4	3.8	3.9	4.7	2.1	3.4
Mustard No-1	6.1	4	5.1	25.3	23.8	24.6	7.5	7.6	7.6	159.4	151.2	155.3	11.6	9.5	10.5	2.9	2.5	2.7	3.8	3.9	3.8
Mustard No-2	7.4	6.3	6.8	21.1	18	19.5	11.5	10.8	11.2	163.7	159.5	161.6	11.3	9.6	10.5	2.6	2.6	2.6	2.7	1.1	1.9
NPJ-193	9.8	7	8.4	19	18	18.5	12.5	10.6	11.6	164.5	160.6	162.6	11.6	10.6	11.1	2.1	1.5	1.8	4.6	3.2	3.9
NPJ-194	6.8	5	5.9	23.8	19.3	21.6	9.5	7.9	8.7	154.8	151.5	153.2	13	9.5	11.3	2.8	1.6	2.2	3.2	2.1	2.7
NRCHB-101	13.4	10.9	12.1	30.8	26.4	28.6	12	11.2	11.6	165.3	159.3	162.3	11.2	10.4	10.8	2.3	1.9	2.1	3.5	1.6	2.6
PM25	12.1	8.3	10.2	15.5	15.5	15.5	14.5	12.5	13.5	154	148	151	10.8	9.2	10	2.5	1.9	2.2	5.8	2.1	4
PM26	8.7	6.6	7.6	36.8	33.4	35.1	11.5	9.9	10.7	154.6	152.3	153.5	10.9	7.8	9.4	2.3	2	2.1	3.8	3.3	3.6
PM-27	10	8.1	9	20.7	17.6	19.2	12.5	9.7	11.1	153	150	151.5	9.4	8.8	9.1	1.9	1.7	1.8	3.2	2.4	2.8
Pusa Bold	8.8	6.1	7.5	35.6	31.9	33.8	16.5	11.3	13.9	151.3	147.7	149.5	10.4	9.4	9.9	4.5	3.8	4.1	10.2	5.3	7.7
Pusa Mahak	11.3	7.2	9.2	30.8	26	28.4	13.5	10.8	8.9	151.1	147.8	149.5	13.1	7.8	10.5	2.4	2.1	2.3	3.8	3.2	3.5
RRE-2012/14	10.8	8.8	9.8	22.6	21.2	21.9	13.5	12.8	13.2	161.7	143.9	152.8	12.3	6.5	9.4	3	1.6	2.3	5.4	2.4	3.9
TM-2	9.1	6.3	7.7	20.8	18	19.4	14.5	10.9	12.7	156.2	143.9	150.1	9.9	10.3	10.1	2.3	2.1	2.2	3.7	1.2	2.4
TM-204	9.5	6.1	7.8	26.6	21.6	24.1	8.8	6.6	7.7	149.8	142.5	146.2	10.3	7.8	9.1	2	1.9	2	3.7	2.6	3.2
Mean	8.7	6.7	7.7	23.9	20.3	22.1	12.2	9.9	11	154.2	144.4	149.3	11.9	9.3	10.6	2.7	2.1	2.4	3.8	2.7	3.3
CD5%	3.5	2.1	2	3.4	2.8	2.1	3.3	2.8	2.1	5.3	6.3	4.1	3.8	3	1.2	0.5	0.3	0.2	1.8	1.1	1

Table 4 (continued): panel 3 of 3

Genotype	Shoot dry wt. (g)			Root-shoot ratio			Biol. yield/plant (g)			Harvest index (%)			Seed yield/plant (g)			NUpE (%)			NUtE (g/g)			NUE (g/g)		
	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled	N40	N0	Pooled
JM13-2	4.5	3.2	3.8	0.4	0.5	0.4	25.48	13.13	17.54	30.6	35.91	35.75	7.78	4.67	6.22	5.04	5.85	5.45	6.59	4.59	5.59	11.01	7.98	9.5
JM13-3	7	7.9	7.5	0.6	0.5	0.5	30.	24.	26.9	21.	20.	22.0	6.6	5.0	5.82	2.4	2.5	2.49	13.	10.	12	9.3	8.6	8.98

					5		93	63	2	5	91	4	1	3			8		45	56		5		
JM13-4	9.5	8.9	9.2	0.5	0.4	0.5	29.28	16.83	24.63	28.5	23.65	24.62	8.32	3.95	6.13	5.25	5.99	5.62	7.64	7.18	7.41	11.78	6.74	9.26
JM13-6	10.3	6.8	8.5	0.4	0.4	0.4	25.97	21.92	23.94	29.4	26.02	27.71	7.65	5.71	6.68	4.22	3.52	3.87	9.58	10.16	9.87	10.83	9.76	10.29
JM15-1	8.1	7	7.5	0.5	0.5	0.5	29.39	19.89	24.64	29.1	35.29	32.2	8.56	7.01	7.78	3.3	2.94	3.12	11.55	13.95	12.75	12.12	11.98	12.05
JM16-1	8.5	5.9	7.2	0.5	0.5	0.5	24.82	15.43	20.13	35.2	50.41	42.8	8.73	7.78	8.25	3.2	3.35	3.28	10.71	10.38	10.54	12.36	13.29	12.83
JM-16-2	6.7	5.9	6.3	0.5	0.4	0.5	19.84	11.51	15.67	40.5	53.98	51.41	7.18	6.19	6.69	5.15	5.88	5.52	6.49	5.69	6.09	10.17	10.58	10.38
JM16-3	6.1	1.8	3.9	0.3	0.7	0.5	31.22	15.54	23.38	19.9	31.73	25.81	6.2	4.79	5.49	2.3	2.61	2.45	11.59	9.76	10.67	8.78	8.18	8.48
JM16-4	4.8	3.8	4.3	0.6	0.5	0.5	27.89	22.88	25.39	27.5	25.25	26.39	7.67	5.79	6.73	3.09	3.48	3.29	9.59	9.06	9.33	10.86	9.89	10.38
JMM-13-57	5.6	6.8	6.2	0.7	0.4	0.5	21.49	19.6	20.54	27.5	20.96	22.74	5.73	4.1	4.91	2.59	2.75	2.67	9.2	7.78	8.49	8.11	7	7.56
JMM-PM27-1	7.3	10.8	9	0.3	0.4	0.4	29.84	19.61	24.72	14.3	19.23	16.77	4.27	3.78	4.02	2.29	2.19	2.24	12.29	10.52	11.41	6.04	6.46	6.25
JMM-PM27-11	5.9	3.2	4.6	0.5	0.6	0.5	27.94	20.71	24.33	24.3	25.65	24.96	6.79	5.1	5.94	3.09	3	3.04	8.93	7.01	7.97	9.61	8.72	9.16
JMM-TM2-1	6.2	5.3	5.7	0.6	0.5	0.5	30.56	16.58	23.57	22.3	31.9	27.12	6.84	5.29	6.06	2.98	2.59	2.79	10.14	10.13	10.14	9.68	9.04	9.36
JMM-TM2-10	6.3	4.6	5.4	0.4	0.5	0.4	29.62	24.94	27.28	27.4	26.97	27.16	8.11	6.72	7.41	3.21	2.68	2.95	9.86	11.89	10.87	11.48	11.49	11.48
JMM-TM2-14	6.9	5.8	6.3	0.6	0.4	0.5	17.81	14.89	16.35	34.2	28.05	28.71	5.97	4.18	5.07	3.15	1.76	2.46	10.88	15.45	13.16	8.45	7.14	7.79
JMM-TM2-160	6.8	4.6	5.7	0.5	0.4	0.4	25.75	21.79	23.77	29.3	26.26	27.79	7.56	5.73	6.64	3.15	2.53	2.84	9.85	12.61	11.23	10.7	9.8	10.25
JMM-TM2-17	9.3	7.8	8.5	0.4	0.5	0.5	19.78	15.55	17.66	24.8	21.69	24.12	4.88	3.39	4.14	2.95	2.11	2.53	9.67	13.34	11.51	6.91	5.8	6.35
JMM-TM2-2	8.7	6.1	7.4	0.4	0.3	0.3	20.91	14.11	17.51	31.4	38.54	34.99	6.5	5.39	5.95	2.73	2.28	2.5	10.91	13.55	12.23	9.2	9.21	9.21
JMM-TM2-20	9.2	5.8	7.5	0.7	0.6	0.6	27.77	20.74	24.25	24.7	28.14	26.44	6.57	5.84	6.21	3.88	3.41	3.64	7.14	7.95	7.54	9.3	9.98	9.64
JMM-TM2-208	10.2	6.7	8.5	0.4	0.5	0.5	24.49	20.65	22.57	36.1	24.03	22.8	7.45	4.95	6.2	4.6	3.44	4.02	7.37	8.86	8.12	10.55	8.46	9.51
JMM-TM2-23	8.8	3.7	6.3	0.5	0.5	0.5	25.15	24.44	24.79	23.8	17.3	20.56	5.94	4.23	5.08	3.88	3.53	3.71	6.81	7.51	7.16	8.41	7.22	7.82
JMM-TM2-28	9.2	7.9	8.5	0.4	0.4	0.4	21.78	11.71	16.75	29	28.01	28.53	6.32	3.27	4.8	3.56	5.03	4.29	8.85	3.73	6.29	8.95	5.59	7.27
JMM-TM2-34	10.7	7.7	9.2	0.5	0.5	0.5	25.92	20.54	23.23	30.4	29.8	30.1	7.87	6.12	6.99	5.17	3.29	4.23	6.1	9.8	7.95	11.14	10.45	10.8
JMM-TM2-38	10.2	6.9	8.6	0.4	0.6	0.5	14.48	13.21	13.85	39.4	28.69	34.02	5.71	3.73	4.72	2.74	2.66	2.7	8.07	8.23	8.15	8.09	6.38	7.23
JMM-TM2-4	8.2	6	7.1	0.3	0.5	0.4	30.56	11.94	21.25	20.9	40.05	30.47	6.39	4.78	5.58	4.1	4.76	4.43	6.69	6.05	6.37	9.04	8.17	8.61
JMM-TM2-47	5.5	6.3	5.9	0.7	0.5	0.6	19.96	14.97	17.46	34.6	28.4	29.79	6.87	4.23	5.55	2.94	3.27	3.11	10.79	9.74	10.27	9.72	7.22	8.47
JMM-TM2-73	10.4	7.8	9.1	0.5	0.5	0.5	26.57	11.13	18.85	24	47.42	35.69	6.37	5.23	5.8	3.03	3.35	3.19	9.03	7.88	8.45	9.01	8.93	8.97
JMM-TM2-15	10.9	7.1	9	0.4	0.5	0.4	15.59	14.6	15.1	25.9	22.98	24.46	4.06	3.34	3.7	2.93	3.27	3.1	10.38	8.82	9.6	5.75	5.7	5.73
KMH-405	11	8.8	9.9	0.5	0.4	0.4	22.86	17.87	20.37	27.6	26.98	27.28	6.32	4.83	5.57	2.54	2.95	2.75	11.85	9.87	10.86	8.95	8.25	8.6
KMR(E)16-1	8.3	5.8	7	0.2	0.4	0.3	17.78	14.76	16.27	33.6	33.13	33.37	5.97	4.89	5.43	2.94	3.28	3.11	8.88	9.29	9.08	8.45	8.36	8.4
Kranti	11.1	8.8	9.9	0.4	0.2	0.3	46.05	39.87	42.96	26.7	27.88	27.28	12.28	11.11	11.69	5.04	5.73	5.38	12.18	9.34	10.76	17.39	18.98	18.18
Mustard No-1	11.5	7.7	9.6	0.4	0.5	0.4	69.4	64.1	66.75	11.1	7.37	9.25	7.71	4.72	6.21	3.13	3.45	3.29	16.81	17.54	17.18	10.92	8.06	9.49
Mustard No-2	8.3	2.3	5.3	0.3	0.5	0.4	23.51	19.5	21.5	32.7	29.62	31.18	7.7	5.79	6.74	2.68	2.85	2.77	13.07	12.61	12.84	10.9	9.89	10.4
NPJ-193	8.3	6	7.1	0.6	0.6	0.5	18.17	17.05	17.61	33.1	30.08	31.6	5.91	5.11	5.51	2.68	2.43	2.55	8.88	10.9	9.89	8.37	8.73	8.55
NPJ-194	7	3.8	5.4	0.5	0.6	0.5	35.68	21.46	28.57	26.1	24.66	25.37	9.33	5.32	7.32	2.86	2.6	2.73	12.17	10.55	11.36	13.21	9.09	11.15
NRCHB-101	5.8	3.8	4.8	0.6	0.5	0.5	31.05	14.1	22.57	28.3	47.17	37.73	8.73	6.66	7.7	2.7	3.09	2.9	12.33	10.2	11.27	12.36	11.39	11.87
PM25	10.2	5	7.6	0.6	0.4	0.5	27.98	18.35	23.16	32.1	34.2	33.16	8.99	6.19	7.59	2.83	3.18	3.01	13.61	10.1	11.85	12.72	10.58	11.65
PM26	11	5.3	8.2	0.4	0.6	0.5	33.79	23.05	28.42	22.2	24.83	23.52	7.5	5.6	6.55	2.85	3.25	3.05	11.19	8.92	10.06	10.61	9.57	10.09
PM-27	7.9	3.4	5.6	0.4	0.7	0.5	21.84	14.05	17.94	30.8	29.12	29.95	6.72	4.07	5.4	2.46	2.85	2.66	7.48	6.47	6.98	9.52	6.96	8.24
Pusa Bold	13.6	9.7	11.6	0.7	0.5	0.6	36.66	29.96	33.31	32	29.43	30.71	11.72	8.82	10.27	5.49	6.38	5.93	8.87	7.73	8.3	16.6	15.08	15.84
Pusa Mahak	15.2	7	11.1	0.3	0.5	0.4	34.18	26.44	30.31	25.5	26.96	26.22	8.71	6.84	7.78	3.1	3.36	3.23	11.94	11.77	11.86	12.33	11.69	12.01
RRE-2012/14	9.3	8.8	9	0.6	0.3	0.4	39.78	29.83	34.8	21.3	20.11	21.99	8.35	5.99	7.17	2.85	2.43	2.64	10.92	9.17	10.05	11.82	10.23	11.03
TM-2	9.6	4	6.8	0.4	0.3	0.4	23.44	19.18	21.31	34.8	29.83	32.34	8.17	5.73	6.95	3.14	3.34	3.24	11.32	10.18	10.75	11.57	9.8	10.68
TM-204	6.6	4.9	5.7	0.5	0.6	0.5	24.1	20.85	22.47	28.2	24.4	27.77	6.74	5.06	5.9	2.76	2.46	2.61	12.61	13.84	13.23	9.54	8.65	9.09
Mean	8.6	6.1	7.3	0.5	0.5	0.5	27.43	20.09	23.73	28.01	29.16	28.51	7.27	5.39	6.33	3.34	3.36	3.35	10.1	9.79	9.94	10.29	9.21	9.75
CD5%	1.8	1.6	1.2	0.1	0.1	0.1	6.83	4.96	4.31	11.32	8.33	6.65	1.48	1.23	0.95	0.7	0.43	0.41	1.86	1.84	1.29	2.1	2.09	0.31

The present investigation revealed that the increased nitrogen rate increased yield attributing traits viz. primary branches, secondary branches, seed yield per plant, number of seeds per siliqua, siliquae per plant, etc. High plant nitrogen content was recorded for Pusa Bold, Kranti, and JM13-4; genotypes with higher plant nitrogen content revealed delayed leaf senescence. Kranti and Pusa Bold recorded the maximum nitrogen use efficiency and seed yield per plant, respectively. Of the forty-four genotypes, Kranti, Pusa Bold, JMM-TM2-47 and RRE2012/14 were the best performing genotypes based on their mean values for various traits including nitrogen use efficiency in both N40 and N0 environments. This study prioritized the screening of nitrogen-use efficient (NUE) genotypes, enabling the successful identification of superior germplasm under nitrogen deficient conditions. Eleven out of forty-four genotypes showing superior performances for NUE and most of the yield traits (Table 5) under both N0 and N40 environments were selected as parents; eight lines (JM13-4, Kranti, JMM-TM2-14, RRE2012/14, JM16-3, Pusa Bold, JMM-TM2-28 and NPJ-194) and three testers (TM-2, NRCHB-101and PM-25) were used to perform line x tester crosses.

The basis on which the eleven parents (eight lines and three testers) were selected for the line × tester mating design is summarised in Table 5.

Table 5: Table showing basis of selection of genotypes for LxT matting design

Genotype	Basis of selection	Class
JM13-4	High plant N, high NUpE, high NUE (AE), high shoot and root dry weight, early flowering and maturity, high NUE at high fertilizer dose,	Line
Kranti	High NUpE, high NUE, high seed yield/plant, medium height, short foot and high 1000 seed weight, high plant N, high shoot dry weight, high root volume, high seeds/siliqua, high biological yield/plant, high secondary branches/plant, high NUE at high fertilizer dose, high secondary branches/plant	Line
JMM-TM2-14	High NUtE, early flowering	Line
RRE2012/14	Early maturity, high shoot dry weight, root and shoot dry weight, high biological yield/plant, high secondary branches/plant, high NUE at high fertilizer dose,	Line
JM16-3	High number of primary branches/ plant, high seeds/siliqua	Line
Pusa Bold	High NUpE, NUE, seed yield/plant, early maturity, medium height, high seed weight and high oil content, high plant N, high shoot and root dry weight, long root, high root volume, high biological yield/plant, high NUE at high fertilizer dose,	Line
JMM-TM2-28	Short foot length, high seeds/siliqua, high number of primary branches/ plant, high secondary branches/plant	Line
NPJ-194	Medium height, short foot length and high seed weight, late leaf senescence, early flowering, high NUE (AE), high NUE at high fertilizer dose, high secondary branches/plant	Line
TM-2	Early maturity and high oil content, high NUE at high fertilizer dose,	Tester
NRCHB101	High NUE at high fertilizer dose, late leaf senescence, high siliquae number /plant, high harvest index, high secondary branches/plant	Tester
PM-25	High seed yield/plant and NUE, early maturity, high seed weight, root dry weight, high root volume, high harvest index, high NUE at high fertilizer dose, high secondary branches/plant	Tester

Genetic diversity

Mahalanobis D^2 statistics grouped forty-four genotypes into three clusters (Table 6); cluster I had forty-one genotypes, cluster II had two genotypes, and cluster III was solitary.

Intra and inter-cluster distances (Table 6) revealed that the maximum intra-cluster distance was recorded for cluster II (1117.21) followed by cluster I (428.68). The maximum inter-cluster distance was observed between cluster III - II (3122.86), followed by cluster III - I (1616.7) and between cluster II - I (1448.7).

Cluster means (Table 6) revealed that cluster I had the genotypes with early flowering (49.2 days), maximum number of secondary branches (11.7), maximum number of seeds per siliqua (11.9) and high nitrogen uptake efficiency (23.7%). Cluster II had the genotypes with early maturity, decreased plant height (134.7 cm), maximum seed yield per plant (9.75 g), long roots (32.9 cm), high root volume (15.25 cc), maximum shoot dry weight (15.3 g), root dry weight (s5.9 g), high root-shoot ratio (0.54), high plant nitrogen content (4.42%) and high harvest index (31.3%). Cluster III had low foot length (6.1 cm), long main raceme (51.0 cm), greater number of primary branches (4.0), more siliquae per plant (159.4), high biological yield per plant (69.4 g), late leaf senescence (95.2 days), high nitrogen utilization efficiency (22.0 g/g) and high nitrogen use efficiency (70.7 g/g).

The grouping of the genotypes into clusters, the average intra- and inter-cluster distances and the cluster means are detailed in Table 6 (parts a to c).

Table 6: Mahalanobis D^2 genetic divergence among the forty-four genotypes of Indian mustard

a. Grouping of the 44 genotypes into three clusters based on Mahalonobis distance

Number of cluster	No. of genotypes	Name of genotypes
Cluster I	41	JM13-3, JM13-4, JM13-6, JM15-1, JM16-1, JM-16-2, JM16-3,JM16-4, JMM-13-57, JMM-PM27-1, JMM-PM27-11, JMM-TM2-1, JMM-TM2-10, JMM-TM2-14, JMM-TM2-160, JMM-TM2-17, JMM-TM2-2, JMM-TM2-20, JMM-TM2-208, JMM-TM2-23, JMM-TM2-28, JMM-TM2-34 , JMM-TM2-38, JMM-TM2-4, JMM-TM2-47, JMM-TM2-73, JMM-TM2-15, KMH-405, KMR(E)16-1, KRANTI, Mustard No-2, NPJ-193, NPJ-194, NRCHB-101, PM25, PM26, PM-27, Pusa Mahak, RRE-2012/14, TM-2, TM-204
Cluster II	2	JM13-2, Pusa Bold
Cluster III	1	Mustard No-1

b. Average intra and inter cluster distance among the clusters of the 44 genotypes

Cluster	I	II	III
I	428.68	1448.7	1616.7
II	1448.69	1117.21	3122.86
III	1616.7	3122.86	0

C. Cluster means of forty-four genotypes

Cluster	I	II	III
DFF	49.2	49.7	49.5
DM	103.4	101.8	104.5
DLSC	90	89.4	95.2
PH	142.5	134.7	137.6
FL	8.7	9.2	6.1
LMR	48.3	47.6	51
NPB	4	3.4	4
NSB	11.7	10.5	9.6
SQP	155.4	127.4	159.4
SSQ	11.9	11.4	11.6
MRL	23.2	33	25.3
RV	12.3	15.3	7.5
SDW	12.3	15.3	7.5
RDW	3.8	5.9	3.8
R/S ratio	0.46	0.54	0.37
BYP	26.2	31.1	69.4
Plant N	2.62	4.42	2.88
HI	28.3	31.3	11.1
SYP	7.13	9.75	7.71
NUpE	23.7	18.6	14.6
NUtE	15.6	12.8	22
NUE	25.8	31.4	70.7

The contribution of total variation was maximum for siliquae per plant (15.5%), biological yield per plant (14.2%), plant nitrogen content (11.2%), root length (10.7%), nitrogen use efficiency (8.1%) and root volume (8.04%), made major contributions, followed by plant height (5.91%), seed yield per plant (4.8%), harvest index (4.6%), nitrogen utilization efficiency (3.8%), days to maturity (2.3%), days to 50% flowering (1.4%) and nitrogen uptake efficiency (1.3%).

Molecular characterization

A total of 80 SSR markers were assessed to identify polymorphisms among the forty-four genotypes (Figure 1). Of the eighty SSR markers, fifty-eight markers generating 158 alleles revealed polymorphism (Table 7). The frequency of major allele (MAF) ranged from 0.41 to 0.89 with an average of 0.65. The highest MAF was detected for OL10-F11a (0.89) followed by BRMS-040 and Ol10B11 (0.88). The number of alleles (Na) per locus varied from 2 to 5 with an average of 2.72 alleles per locus. Out of total 58 polymorphic markers, 33 markers had three alleles, 21 had two alleles, three markers had four alleles and one marker viz. CN-52 (B-16) had five alleles. Effective number of alleles (Ne) ranged between 1.23 and 3.16 with an average of 1.97. The maximum effective number of alleles was noted in BRMS-033 (3.16), followed by CN-52 (B-16) (2.95) and PBCESSRJU1 (2.94). The Shannon's information index (I) ranged between 0.34 and 1.29 with a mean of 0.76. The highest information index was found in CN-52 (B-16) (1.29), followed by BRMS-033 (1.22) and PBCESSRJU1 (1.21); expected heterozygosity (He) ranged from 0.19 to 0.68, with an overall mean of 0.47. The highest expected heterozygosity was observed for BRMS-033 (0.68), followed by CN-52 (B-16) and PBCESSRJU1 (0.66 each). The polymorphism information content (PIC) scores ranged from 0.17 to 0.62 with an average of 0.40. The highest polymorphism information content score was recorded for BRMS-033 (0.62), CN-52 (B-16) (0.62) and PBCESSRJU1 (0.61). In contrast, the lowest PIC score (0.17) was recorded for OL10-F11a. The allelic and diversity statistics for the 58 polymorphic SSR markers are tabulated in Table 7.

Table 7: SSR markers with their number of different alleles amplified (Na), major allele frequency (MAF), number of effective alleles (Ne), Shannon's informative index (I), observed heterozygosity (Ho), expected heterozygosity (He) and polymorphism information content (PIC) in the forty-four genotypes.

S.N.	Primer	Na	MAF	Ne	I	Ho	He	PIC
1	BRMS-003	3	0.692	1.787	0.705	0	0.44	0.364
2	Ol10B11	2	0.878	1.273	0.371	0	0.214	0.191
3	E05	2	0.791	1.495	0.513	0	0.331	0.276
4	E07	3	0.619	2.014	0.808	0	0.503	0.416
5	BRMS-017	3	0.614	2.215	0.934	0	0.549	0.489
6	SORF-73 (B-27)	2	0.525	1.995	0.692	0	0.499	0.374
7	CN-52 (B-16)	5	0.512	2.949	1.294	0	0.661	0.617
8	Ra2-E03	3	0.86	1.331	0.493	0	0.249	0.233
9	BRMS-093	2	0.581	1.948	0.68	0	0.487	0.368
10	SR_94102	3	0.605	2.188	0.911	0	0.543	0.474
11	SSR_Na10-D11	3	0.591	2.262	0.937	0	0.558	0.49
12	BRMS-240	3	0.86	1.331	0.493	0	0.249	0.233

13	SSR_Na10D09	3	0.568	2.39	0.978	0	0.582	0.516
14	NI-F02a	2	0.585	1.943	0.679	0	0.485	0.368
15	BRMS_324	2	0.568	1.963	0.684	0	0.491	0.37
16	SR_9222	3	0.614	2.185	0.918	0	0.542	0.479
17	sR7178	3	0.591	2.262	0.937	0	0.558	0.49
18	Ol10B01	3	0.86	1.331	0.493	0	0.249	0.233
19	Ni3-B07	3	0.568	2.39	0.978	0	0.582	0.516
20	sS1702	2	0.585	1.943	0.679	0	0.485	0.368
21	Ol10-H02	3	0.692	1.787	0.705	0	0.44	0.364
22	BRMS-040	2	0.878	1.273	0.371	0	0.214	0.191
23	Ni2B03	2	0.791	1.495	0.513	0	0.331	0.276
24	sORA43	3	0.619	2.014	0.808	0	0.503	0.416
25	BRMS-011	3	0.614	2.215	0.934	0	0.549	0.489
26	Ni2D10	2	0.841	1.365	0.438	0	0.268	0.232
27	PBCESSRJU13	2	0.705	1.713	0.607	0	0.416	0.33
28	NI02-D08a	3	0.581	2.021	0.77	0	0.505	0.399
29	EJU1	3	0.614	2.215	0.934	0	0.549	0.489
30	NI03-H07a	2	0.548	1.982	0.689	0	0.495	0.373
31	PBCESSRJU1	4	0.488	2.944	1.207	0	0.66	0.606
32	PBCESSRJU2	3	0.581	2.021	0.77	0	0.505	0.399
33	PBCESSRJU3	3	0.595	2.125	0.864	0	0.529	0.446
34	PBCESSRJU4	4	0.5	2.844	1.18	0	0.648	0.592
35	Ra2-E11	3	0.614	2.021	0.807	0	0.505	0.416
36	Ra2-E12	2	0.705	1.713	0.607	0	0.416	0.33
37	G02	3	0.69	1.789	0.703	0	0.441	0.363
38	Ra2-D04	3	0.705	1.783	0.734	0	0.439	0.375
39	OL10-A03a	2	0.565	1.967	0.685	0	0.491	0.371
40	OL10-F11a	2	0.895	1.232	0.336	0	0.188	0.171
41	RA02-A04a	3	0.605	2.099	0.855	0	0.524	0.441
42	RA02-E01a	2	0.548	1.982	0.689	0	0.495	0.373
43	SSR_Na10-D11	2	0.591	1.936	0.677	0	0.483	0.367
44	BrgMS710	3	0.659	1.948	0.812	0	0.487	0.417
45	SR_7223	3	0.568	2.367	0.968	0	0.577	0.509
46	BrgMS339	2	0.721	1.673	0.592	0	0.402	0.321
47	BRMS-019	2	0.714	1.69	0.598	0	0.408	0.325
48	cnu_m60a	3	0.659	1.888	0.752	0.023	0.47	0.387
49	MB5	2	0.698	1.73	0.613	0	0.422	0.333
50	Na10-C01c	3	0.682	1.809	0.707	0	0.447	0.366
51	Ni3C05	3	0.705	1.844	0.804	0	0.458	0.411
52	Ni3-G07	3	0.705	1.844	0.804	0	0.458	0.411
53	BN6A3	3	0.636	2.118	0.905	0	0.528	0.471
54	BRMS-033	4	0.405	3.161	1.22	0	0.684	0.622
55	CB-10065	3	0.568	2.367	0.968	0	0.577	0.509
56	ENA17	3	0.659	2.029	0.877	0	0.507	0.455
57	cnu_m62a	2	0.655	1.825	0.644	0.024	0.452	0.35
58	NA10-C01a	3	0.545	2.344	0.945	0	0.573	0.494
Mean		**2.74**	0.65	**1.97**	**0.76**	**0.00**	**0.47**	**0.40**

Jaccard's similarity coefficient ranged from 0.133 to 0.952; genotypes Pusa Mahak and JM16-4 revealed the least degree of resemblance, whereas JMM-TM2-15 and KMH-405 exhibited the maximum degree of similarity. A dendrogram based on Jaccard's coefficient of similarity using UPGMA (Figure 2) revealed 44 genotypes were split into two major clusters, cluster A and cluster B. Cluster A comprised of two sub-clusters; sub-cluster I had JMM-TM2-20, JMM-TM2-2, JM16-3, NPJ-194, Pusa Mustard No 1, PM-27, PM-26, JMM-TM2-17, JMM-13-57, JMM-TM2-34, JMM-TM2-23, JMM-TM2-38, and JM16-4, while sub-cluster II had JM13-4, JM13-3, JM13-2, JM16-1, JM13-6, JMM-TM2-14, JMM-TM2-10, JM15-1, JM16-2, JMM-TM2-1, JMM-PM27-11, JMM-PM27-10, JMM-TM2-47, JMM-TM2-38, KMH-405, JMM-TM2-15, Kranti, JMM-TM2-73, JMM-TM2-4, JMM-TM2-28, JMM-TM2-160, PM25, NRCHB-101, NPJ-193,N0-2, and KMR(E)16-1. Cluster B had TM2, RRE-2012/14, TM-204, Pusa Bold, Pusa Mahak. The parental genotypes of LxT design emerged from different clusters. JM16-3 and NPJ-194 were in sub-cluster I of cluster A, whereas JM13-4, Kranti, JMM-TM2-14, JMM-TM2-28, NRCHB-101 and PM-25 were in sub-cluster II. Genotypes RRE2012/14, Pusa Bold, and TM-2 were grouped in cluster B.

Genetic Architecture, Combining Ability, and Heterosis via Line × Tester Analysis

Analysis of variance

The analysis of variance (Table 8) for the parents and F1s revealed high significant variances for all the traits except days to 50% flowering, indicating substantial amount of variation present among the genotypes. Mean squares due to parents were significant for all the traits except days to leaf senescence, primary branches, secondary branches, length of

main raceme and seed yield per plant. Simultaneously, mean squares due to parent vs. hybrids were significant for the traits under study except for days to leaf senescence, plant height, length of main raceme, foot length, seeds per siliqua, thousand seed weight, R/S ratio and nitrogen use efficiency.

The mean squares from the analysis of variance for the line × tester experiment, together with the percentage contribution of lines, testers and their interactions to the sum of squares of crosses, are given in Table 8.

Table 8: Analysis of variance for Line x Tester cross

Source	df	Days to 50% flowering	Days to 75% maturity	Days to leaf senescence	PH	PB	SB	LMR	FL	SQP	SSQ
Replications	1	2.41	2.06	1.73	0.69	0.42	19.74**	0.74	0.22	7.83	3.4
Genotypes	34	1.3	5.10**	16.54**	279.26**	0.86**	6.54**	34.05**	7.84**	451.01**	5.54**
Parents	10	1.78	5.35**	10.54	367.35**	0.17	2.67	18.08	7.36**	912.57**	4.78*
P vs C	1	1.55	19.22**	21.34	9.67	4.58**	68.64**	21.58	1.78	553.63**	0.7
Crosses	23	1.09	4.38**	18.94**	252.67**	1.00**	5.53**	41.54**	8.32**	245.87**	6.08**
Lines	7	0.76	3.52**	14.14	410.73**	2.21**	5.08**	28.27	9.38**	237.38**	12.36**
Testers	2	1.27	8.40**	85.15**	519.16**	0.65	1.99	85.58**	4.71*	224.40**	0.69
Lines x Testers	14	1.22	4.23**	11.88	135.58**	0.45	6.26**	41.88**	8.31**	253.17**	3.72*
Error	34	1.03	0.91	7.14	11.88	0.25	1.3	12.52	1.41	6.54	1.53
CV%		2	0.92	3.04	2.38	12.27	9.72	7.4	14.06	1.65	10.44
% Contribution to sums of squares of crosses											
Lines		21.3	24.5	22.7	49.5	67	27.9	20.7	34.3	29.4	61.8
Testers		10.2	16.7	39.1	17.9	5.7	3.1	17.9	4.9	7.9	1
Lines x Testers		68.6	58.8	38.2	32.7	27.3	68.9	61.4	60.8	62.7	37.2

** Significant at P=0.05; ** Significant at P=0.01*

Combining ability analysis

Mean squares due to lines (GCA) were significant for the traits except days to leaf senescence, length of main raceme and thousand seed weight. Mean squares due to testers (GCA) were significant for the characters other than primary branches, secondary branches, number of seeds per siliqua, thousand seed weight and nitrogen utilisation efficiency. Also, significant mean squares due to line × tester interactions (SCA) were observed for the traits except days to leaf senescence, primary branches, secondary branches, seeds per siliqua, and thousand seed weight. The relative contribution of lines, testers, and their interactions to the sum of squares (SS) due to crosses varied substantially by trait. Lines contributed maximally to seed yield per plant (68.3%), primary branches (67.0%), and biological yield per plant (66.4%), but minimally to shoot dry weight (12.1%). In contrast, testers' key contributions were restricted to shoot dry weight (53.4%), days to leaf senescence (39.1%), and root-shoot ratio (36.4%). The line × tester components predominantly governed secondary branches per plant (68.9%), thousand-seed weight (73.5%), and root volume (71.1%). The lowest percentage contributions for lines, testers, and interactions were observed in shoot dry weight (12.1%), seeds per siliqua (1.0%), and oil content (17.6%), respectively (Figure 3).

Mean performance of parents and GCA effects

The mean performances and GCA effects of eight lines and three testers (Table 9a) revealed that the earliest maturing line was JM13-4 (102 days) followed by JMM-TM2-14 (103 days), Pusa Bold (103.5 days) and JMM-TM2-28 (103.5 days). Among the lines, JM13-4 had a significantly favourable negative GCA effect of (-1.08), while Kranti had a significant positive GCA effect. NPJ-194 registered the maximum days to leaf senescence (93.0 days), followed by Kranti, Pusa Bold, and TM2 (88.0-88.5 days). Non-significant effects were noted for the lines; however, the tester PM25 displayed a significantly positive effect for GCA (2.58). Pusa Bold recorded the shortest height (122.1 cm) followed by NPJ-194 (135.21 cm). The lines Pusa Bold and NPJ-194 showed a significantly favourable negative GCA effect, whereas JM13-4 and JMM-TM2-14 displayed significant positive GCA. Testers such as TM2 and PM25 revealed significantly negative GCA effect. RRE2012/14 had maximum numbers of primary branches (4.2), while NPJ-194 obtained maximum number of secondary branches (12.2). Kranti was the only line showing positive GCA for both traits, whereas, lines showing significant negative GCA were JM16-3 and Pusa Bold for primary branches. No tester showed any GCA for branching traits. Maximum length of main raceme was recorded for JM16-3 (51.3 cm) with significant positive GCA among lines, while PM25 had positive GCA among testers. NPJ-194 revealed maximum mean value for foot length (12.5 cm), significant negative GCA effects were recorded in the Kranti and RRE2012/14 cultivars, whereas Pusa Bold and NPJ-194 were found to have significant positive GCA effects. Number of siliqua per plant was maximum for Pusa Bold (177.1), followed by NRCHB-101 cultivar (174.1). RRE2012/14, JM16-3, and PM25 were found to have significant positive GCA effects for this particular trait. JMM-TM2-28 cultivar had the maximum seeds per siliqua, and Kranti and JMM-TM2-28. Maximum oil content was observed in RRE2012/14 (36.9%) having positive GCA effects followed by Kranti; among testers, only PM25 had positive GCA effects. Maximum root length was recorded for Kranti (28.8 cm) followed by JMM-TM2-28 (27.1 cm), with significant positive GCA effects; PM25 was the best general combiner among testers. JMM-TM2-28 (28.2 cc) and NPJ-194 (26.2 cc) recorded the maximum root volume and significant positive GCA effects, and NRCHB-101 recorded positive GCA among testers. The maximum root dry weight was observed in TM2 and it was the best general combiner among testers, and Kranti and JMM-TM2-14 showed positive GCA effects. JM13-4 recorded the maximum shoot dry weight, but the maximum root-shoot ratio was observed in JMM-TM2-28. JMM-TM2-14 recorded the maximum biological yield, and Kranti, RRE2012/14 and JMM-TM2-28 were good general combiners among lines, while TM2 and PM25 among testers. JM13-4 recorded the maximum harvest index; maximum seed yield per hectare was registered in RRE2012/14, which was statistically equivalent to JMM-TM2-14, JMM-TM2-28, Pusa Bold, PM25 and NRCHB-101. Though there was no significant variation between the parent plants regarding seed yield per plant, Kranti and RRE2012/14 showed favourable GCA effects, suggesting that these

two could be used as better yield donors. NUE was maximum for RRE2012/14, positive GCA effects were recorded for Kranti, RRE2012/14 and JM16-3 among the lines, while NRCHB-101 appeared to be the best general combiner among the testers. The line RRE 2012/14 scored desirable traits for twelve characters, JMM-TM2-28 for eleven traits, and Kranti recorded desirable traits for seven traits. Among the testers, PM25 had desirable traits for eight traits followed by TM2 for seven traits. Among the lines, Kranti recorded significant positive GCA effects for most of the traits followed by RRE 2012/14 with favourable GCA effects for traits including NUE. Kranti recorded positive significant GCA effects for twelve traits followed by RRE 2012/14 for eight traits. Among the testers, PM 25 recorded favourable GCA effects for the seven traits. The lines Kranti and RRE2012/14 and the testers PM25 and NRCHB101 demonstrated both high mean performance and strong General Combining Ability (GCA). They are identified as excellent general combiners for improving multiple agronomic traits simultaneously.

Table 9a presents the mean values and general combining ability (GCA) effects of the lines and testers for the traits studied.

Table 9: Mean values and GCA/SCA effects of lines and testers for the traits under study
a. Mean values and GCA effects of lines and testers for the traits under study

Genotype	DM		DLSC		PH		PB		SB		LMR	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Lines												
JM13-4	102	-1.08**	86	-0.92	147.77	11.07**	3.3	0.01	10.9	0.91	47.68	-1.88
Kranti	105.5	0.92*	88.5	-1.58	135.64	-2.51	4	1.37**	8.9	1.18*	50.29	-2.05
JMM-TM2-14	103	-0.75	85.5	-1.25	141.65	5.10**	3.7	-0.3	9.2	0.21	45.04	-1.69
RRE 2012/14	106	0.75	85	-1.42	144.15	2.53	4.2	-0.03	11.1	-0.85	47.62	2.32
JM16-3	106	0.25	86.5	2.08	147.11	0.92	3.5	-0.46*	8.9	-1.15*	51.3	3.47*
Pusa Bold	103.5	-0.42	88	1.25	122.05	-5.47**	3.5	-0.51*	11.1	-1.09*	43.15	0.78
JMM-TM2-28	103.5	0.75	87	-0.08	143.16	4.66**	3.9	-0.29	9.3	0.61	50.28	0.92
NPJ -194	107	-0.42	93	1.92	135.21	-16.30**	3.8	0.21	12.2	0.18	41.71	-1.87
CD5% / SE(gi)	1.9	0.39	5.4	NS	7.01	1.41	2.31	0.2	2.31	0.46	7.19	1.44
Testers												
TM2	104.5	-0.48	88.5	-0.73	147.1	-4.70**	3.4	-0.03	10.1	-0.4	45.35	-2.2
NRCHB-101	106	-0.35	86.5	-1.85**	171.54	-1.63	3.5	0.22	11.5	0.27	47.29	-0.22
PM-25	103	0.83**	85	2.58**	164.82	-6.33**	4	-0.19	9.6	0.12	47.18	2.41**
CD5% / SE(gi)	1.9	0.24	5.4	0.67	7.01	0.9	2.31	0.13	2.314	0.28	7.192	0.88

Table 9 (continued): part a, panel 2 of 3

Genotype	Siliquae/plant		Seeds/siliqua		1000 seed wt. (g)		Oil content (%)		Foot length (cm)		Main root length (cm)		Root volume (cc)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Lines														
JM13-4	101.7	-1.11	10.9	-1.11*	3.86	-0.18	34.4	-1.07*	9.1	-0.46	23.21	-1.08**	13.95	-2.81**
Kranti	146.4	-3.64**	11.8	2.9**	4.15	0.1	36	2.12**	8.8	-1.26*	28.82	5.43**	17.43	0.46
JMM-TM2-14	136.6	0.19	9.8	-1.04*	3.73	0.09	35	-0.95	10.1	-0.68	22.99	-1.38**	13.88	-0.19
RRE 2012/14	146.7	13.14**	11	-0.79	3.96	0.01	36.9	2.91**	9.6	-1.32*	24.74	1.97**	24.97	0.89
JM16-3	134.2	3.55**	9.8	0.26	4.18	-0.07	29.9	-0.54	6.6	0.49	17.89	-3.82**	8.29	-1.33**
Pusa Bold	177.1	-0.98**	11	-0.23	3.67	0.01	36	-0.25	5.5	1.25*	20.63	-3.71**	18.55	-0.17
JMM-TM2-28	156.8	-8.21**	15.2	1.22*	3.86	-0.01	35.3	-1.15	6.5	-0.26	27.1	2.11**	28.15	1.75**
NPJ -194	164.1	-2.95**	12.2	-1.21*	4.22	0.05	34.1	-1.07	12.4	2.25**	24.78	0.47	26.21	1.41**
CD5% / SE(gi)	5.2	1.04	2.5	0.5	0.34	NS	2.4	0.49	2.4	0.48	1.86	0.37	2.38	0.48
Testers														
TM2	155.8	-4.24**	11.9	-0.09	4.45	-0.02	33	-0.3	9.5	-0.58	19.89	0.18	13.04	-0.64*
NRCHB-101	174.1	2.87**	11.7	0.24	3.48	0.02	32.8	-1.05**	8.8	0.49	22.84	-0.84**	23.7	0.88**
PM-25	163.7	1.37*	13.3	-0.15	4	0	36	1.35**	8.4	0.09	22.86	0.66**	19.16	-0.24
CD5% / SE(gi)	5.2	0.64	2.5	NS	0.34	NS	2.4	0.3	2.4	0.33	1.86	0.23	2.38	0.29

Table 9 (continued): part a, panel 3 of 3

Genotype	Root dry wt. (g)		Shoot dry wt. (g)		Root-shoot ratio		BYP (g)		HI (%)		SYP (g)		SYH (Kg)		NUE (Kg/Kg)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Lines																
JM13-4	2.25	0.03	10.33	-0.43	0.22	0.02	21.12	-1.58**	32.46	-0.19	6.86	-0.46	563.5	-1.68	21.13	-0.06
Kranti	3.07	0.81**	7.89	0.56	0.39	0.064*	33.82	6.45**	24.05	-2.04*	8.11	1.15**	577.4	13.0**	21.65	0.49**
JMM-TM2-14	2.81	0.25*	7.33	0.73	0.38	-0.02	37.62	-3.72**	19.4	-1.07	7.3	-1.54**	588.4	-31.1**	22.07	-1.16**
RRE 2012/14	3.44	-0.07	6.09	-0.85	0.58	0.028	35.64	6.96**	21.38	1.67	7.63	2.88**	598.5	9.84*	22.45	0.37*
JM16-3	2.3	-0.47**	9.89	0.57	0.23	-0.068*	21.91	-4.89**	30.8	3.48**	6.76	-0.46	573.3	8.13*	21.5	0.30*
Pusa Bold	2.93	-0.36**	7.23	-0.89*	0.41	-0.007	27.65	-4.12**	24.75	1.51	6.84	-0.74*	578.7	-5.45	21.7	-0.2
JMM-TM2-28	3.33	-0.12	4.88	-0.33	0.69	0.009	31.44	1.77**	23.25	-3.80**	7.31	-0.74*	589.4	2.5	22.1	0.09
NPJ -194	3.29	-0.06	7.11	0.65	0.47	-0.026	28.29	-0.88*	27.84	0.45	7.85	-0.09	562.1	4.71	21.08	0.18
CD5% / SE(gi)	0.56	0.11	2.11	0.42	0.13	0.025	1.9	0.38	5.02	1.01	1.59	0.32	18.1	3.63	0.68	0.14
Testers																
TM2	3.47	0.19**	8.23	0.71**	0.43	-0.014	27.15	1.63**	26.84	-2.77**	7.29	-0.51*	563.7	-0.7	21.14	-0.03
NRCHB-101	3.3	0.03	6.83	1.20**	0.48	-0.055**	24.88	-2.48**	28.69	2.86**	7.14	0.15	595.8	10.3**	22.34	0.39**
PM-25	3.01	-0.22**	7.67	-1.91**	0.39	0.068**	29.45	0.85**	26.23	-0.1	7.73	0.35	579.8	-9.61**	21.74	-0.36**
CD5% / SE(gi)	0.56	0.07	2.11	0.26	0.13	0.015	1.9	0.23	5.02	0.62	1.59	0.2	18.1	2.22	0.68	0.08

The mean performance and SCA effects (Table 9b) revealed a high degree of variation among all the F_1 hybrids for all the traits examined. JM16-3 × NRCHB101 was the earliest maturing hybrid with a significantly negative SCA effect (-1.81), while a significantly positive SCA effect was noted for JM13-4 × NRCHB101, JM16-3 × TM2, JMM-TM2-28 × NRCHB101 and NPJ194 × PM25 for days to maturity. NPJ194 × PM25 had the highest days to leaf senescence (96.0 days), while the only significantly negative SCA effect was noted for NPJ194 × TM2 (-5.10). Reduced plant height was observed in NPJ194 × NRCHB101 (124.2 cm), while significantly negative SCA effects for plant height were obtained in JM13-4 × TM2, RRE2012/14 × TM2, Pusa Bold × TM2, and JMM-TM2-14 × PM25. Maximum primary branches was for Kranti × NRCHB101 (6.7), but SCA values for this character were not significant. In secondary branches, the maximum was recorded for JM13-4 × PM25 (15.5). SCA was significantly positive in the combinations JM13-4 × PM25, RRE2012/14 × TM2, NPJ194 × TM2 and Pusa Bold × NRCHB101. Main raceme was maximum for JM16-3 × PM25 (56.3 cm); significant SCA for this character was recorded in JM13-4 × TM2. Foot length was maximum for JM13-4 × TM2 (4.9 cm), which was also significantly negative followed by NPJ194 × NRCHB101. The maximum oil content was observed in Kranti × PM25 (39.0%). but no hybrid showed significant SCA for oil content. Regarding the root traits, Kranti × TM2 had the maximum root dry weight (5.12 g) with positive SCA; for shoot dry weight, the

maximum value with positive SCA was recorded for NPJ194 × TM2(12.2 g), and JM13-4 × PM25 had the favourable SCA. For root-shoot ratio, the maximum was obtained in Kranti × PM25 (0.61); JM13-4 × TM2 had significant positive SCA effects for root-shoot ratio. Kranti × TM2 and JMM-TM2-28 × TM2 had the maximum root length (31.5 cm) with favourable SCA effects. The maximum root volume was for RRE2012/14 × NRCHB101 (31.4 cc) and positive SCA effects were recorded for Kranti × TM2, RRE2012/14 × NRCHB101, JM16.

Table 9b presents the mean values and specific combining ability (SCA) effects of the twenty-four crosses for the traits studied.

Table 9 (continued): part b
b. Mean values and SCA effects of crosses for the traits under study

Cross	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
	DM		DLSC		PH		PB		SB		LMR		FL (cm)	
JM13-4 x TM 2	100.5	-1.35	86	-0.77	144.3	-6.73**	4.9	0.66	12.3	-0.6	50.3	6.15*	4.9	-2.35*
Kranti x TM2	105	1.15	85.5	-0.6	137.4	-0.03	5	-0.6	14.5	1.34	46.1	2.21	6.3	-0.19
JMM-TM2-14 x TM2	102.5	0.31	87.5	1.06	149	3.97	4	0.07	11.1	-1.1	41.3	-3	8.3	1.22
RRE2012/14 x TM2	105	1.31	86	-0.27	136.2	-6.31*	4	-0.2	13.7	2.57**	48.3	-0.04	7.3	0.84
JM16-3 x TM2	105	1.81*	93.5	3.73	146.5	5.62*	3.7	-0.07	9.6	-1.24	48.4	-1.08	9.3	1.06
Pusa Bold x TM2	101.5	-1.02	91	2.06	125.3	-9.23**	3.6	-0.17	9.9	-1	47.8	1.03	7.5	-1.54
JMM-TM2-28x TM2	102.5	-1.19	87.5	-0.1	154.2	9.53**	3.7	-0.24	11.1	-1.50*	46.3	-0.64	6.9	-0.57
NPJ194x TM2	101.5	-1.02	84.5	-5.10*	126.8	3.17	5	0.56	13.7	1.53*	39.5	-4.63	11.5	1.54
JM13-4x NRCHB101	103.5	1.52*	88	2.35	164.1	9.97**	4	-0.48	12.1	-1.47	37.8	-8.30**	9.6	1.25
Kranti x NRCHB101	103.5	-0.48	85.5	0.52	136.8	-3.76	6.7	0.85	12.5	-1.34	40.3	-5.60*	8.4	0.81
JMM-TM2-14 x NRCHB101	101.5	-0.81	85.5	0.19	155.6	7.46**	3.8	-0.38	14.3	1.43	50	3.72	6.3	-1.81
RRE2012/14 x NRCHB101	103	-0.81	85	-0.15	141.6	-3.94	4.7	0.25	11.3	-0.51	52.3	1.99	7.7	0.17
JM16-3 x NRCHB101	101.5	-1.81*	85.5	-3.15	141.5	-2.47	4	-0.02	12.1	0.59	50.3	-1.12	9.7	0.42
Pusa Bold x NRCHB101	103.5	0.85	86.5	-1.31	136.2	-1.34	3.7	-0.27	13.5	1.93*	53.3	4.52	11.5	1.46
JMM-TM2-28 x NRCHB101	106	2.19**	86	-0.48	144.3	-3.41	4.5	0.32	14.7	1.43	51.5	2.61	10.5	1.95*
NPJ194 x NRCHB101	102	-0.65	90.5	2.02	124.2	-2.51	4.4	-0.28	10.8	-2.04*	48.3	2.18	6.8	-4.26**
JM13-4 x PM25	103	-0.17	88.5	-1.58	158.8	-3.24	3.9	-0.18	15.5	2.08**	50.9	2.15	9.1	1.1
Kranti x PM25	104.5	-0.67	89.5	0.08	152.3	3.79	5.2	-0.25	13.7	0.01	51.9	3.39	6.5	-0.61
JMM-TM2-14x PM25	104	0.5	88.5	-1.25	144.7	-11.4**	4.1	0.31	12.4	-0.33	48.2	-0.73	8.3	0.59
RRE2012/14x PM25	104.5	-0.5	90	0.42	163.8	10.25**	4	-0.05	9.6	-2.06**	51	-1.95	6.1	-1.01
JM16-3 x PM25	104.5	0	92.5	-0.58	148.8	-3.15	3.7	0.09	12	0.64	56.3	2.2	7.4	-1.48
Pusa Bold x PM25	104	0.17	91.5	-0.75	156.1	10.57**	4	0.44	10.5	-0.92	45.8	-5.55*	9.8	0.07
JMM-TM2-28x PM25	104	-1	91.5	0.58	149.5	-6.12*	3.7	-0.08	13.2	0.08	49.5	-1.98	6.8	-1.37
NPJ194 x PM25	105.5	1.67*	96	3.08	134	-0.66	4	-0.28	13.2	0.51	51.2	2.46	13.4	2.72**
CD5%/ SE(sij)	1.9	0.67	5.4	1.89	7	2.44	1	NS	2.1	0.74	7.2	2.5	2.4	0.84

Table 9 (continued): part b, panel 2 of 3

Cross	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
	Oil (%)		RDW (g)		SDW (g)		RS Ratio		MRL (cm)		RV (cc)		SQP (No.)	
JM13-4 x TM 2	33.9	-0.24	3.82	-0.11	6.9	-2.68**	0.55	0.13**	21.2	-2.16**	19.3	-4.12**	145.7	-5.66**
Kranti x TM2	38.4	1.06	5.12	0.42*	10.9	0.28	0.47	0.01	21.1	1.61*	30.2	3.58**	156.3	7.46**
JMM-TM2-14 x TM2	34.7	0.46	3.88	-0.27	9.7	-1.09	0.4	0.02	31.5	-1.41*	27	1	167.5	14.84**
RRE2012/14 x TM2	38	-0.12	4.08	0.25	8.9	-0.28	0.46	0.03	25.3	-1.78**	24.9	-2.17*	163.1	-2.52
JM16-3 x TM2	33.2	-1.42	3.47	0.04	11.5	0.88	0.3	-0.03	24.7	-0.03	25.2	0.28	153.8	-2.23
Pusa Bold x TM2	35.4	0.45	3.38	-0.15	10.3	1.2	0.33	-0.06	27.7	-0.31	27.3	1.23	149.7	-1.8
JMM-TM2-28x TM2	33.1	-0.9	3.72	-0.07	9.9	0.2	0.38	-0.03	23.4	4.32**	26.8	-1.19	141.2	-3.06
NPJ194x TM2	34.8	0.71	3.73	-0.11	12.2	1.49*	0.3	-0.07	27.4	-0.22	29	1.39	142.5	-7.03**
JM13-4x NRCHB101	34.7	1.28	3.48	-0.28	9.8	-0.32	0.36	-0.02	19.2	1.76*	23.1	-1.79*	156.6	-1.87
Kranti x NRCHB101	35.5	-1.05	4.1	-0.44*	11.7	0.6	0.35	-0.07	21.4	-1.76*	23.5	-4.67**	153.3	-2.64
JMM-TM2-14 x NRCHB101	33.8	0.34	4.28	0.3	11.2	-0.1	0.38	0.04	24.7	0.69	26.1	-1.43	174.2	14.43**
RRE2012/14 x NRCHB101	38.4	1.05	3.76	0.1	10.1	0.42	0.37	-0.01	23.8	1.42*	31.4	2.79**	173.2	0.51
JM16-3 x NRCHB101	34	0.14	3.06	-0.2	11.1	-0.02	0.28	-0.01	24.9	-0.97	29.5	3.11**	163.3	0.17
Pusa Bold x NRCHB101	32.9	-1.3	3.84	0.47*	8.8	-0.8	0.43	0.08	31.1	1.04	28.3	0.71	153	-5.60**
JMM-TM2-28 x NRCHB101	33.6	0.33	3.63	0.01	10.9	0.74	0.33	-0.03	24.9	-1.44*	30.8	1.32	149.5	-1.87
NPJ194 x NRCHB101	32.6	-0.79	3.72	0.05	10.7	-0.51	0.37	0.03	27.9	-0.74	29.1	-0.03	153.5	-3.13
JM13-4 x PM25	34.7	-1.04	3.91	0.39	10	3.00**	0.39	-0.11*	22.7	0.4	29.7	5.92**	164.5	7.53**
Kranti x PM25	39	-0.01	4.32	0.02	7.1	-0.88	0.61	0.06	21.1	0.15	28.2	1.09	149.6	-4.82*
JMM-TM2-14x PM25	35.1	-0.8	3.71	-0.03	9.3	1.19	0.4	-0.06	24.8	0.72	26.8	0.43	129	-29.3**
RRE2012/14x PM25	38.8	-0.93	3.08	-0.35	6.4	-0.14	0.49	-0.02	27	0.36	26.9	-0.62	173.2	2.01
JM16-3 x PM25	37.6	1.28	3.18	0.16	7.1	-0.85	0.46	0.04	21.2	1	21.9	-3.39**	163.7	2.06
Pusa Bold x PM25	37.4	0.85	2.81	-0.32	6.1	-0.4	0.46	-0.02	21.1	-0.73	24.5	-1.94*	164.5	7.40**
JMM-TM2-28x PM25	36.3	0.57	3.43	0.06	6.1	-0.94	0.56	0.07	31.5	-2.88**	28.2	-0.13	154.8	4.93*
NPJ194 x PM25	35.8	0.07	3.49	0.06	7.1	-0.98	0.49	0.04	25.3	0.96	26.7	-1.35	165.3	10.16**
CD5%/ SE(sij)	2.4	NS	0.56	0.19	2.1	0.73	0.13	0.04	1.9	0.65	2.4	0.83	5.2	1.81

Table 9 (continued): part b, panel 3 of 3

Cross	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
	SSQ (No.)		BYP (g)		HI (%)		SYP (g)		TSW (g)		SYH (Kg/ha)		NUE (Kg/Kg)	
JM13-4 x TM 2	11.7	0.99	28.2	-2.69**	27.9	0.96	7.9	-0.25	4	0.17	601.5	-13.68*	22.55	-0.51*
Kranti x TM2	13.7	-1.03	41.3	2.39**	21.2	-3.85*	8.8	-0.96	4.18	0.08	628.6	-1.19	23.57	-0.04
JMM-TM2-14 x TM2	11.8	1.02	34.7	5.94**	22.3	-3.74*	7.7	0.66	4.26	0.17	608.8	23.01**	22.83	0.86**
RRE2012/14 x TM2	12.3	1.24	37.1	-2.33**	26.8	-1.97	10	-1.51*	3.89	-0.13	616.6	-10.01	23.12	-0.38
JM16-3 x TM2	13	0.92	24.9	-2.70**	37.2	6.67**	9.3	1.15*	4.11	0.17	627.4	2.44	23.53	0.09
Pusa Bold x TM2	10.1	-1.49	29.3	0.95	28.6	-0.01	8.4	0.53	3.72	-0.29*	600	-11.4	22.5	-0.43
JMM-TM2-28x TM2	12.6	-0.44	35	0.73	22.7	-0.57	8	0.1	4.02	0.02	613.8	-5.51	23.02	-0.21
NPJ194x TM2	9.4	-1.21	29.3	-2.29**	30	2.5	8.8	0.28	3.88	-0.18	637.9	16.34*	23.92	0.61*
JM13-4x NRCHB101	8.7	-2.33*	27.2	0.36	34.1	1.58	9.3	0.47	3.56	-0.31*	631.1	4.9	23.66	0.18
Kranti x NRCHB101	16.4	1.39	37.4	2.54**	29.8	-0.87	11.2	0.77	4.14	-0.01	645.4	4.57	24.2	0.17
JMM-TM2-14 x NRCHB101	11.5	0.4	22.9	-1.77*	34.3	2.61	7.9	0.14	4.08	-0.06	611.5	14.72*	22.93	0.55*
RRE2012/14 x NRCHB101	9.9	-1.46	35.6	0.29	32	-2.41	11.4	-0.74	4.1	0.03	632.5	-5.17	23.72	-0.19
JM16-3 x NRCHB101	12.2	-0.2	24	0.48	31.9	-4.29*	7.7	-1.11	3.89	-0.09	630.9	-5.11	23.66	-0.19
Pusa Bold x NRCHB101	13.1	1.21	24.8	0.55	32.9	-1.38	8.2	-0.35	4.22	0.16	624.9	2.54	23.43	0.1
JMM-TM2-28 x NRCHB101	13.8	0.43	27.2	-2.92**	31.5	2.59	8.6	0.07	4.16	0.12	616.4	-13.93*	23.12	-0.52*
NPJ194 x NRCHB101	11.5	0.56	28	0.47	35.3	2.17	9.9	0.74	4.27	0.16	630	-2.51	23.63	-0.09
JM13-4 x PM25	12	1.34	32.5	2.33**	27	-2.54	8.8	-0.22	3.99	0.14	615	8.79	23.06	0.33
Kranti x PM25	14.3	-0.36	33.2	-4.93**	32.4	4.72*	10.8	0.19	4.05	-0.07	617.5	-3.39	23.16	-0.13
JMM-TM2-14x PM25	9.3	-1.42	23.8	-4.17**	29.8	1.13	7.1	-0.8	4.01	-0.11	539.1	-37.73**	20.22	-1.41**
RRE2012/14x PM25	11.2	0.22	40.7	2.04**	35.8	4.38*	14.6	2.24**	4.14	0.1	632.9	15.18*	23.73	0.57*
JM16-3 x PM25	11.3	-0.72	29.1	2.23**	30.9	-2.38	9	-0.03	3.88	-0.08	618.7	2.67	23.2	0.1
Pusa Bold x PM25	11.8	0.27	26.1	-1.51*	32.7	1.39	8.5	-0.19	4.18	0.14	611.3	8.86	22.92	0.33
JMM-TM2-28x PM25	13	0.01	35.7	2.19**	23.9	-2.03	8.5	-0.17	3.88	-0.14	629.9	19.44**	23.62	0.73**
NPJ194 x PM25	11.2	0.65	32.7	1.82**	25.5	-4.67*	8.3	-1.02	4.1	0.02	598.8	-13.82*	22.45	-0.52*
CD5%/ SE(sij)	2.5	0.87	1.9	0.66	5	1.75	1.6	0.55	0.34	0.12	18.1	6.29	0.68	0.24

The maximum number of siliquae per plant was obtained for JMM-TM2-14 × NRCHB101 (174.2) and the significant positive SCA effect was noticed in JMM-TM2-14 × TM2, JMM-TM2-14 × NRCHB101, JM13-4 × PM25, Kranti × TM2 and NPJ194 × PM25. Kranti × NRCHB101 produced the maximum number of seeds per siliqua (16.4), but there was no significant positive SCA effect. Biological yield was maximum in Kranti × TM2 (41.3 g) and the hybrid

recorded significant positive SCA, along with JMM-TM2-14 × TM2, Kranti × NRCHB101, JM13-4 × PM25, RRE2012/14 × PM25, JM16-3 × PM25, JMM-TM2-28 × PM25 and NPJ194 × PM25. Harvest index was maximum for JM16-3 × TM2 (37.2%) with significant positive SCA effect. Seed yield per plant was maximum for RRE2012/14 × PM25, while Kranti × NRCHB101 had the maximum seed yield per hectare (645.4 kg ha^{-1}) and maximum NUE (24.2 kg kg^{-1}). Significant positive SCA effects for both NUE and seed yield per hectare were obtained in JMM-TM2-14 × TM2, NPJ194 × TM2, JMM-TM2-14 × NRCHB101, RRE2012/14x PM25 and JMM-TM2-28 × PM25. Cross-evaluation revealed that the hybrids Kranti × PM25, RRE2012/14 × PM25, and RRE2012/14 × NRCHB101 exhibited superior mean performance for the majority of traits governing seed yield and NUE. Furthermore, under recommended nitrogen regimes (N40), Kranti × TM2, RRE2012/14 × PM25, and JM13-4 × PM25 emerged as the most elite combinations specifically for optimizing both yield and NUE.

Genetic variability parameters

The study revealed that the SCA variances were mostly higher than the GCA variances for most of the traits (Table 10) except shoot dry weight, oil content, days to leaf senescence, and NUE. Moderate GCV and PCV were recorded for foot length, shoot dry weight, root-shoot ratio and NUE, while rest of the traits exhibited low variances. High broad sense heritability was recorded for plant height, siliquae/ plant, oil content, root dry weight, shoot dry weight, main shoot length, root volume, biological yield per plant, harvest index, seed yield/ plant, seed yield/ha and NUE. High narrow-sense heritability was for oil content, shoot dry weight, maximum root length, and biological yield per plant. Moderate narrow sense heritability was observed for days to leaf senescence, plant height, root dry weight, root-shoot ratio, harvest index, seed yield per plant, and seed yield per hectare. Average degree of dominance of genes was less than unity, for the traits days to leaf senescence, primary branches per plant, oil content, root dry weight, shoot dry weight, root-shoot ratio, root length, biological yield per plant, and seed yield per plant; conversely, it was greater than unity for days to maturity, main shoot length, seeds per siliqua, harvest index, and NUE. Additive genetic variance was non-significant for secondary branches, foot length, siliquae per plant, thousand seed weight, and root volume. Therefore, for these characters narrow sense heritability and average degree of dominance could not be estimated.

The estimates of the genetic parameters derived from the line × tester analysis are presented in Table 10.

Table 10: Estimates of genetic parametersobtained from line x tester analysis

Character	GCV (%)	PCV (%)	Additive genetic variance	Dominance variance	h^2 (bs) (%)	h^2 (ns) (%)	Average degree of dominance
Days to maturity	0.73	0.98	0.31	1.66	55.7	15.32	2.3
Days to leaf senescence	2.28	3.13	6.87	2.37	53	45.19	0.59
Plant height (cm)	4.65	4.94	59.89	61.85	88.43	58.32	1.02
Primary branches/ plant	8.26	11.98	0.18	0.1	47.53	37.2	0.75
*Secondary branches/ plant	5.39	8.28	-	2.59	42.46	-	-
Main shoot length (cm)	4.69	7.03	2.73	14.68	44.58	12.1	2.32
*Foot length (cm)	10.24	14.27	-	3.45	51.5	-	-
*Siliquae/ plant	3.47	3.66	-	123.32	89.81	-	-
Seeds/ siliqua	6.14	9.6	0.51	1.09	40.89	19.74	1.46
*1000 seed wt. (g)	0.98	3.08	-	0.02	10.11	-	-
Oil content (%)	3.7	4.42	3.15	0.46	69.98	65.2	0.38
Root dry wt. (g)	7.6	9.43	0.11	0.06	64.94	50.57	0.75
Shoot dry wt. (g)	16.82	18.78	3.59	1.54	80.22	66.08	0.65
Root-shoot ratio	13.49	17.09	0.005	0.004	62.31	44.89	0.88
Main root length (cm)	7.46	7.92	4.81	3.59	88.72	64.62	0.86
*Root volume (cc)	5.17	6.19	-	9.39	69.73	-	-
Biological yield/ plant (g)	10.44	10.66	14.72	10.51	95.82	70.61	0.85
Harvest index (%)	9.56	11.34	8.85	12.31	71.12	41.94	1.18
Seed yield/ plant (g)	9.59	11.58	0.94	0.8	68.68	48.14	0.92
Seed yield/ha (kg)	1.92	2.19	142.38	258.25	77.42	40.6	1.35
NUE (g/g)	1.96	2.37	0.02	0.06	68.45	29.86	1.61

**Additive genetic variance is negative*

Estimates of heterosis

Estimates of standard heterosis percentage (Table 11), compared to performance of the standard variety NRCHB-101 (recommended mustard variety for Assam) revealed that, for days to maturity, fifteen crosses showed negative heterosis (-5.50 to 0%); maximum significant negative heterosis was observed for JM13-4 x TM 2 (-5.50%). For days to leaf senescence, three crosses, viz. NPJ194 x PM25, JM16-3 x PM25 and JM16-3 x TM2 exhibited significant positive heterosis. For plant height all the hybrids expressed significant negative heterosis, as the standard variety NRCHB 101 was the tallest genotype. Six crosses showed positive significant heterosis for primary branches per plant; maximum heterosis was recorded for Kranti x NRCHB101. For secondary branches/ plant four crosses viz. JM13-4 x PM25, JMM-TM2-28 x NRCHB101, Kranti x TM2, and JMM-TM2-14 x NRCHB101 revealed positive significant heterosis. Negative heterosis is desirable for length of the main raceme and was significant for NPJ194 x TM2 and JM13-4 x NRCHB101; only JM16-3 x PM25 had significant positive heterosis (19%). For number of siliquae per plant all the crosses revealed negative heterosis; for number of seeds per siliqua, Kranti x NRCHB101 and Kranti x PM25 had positive heterosis. For oil content, eleven crosses revealed positive heterosis ranging from the value 7.9% to 18.8% heterosis; maximum was recorded for Kranti x PM25 (18.8%). Maximum thousand seed weight was obtained for NPJ194 x NRCHB101 (22.6%); maximum root dry weight was recorded for Kranti x TM2 (59.3%); positive significant heterosis for shoot dry weight was observed for fifteen crosses, ranging from 36.5% to 71.1%. Kranti x PM25 (27.1%) revealed positive heterosis for root-shoot ratio. Significant positive heterosis for root volume was recorded for sixteen crosses ranging from 10.1% (JMM-TM2-14 x NRCHB101) to 32.5% (RRE2012/14 x NRCHB101); maximum root length with highest positive heterosis was for Kranti x TM2 (40.6%) followed by fourteen cross combinations. The maximum heterosis for biological yield per plant was exhibited by Kranti x TM2 (66.2%); for harvest index, JM16-3 x TM2(29.8%), followed by RRE2012/14 x PM25 (24.8**) had maximum positive heterosis. For seed yield per plant,

twelve crosses expressed significant positive heterosis, the highest heterosis percentage was observed in the cross RRE2012/14 x PM25 (104%);. for seed yield per hectare, maximum significant positive heterosis was exhibited for Kranti x NRCHB101 (8.3%). Maximum positive heterosis for NUE was recorded for JMM-TM2-28 x NRCHB101 followed Kranti x PM25 and JM13-4 x PM25. Out of the twenty-four crosses, JM13-4 x PM 25, Kranti x TM2, JM16-3 x TM-2, RRE 2012/14 x PM 25, NPJ-194 x TM-2, Kranti x NRCHB101, Kranti x PM25, RRE2012/14 x PM25 and JMM-TM2-28 x PM25 were positively heterotic for yield traits (Table 12). Occurrence of heterosis either positive or negative was commonly observed in these crosses of Indian mustard.

The estimates of standard heterosis (%) of the crosses over the check variety NRCHB-101 are presented in Table 11.

Table S11: Estimates of standard heterosis percentage for phenological and morphological traits

Cross	DM	DLS	PH	PB	SB	LMR	FL	SQP	SSQ	OIL
JM13-4 x TM 2	-5.2**	-0.6	-15.9**	40.0**	7	6.3	-44.2**	-16.3**	0	3.3
Kranti x TM2	-0.9	-1.2	-19.9**	42.9**	26.2*	-2.4	-28.8*	-10.2**	17	17.0**
JMM-TM2-14 x TM2	-3.3**	1.2	-13.1**	14.3	-3.5	-12.7	-6.2	-3.8*	0.9	5.8
RRE2012/14 x TM2	-0.9	-0.6	-20.6**	14.3	19.2	2.1	-17.7	-6.3**	4.9	15.8**
JM16-3 x TM2	-0.9	8.1*	-14.6**	5.7	-16.5	2.3	5.1	-11.7**	11.1	1.3
Pusa Bold x TM2	-4.3**	5.2	-27.0**	1.4	-13.9	1.1	-15.6	-14.0**	-13.7	7.9*
JMM-TM2-28 xTM2	-3.3**	1.2	-10.1**	5.7	-3.5	-2.2	-21.8	-18.9**	7.7	1
NPJ194 x TM2	-4.3**	-2.3	-26.1**	42.9**	19.1	-16.5*	30.5*	-18.2**	-19.7	6.2
JM13-4 x NRCHB101	-2.4*	1.7	-4.4*	14.3	5.2	-20.1*	8.8	-10.1**	-25.6*	5.6
Kranti x NRCHB101	-2.4*	-1.2	-20.3**	91.4**	8.7	-14.7	-5.4	-11.9**	40.5**	8.3*
JMM-TM2-14 x NRCHB101	-4.3**	-1.2	-9.3**	8.6	24.3*	5.7	-28.4*	0.1	-1.7	3.2
RRE2012/14 x NRCHB101	-2.8**	-1.7	-17.4**	34.3*	-1.7	10.5	-13.2	-0.5	-15.4	17.1**
JM16-3 x NRCHB101	-4.3**	-1.2	-17.5**	14.3	5.2	6.4	10.1	-6.2**	4.3	3.8
Pusa Bold x NRCHB101	-2.4*	0	-20.6**	5.7	17.4	12.6	30.5*	-12.1**	12.2	0.3
JMM-TM2-28x NRCHB101	0	-0.6	-15.9**	28.6	27.8**	8.9	18.8	-14.1**	17.9	2.5
NPJ194 x NRCHB101	-3.8**	4.6	-27.6**	25.7	-6.1	2.1	-22.9	-11.8**	-1.7	-0.6
JM13-4 x PM25	-2.8**	2.3	-7.4**	11.4	34.8**	7.6	2.5	-5.5**	2.6	5.9
Kranti x PM25	-1.4	3.5	-11.2**	48.6**	19.1	9.8	-26	-14.1**	22.2*	18.8**
JMM-TM2-14 x PM25	-1.9*	2.3	-15.7**	16.8	7.7	1.9	-5.8	-25.9**	-20.5	7
RRE2012/14 x PM25	-1.4	4.1	-4.5*	14.3	-16.5	7.8	-31.1*	-0.5	-4.3	18.4**
JM16-3 x PM25	-1.4	6.9*	-13.3**	5.7	4.3	19.0*	-16	-6.0**	-3.4	14.6**
Pusa Bold x PM25	-1.9*	5.8	-9.0**	14.3	-8.7	-3.1	10.3	-5.5**	0.9	14.1**
JMM-TM2-28 x PM25	-1.9*	5.8	-12.8**	5.7	14.8	4.8	-23.2	-11.1**	11.1	10.5**
NPJ194 x PM25	-0.5	11.0**	-21.9**	14.3	14.8	8.2	51.5**	-5.1**	-4.3	9.3*

Table S11 (continued): panel 2 of 2

Cross	TSW	RDW	SDW	RSR	RV	MRL	BYP	HI	SYP	SYH	NUE
JM13-4 x TM 2	14.8**	15.8	1.5	15.4	-18.7**	-4.4	13.4**	-2.9	10.3	0.9	-49.5**
Kranti x TM2	20.1**	55.2**	59.3**	-1.8	27.6**	40.6**	66.2**	-26.1**	22.9*	5.5**	-56.4**
JMM-TM2-14 x TM2	22.3**	17.4*	41.7**	-16.2	14.0**	-2.5	39.5**	-22.3*	7.9	2.2	-35.6*
RRE2012/14 x TM2	11.8*	23.5**	30.5	-3.9	5.2	10.6*	49.2**	-6.6	39.4**	3.5*	-13.5
JM16-3 x TM2	18.0**	5	68.2**	-36.7**	6.1	-7.1	0.1	29.8**	29.8*	5.3**	-51.0**
Pusa Bold x TM2	6.9	2.4	51.5**	-31.5*	15.0**	-7.8	17.9**	-0.3	17.4	0.7	-47.7**
JMM-TM2-28 xTM2	15.4**	12.6	45.2**	-21.7	12.9*	37.9**	40.6**	-20.8*	11.3	3	-43.1**
NPJ194 x TM2	11.4*	12.9	78.3**	-36.6**	22.4	10.9*	17.9**	4.7	23.0*	7.1**	-31.6*
JM13-4 x NRCHB101	2.3	5.5	43.2**	-25.6	-2.5	8.3*	9.2*	18.9*	29.7*	5.9**	0.5
Kranti x NRCHB101	18.8**	24.2**	71.1**	-26.9*	-0.8	21.3**	50.2**	3.9	56.4**	8.3**	-10.2
JMM-TM2-14 x NRCHB101	17.1**	29.5**	63.3**	-20.4	10.1*	2.2	-8.0*	19.4*	9.9	2.6	34.5*
RRE2012/14 x NRCHB101	17.7**	13.9	47.7**	-22.4	32.5**	20.1**	43.2**	11.5	59.5**	6.2**	56.6**
JM16-3 x NRCHB101	11.8**	-7.3	62.1**	-42.4**	24.5**	-15.7**	-3.7	11.3	7.4	5.9**	28.6*
Pusa Bold x NRCHB101	21.1**	16.4	29.3	-9.4	19.2**	-6.4	-0.2	14.5	14.2	4.9**	-9.2
JMM-TM2-28x NRCHB101	19.5**	9.8	60.1**	-30.4*	29.9**	8.2*	9.5*	9.8	20.1	3.5*	69.4**
NPJ194 x NRCHB101	22.6**	12.6	56.0**	-23.5	22.8**	4.1	12.4**	23.2*	38.6**	5.7**	-19.8
JM13-4 x PM25	14.5**	18.5*	46.2**	-18.4	25.4**	8.9*	30.5**	-5.8	22.8*	3.2*	67.6**
Kranti x PM25	16.4**	30.9**	3.8	27.1*	18.8**	36.3**	33.6**	13.1	51.1**	3.6*	69.1**
JMM-TM2-14 x PM25	15.1**	12.3	36.5*	-17.2	13.3*	9.0*	-4.2	4	-0.5	-9.5**	29.5*
RRE2012/14 x PM25	18.8**	-6.8	-5.9	2.6	13.4*	22.0**	63.6**	24.8**	104.0**	6.2**	47.0**
JM16-3 x PM25	11.5*	-3.6	4.3	-4.7	-7.7	-0.5	16.8**	7.6	25.4*	3.8*	17.3
Pusa Bold x PM25	20.0**	-15	-10.5	-4.9	3.3	-7.6	4.9	13.9	19.3	2.6	-48.2**
JMM-TM2-28 x PM25	11.5*	3.9	-10.1	16.4	19.1**	8.5*	43.4**	-16.6	19.6	5.7**	42.9**
NPJ194 x PM25	17.7**	5.8	3.7	2.8	12.5*	18.1**	31.3**	-11	16.8	0.5	5.3

The superior cross combinations, with their mean performance, SCA effects and heterosis for yield-attributing traits, are summarised in Table 12.

Table 12: Superior cross combinations with mean, SCA and heterosis for yield attributing traits

Superior cross	Yield attributing trait	Mean	SCA effect	Heterosis (%)
JM13-4 x PM 25	Secondary branches (No.)	15.5	2.08**	34.8**
Kranti x TM2	Biological yield (g/plant)	41.34	2.39**	66.2**
JM16-3 x TM-2	Harvest index (%)	37.25	6.67**	29.8**
RRE 2012/14 x PM 25	Biological yield (g/plant)	40.72	2.04**	63.6**
NPJ-194 x TM-2	Seed yield (kg/ ha)	637.87	16.34*	42.06**
Kranti x NRCHB101	Biological yield (g/ plant)	38.48	2.54**	12.5**
Kranti x PM25	N use efficiency (kg/kg)	31.49	7.75**	69.09**
RRE2012/14 x PM25	Seed yield (kg/ha)	632.93	15.18*	37.12**
JMM-TM2-28 x PM25	Seed yield (kg/ha)	629.85	19.44**	34.04**

** Significant at P=0.05, ** Significant at P=0.01*

Discussion

ANOVA revealed significant genotypic variations in both environments; ANOVA over the environments revealed the presence of highly significant genotypic, environmental and GXE effects for majority traits demonstrating highly influenced the phenotypic expressions of the plants for growth, NUE and yield traits. Significant genotype-by-nitrogen interactions confirm that distinct plant varieties are specialized for either nitrogen-deficient or optimal nitrogen environments (Fageria & Baligar, 2005; Hirel et al., 2007; Xu et al., 2012). High plant N, delayed leaf senescence and increased photosynthetic activity were in agreement with Zaneet al. (2002); increased leaf N content delays N efflux from the leaf. Moll et al. (1982) explained primary components of NUE as NUpE and NUtE; due to the presence of early developed extensive root system, lower NUE in oilseed rape prevails as a result of lower NUtE (rather than NUpE)(Kamh et al., 2005). Earlier studies revealed genotypic variation for NUE, NUpE and NUtE in rapeseed (Fageria et al., 2008; White and Brown, 2010). NUpE is associated with root growth and N uptake rates. Excessive nitrogen

uptake (high NupE) without efficient utilization (low NUtE) can lead to luxury consumption and environmental issues. Conversely, efficient utilization efficiency (high NUtE) without sufficient uptake (low NUpE) limits potential yield. Thus; improving NUE often requires balancing both NUpE and NUtE. However, the study emphasised on nitrogen use efficient genotypes and it was observed that some efficient genotypes performed well at N0 conditions. Application of nitrogen increased growth, biomass production, yield attributes, and seed yield through vegetative growth and photosynthesis (Gan et al., 2008; and Keerthi et al., 2017).

High difference between GCV and PCV was observed for root dry weight, indicating the trait was highly influenced by environment (Bind et al., 2014). High GCV and PCV indicated a high amount of variability present among genotypes for improvement of the traits as also reported by Akbari and Niranjana (2021) for yield per plant and biomass yield per plant. Low GCV and PCV for plant height, and number of siliquae per plant were also reported by Patel et al., (2012) and Yadav et al., (2019). High heritability suggests a high component of the heritable portion of variation, which can be exploited for crop improvement. High heritability was observed for most of the traits as also reported by Shekhawat et al., (2014) and Rout et al., (2019). A high heritability does not always mean high genetic gain, and therefore, heritability coupled with genetic advances should predict the ultimate effect of selecting superior genotypes (Sarma et al., 2003). High heritability coupled with high GAM for various traits under study implies the predominance of additive gene action, as also reported by Pal et al., (2019), offering crop improvement opportunities through direct selection. High to moderate heritability accompanied by high to moderate genetic advance indicated that heritability was due to additive gene action and simple selection may be effective for that character (Meena and Kumar, 2023). High heritability coupled with low GAM was also reported by Kumar et al. (2023). The high significant positive correlation between seed yield per plant and NUE revealed that seed yield was governed by the crop's ability to convert available nitrogen into economic yield as explained by Moll et al., (1982) and Xu et al., (2012) how the variations in maternal sink capacity (seed yield) are strictly linked to metabolic nitrogen utilization efficiency. Thus, selection of enhanced NUE may result in increased seed yield, facilitating the development of low-input, high-yielding cultivars (Han et al., 2015).

Mahalanobis D^2 statistics grouped the genotypes into three distinct clusters; the grouping of genotypes indicated that geographical distribution need not necessarily be the indicator of genetic divergence, as also reported by Verma (2006); and Fu and Guel (2010), as the pedigree of breeding lines may influence the clustering pattern Chandra et al. (2018). The inter-cluster distances were larger than the intra-cluster distance, indicating that wider diversity existed among the genotypes belonging to distantly spaced groups. Hybridization between widely diverse parents selected from diverse clusters may produce broad variability with high heterotic effect (Akabari and Niranjana, 2015). The genotypes grouped into the same cluster displayed low degree of divergence, and when crosses are made among the genotypes of the same cluster, no transgressive segregant is expected from such combinations (Akbari and Niranjana, 2015; Singh et al., 1979). Therefore, hybridization programs should always be formulated in such a way that the parents belonging to different clusters with maximum divergence could be utilized to get desirable transgressive segregant (Kumar and Yadav, 2007).

Fifty-eight polymorphic SSR markers revealed 158 alleles; the allelic richness per locus recorded was 2.72, which was higher than that reported by Thakur et al. (2017), with an average of 2.17. The increased number of alleles may be due to the inclusion of genetically diverse germplasm lines. The average major allele frequency was 0.65; if the MAF is high (close to 1.0), it implies low diversity, while lower MAF values suggest higher genetic variation. Sharma et al. (2021) also reported an average MAF value of 0.62 in Indian mustard. The average number of effective alleles was 1.97, which was lower than the mean number of alleles (2.72), indicating unequal allele frequencies, where a few common alleles contribute most of the diversity; higher Ne indicates greater genetic diversity and a healthier population. Shannon's information index ranged from 0.34 to 1.29 with an average of 0.76, and high values of Shannon's information index suggest substantial genetic diversity in the mustard germplasm under study (Sharma et al., 2008). Average expected heterozygosity was 0.47, whereas observed heterozygosity was almost zero (0.001), indicating that the genotypes are largely homozygous, as expected for inbred or pure-line material in a self-pollinating crop such as mustard; thus the diversity in this germplasm lies mainly between genotypes rather than within them. The polymorphic information content (PIC) values ranged from 0.17 to 0.62, with an average of 0.40, indicating that the marker set was moderately informative overall. Eight markers had PIC values above 0.50 and were highly informative, 43 markers were reasonably informative (0.25–0.50), and seven markers were slightly informative (<0.25). The markers BRMS-033 (PIC 0.62), CN-52 (B-16) (0.62), PBCESSRJU1 (0.61) and PBCESSRJU4 (0.59) revealed high allelic diversity and polymorphism, and thus were very useful for genotype characterization and marker-assisted breeding. Similar findings were reported by Abbas et al. (2009), Chandra et al. (2013), Thakur et al. (2015) and Singh et al. (2021). The Jaccard's similarity coefficient based on UPGMA clustering revealed two main groups of genotypes, with Line x Tester parents belonging to various clusters, and this reflects great genetic divergence between them. The conformity of molecular diversity and breeding efficiency implies that these genetically diverse parents serve as an excellent source for development of high-yielding nitrogen efficient cultivars of mustard.

ANOVA for the traits under Line x Tester mating design corroborated Kaur et al. (2020); Gami and Chauhan (2013); Ram et al. (2023) and Gupta et al. (2003). Mean squares due to lines (GCA), testers (GCA) and line × tester interactions (SCA) were significant for most of the traits as also reported by Rameeh (2012;2016). Mean performance and GCA effects of eight lines and three testers were in agreement with Meena et al. (2018); Adhikary et al. (2018); Choudhary et al. (2020) and Kumar et al. (2021). GCA variance gives additive genetic variance and additive x additive interaction, while SCA variance includes non-additive genetic variance including dominance and non-additive epistasis. In the present study, the SCA variances were mostly higher than the GCA variances for most of the traits, suggesting the primary role of non-additive gene action in the expression of those traits, which include dominance and epistasis involving dominance. In such situation, heterosis breeding would help to utilize the maximum proportion of non-

additive genetic variation. Recurrent selection can be used for crop improvement to utilize additive, additive x additive epitasis as well as non-additive genetic component consisting of dominance, additive x dominance and dominance x dominance (Singh et al., 2022). The predominant role of non-additive gene action in the inheritance of seed yield per plant was observed by Ghosh et al. (2019). The average degree of dominance was greater than one for most of the traits, indicating that the genes for these traits showed over dominance as also reported by Patel et al. (2012) and Bind et al., (2014). Conversely, average degree of dominance of genes for some traits was less than unity, implying preponderance of additive gene action. High estimates of H2bs and H2ns were recorded; high H2ns indicates that the traits were preponderantly governed by additive gene action and simple selection for such characters would be rewarding. For seed yield, the average degree of dominance was 0.92, indicating the presence of incomplete to nearly complete dominance. Similarly, the average degree of dominance approached unity for plant height (1.02), suggesting complete dominance governing the expression of these traits, demonstrating that the magnitude of dominance genetic variance is roughly equal to the additive genetic variance. Evaluation of 24 mustard hybrids against the recommended variety NRCHB-101 revealed significant heterotic potential for yield traits, with RRE2012/14 x PM25 showing the maximum seed yield heterosis (104%) and Kranti x NRCHB-101 yielding 8.3% higher per hectare. Key hybrids, particularly Kranti x TM2 and Kranti x NRCHB101, exhibited strong positive heterosis for root biomass, nutrient use efficiency, and yield components, making them suitable candidates for breeding programs targeting superior adaptability.

Conclusion

The present study demonstrated substantial genetic variability for nitrogen use efficiency (NUE), and yield-related traits in Indian mustard, providing a strong foundation for breeding nitrogen-efficient cultivars. The integration of morpho-physiological, biochemical, molecular, and genetic analyses enabled the identification of superior nitrogen-efficient genotypes and their effective utilization in a Line × Tester breeding programme. Among the parental lines, Kranti, RRE2012/14, PM25, and NRCHB-101 emerged as excellent combiners, while hybrids such as Kranti × TM2, Kranti × PM25, Kranti × NRCHB-101, RRE2012/14 × PM25, RRE2012/14 × NRCHB-101, and JM13-4 × PM25 exhibited superior performance for NUE, root traits, and seed yield, making them promising candidates for future breeding programmes. The observed phenotypic and molecular diversity provides opportunities to broaden the genetic base of Indian mustard, while the informative SSR markers identified in this study offer valuable resources for parental selection and marker-assisted breeding. The identified nitrogen-efficient parents and hybrids can be exploited to develop high-yielding cultivars with improved nitrogen-use efficiency, thereby reducing fertilizer inputs without compromising productivity. Future efforts should focus on validating the superior parents and hybrids under multi-location trials and elucidating the genomic architecture underlying nitrogen use efficiency through high-density molecular mapping and genomic-assisted selection. Such approaches will accelerate the development of stable, climate-resilient Indian mustard cultivars with enhanced nitrogen-use efficiency and productivity under reduced nitrogen inputs.

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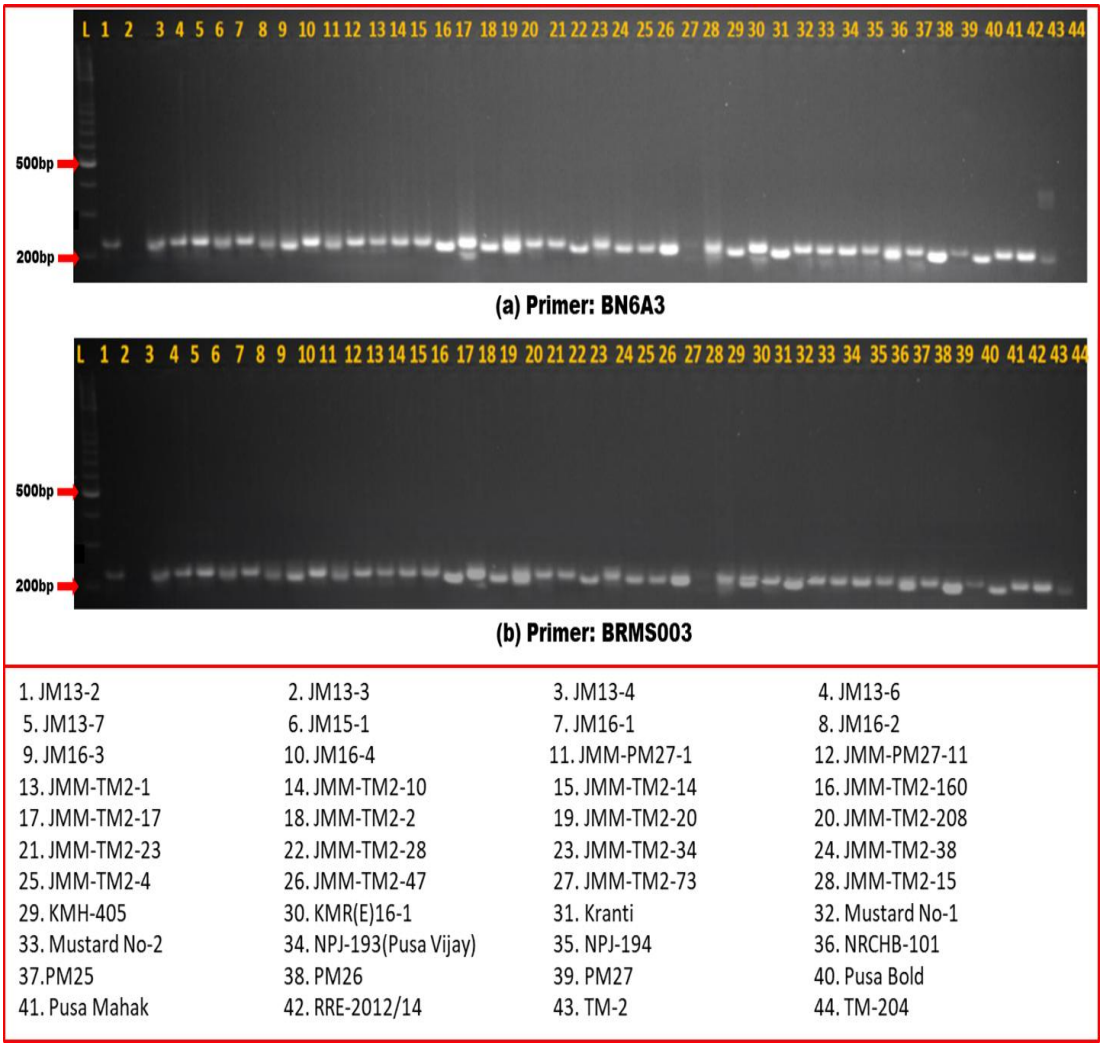

Figure 1: PCR amplification of the 44 genotypes: (a) BN6A3 and (b) BRMS003 showing polymorphism

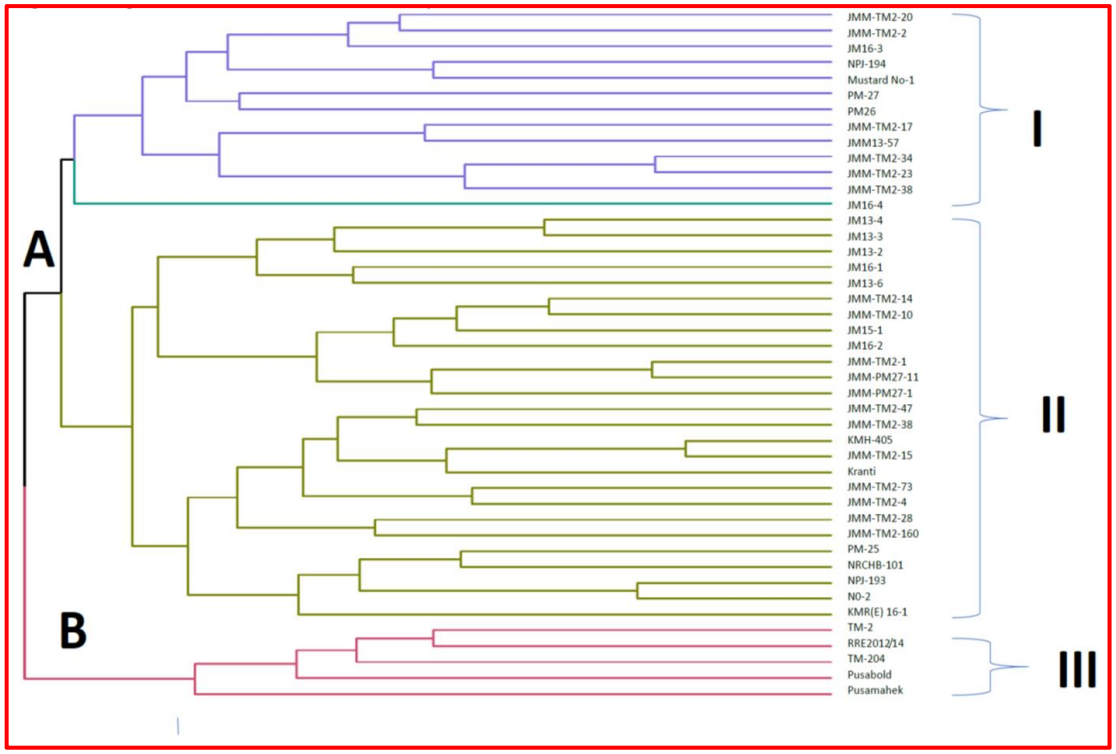

Figure 2: Clustering of genotypes using UPGMA based on Jaccard's coefficient similarity estimated from 58 polymorphic SSR markers

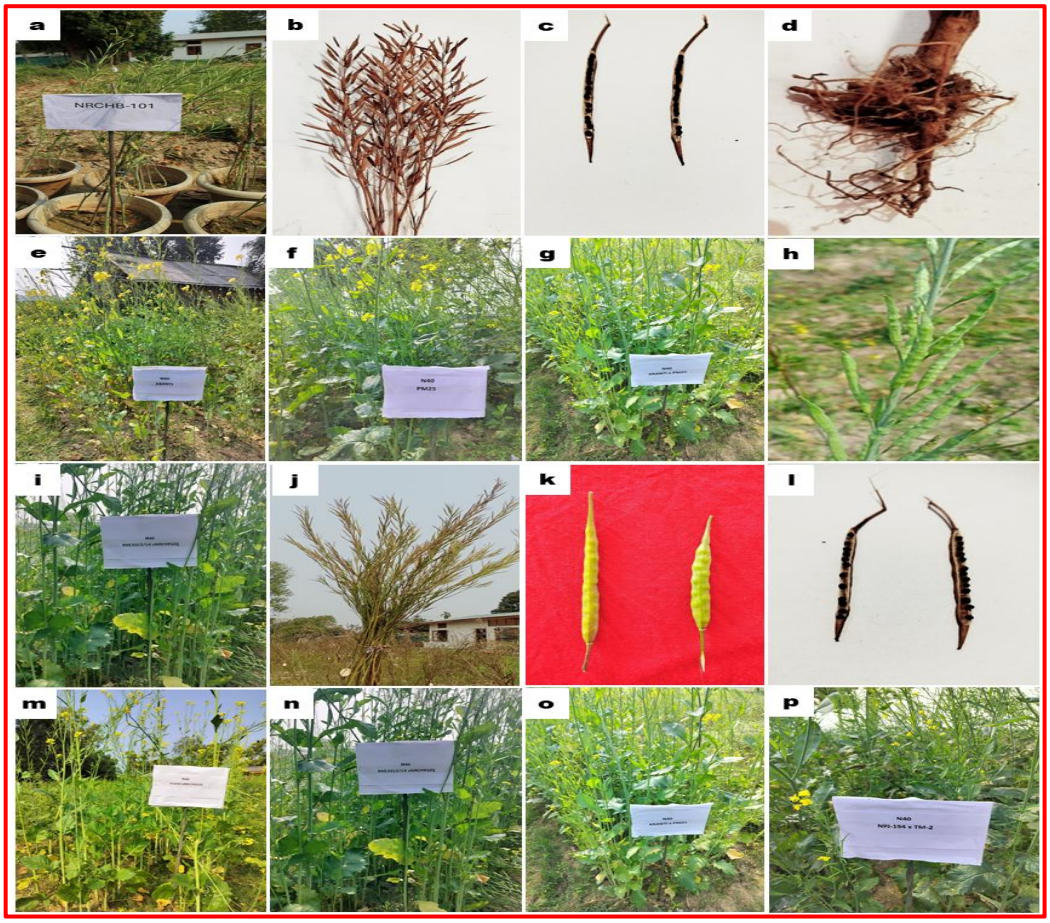

Figure 3: Performances of some of the parents and hybrids: a) performance of NRCHB-101 under pot culture; b) no. of siliquae per plant in NRCHB-101; c) root length of NRCHB-101 under pot culture; d) no. of seeds per siliqua n NRCHB-101; e) performance of Kranti (Line) at N40 dose under field conditions; f) performance of PM-25 (Tester) at N40 dose under field conditions; g) performance of F1 Cross Kranti x PM-25 at N40 dose under field conditions; h) Siliquae per plant of Kranti x PM-25 at N40 dose under field conditions; i) performance of F1 RRE2012/4X NRCHB-101 at N40 dose under field conditions; j) harvesting of F1 RRE2012/4X NRCHB-101 at N40 dose under field conditions; k) siliqua of RRE2012/4X NRCHB-101 at N40 dose under field conditions; l) no. of seeds per siliqua in RRE2012/4X NRCHB-101 at N40 dose under field conditions; (m-p) Performances of hybrids viz. Kranti x NRCHB101, RRE2012/14 x NRCHB101, Kranti x PM25 and NPJ-194 x TM-2 at N40 dose under field conditions in Rabi 2023-24.

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