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Deciphering the Morpho-Biochemical Determinants of Host Preferences and Population Dynamics in Lipaphis erysimi (Kaltenbach) Across Diverse Brassica juncea Germplasm

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Article

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Abstract

The mustard aphid (*L. erysimi* Kalt.) is the most significant biotic factor that restricts the production of *B. juncea* in various agroecological situations. In this study, the key morpho-physicochemical components conferring resistance and their role in aphid preference and population build-up were investigated under open field and control conditions. Twenty diverse *B. juncea* germplasm were screened for aphid infestation under natural and artificial conditions in two consecutive *Rabi* seasons (2023-24 and 2024-25). Significant variation was noticed for aphid population build-up, multiplication rate, and resistance index among the germplasm. KRANTI, PM-25 and PDL-1 germplasm gave significantly less aphid infestation, while TARAMERA, PM-29 and LACMA-SP523N5 were highly susceptible and preferred to aphid infestation. Biochemical parameters showed a strong association with resistance: Sugars exhibited a significant positive correlation with aphid population, indicating a stronger host preference. Phenols and glucosinolates exhibit a significant negative correlation with aphid population and act as defensive compounds. High activities of peroxidase (PO), phenylalanine ammonia-lyase (PAL), and tyrosine ammonia-lyase (TAL) also contribute to resistance induction by activating secondary metabolite defence pathways. Wax content under morphological characters showed a significant negative association with aphid multiplication rate and limited or no effect of plant height, stem diameter, and shoot length on the infestation level. Multivariate analyses indicated that biochemical compounds (sugars, phenols and glucosinolates) explain the major share of variance for aphid resistance, whereas morphological parameters also play a secondary role for the resistance to the mustard aphid. Germplasm was clustered in three classes, viz., resistant, moderately resistant and susceptible, according to their defence characteristics. The findings of this study suggest that screening of different *B. Juncea* germplasm for determining resistance, may be a more viable option to minimise the crop losses caused by *L. erysimi.* Moreover, the findings of the present study could also help to formulate the breeding programmes aimed at developing cultivars resistant to this notorious pest.

Introduction

Rapeseed-mustard is cultivated worldwide for its food and oil value, as well as its nutritional, medical, bio-industrial, and crop-rotation benefits[1]. Due to its adaptability across diverse agroclimatic zones, *B. juncea* (L.) Czern & Coss. remains the most popular Brassica species in India, accounting for the majority of the crop's cultivation. India ranks among the top three producers of rapeseed-mustard, along with China and Canada, and contributes about 14% of the world's rapeseed-mustard production, underscoring its significant role in the global oilseed market. Rapeseed-mustard contributes a substantial share of global oilseed production and serves multiple purposes, including traditional medicine, animal feed, and culinary applications, making it the third-largest source of edible oil in the world, behind soybean and palm oil[2]. In India, the area, production and productivity under rapeseed and mustard have continued to increase over the last few years. During 2024-25, rapeseed and mustard were grown on 8.63 million ha, with production and productivity of 12.61 million tonnes and 1461 kg/ha, respectively[3]. The average productivity of mustard in India is significantly lower than the world average, at about three-fourths of it. Furthermore, the production and productivity of rapeseed mustard vary greatly due to various biotic and abiotic stresses encountered across India's diverse agroecological zones[4].

Insect pests are a major obstacle to increasing global oilseed crop production, with around 50 insect species known to infest *B. species* in India[5]. Among these, the mustard aphid, *Lipaphis erysimi* (Kalt.) (Homoptera: Aphididae), is the major yield-reducing factor in rapeseed mustard, causing 10.2–61.1% preventable yield[8]. Several studies have reported variable plant damage severity by the mustard aphid, *L. erysimi*, from 10 to 90% and yield loss from 65 to 96% in different *Brassica* spp. depending on crop growth stage, speed of population increase, and agro-climatic conditions [9–11]. Aphids get the majority of their nourishment from plant sap via their piercing and sucking mouthparts and stylets. The styles are used for exploring and probing plant tissue to locate elements. Aphids can breach the simplest and use intracellular compartments without injuring them, allowing them to obtain phloem sap. They have a short life cycle and can reproduce both asexually and sexually[12]. As a result, an outbreak could occur in a short period if the conditions are favourable. The phloem sap-feeding mechanism, ubiquitous distribution, shorter generation time, and a highly efficient mode of reproduction (parthenogenesis and viviparity) make this aphid species attain the global status of a strong yield-reducing factor, resulting in retarded growth of the plant, poor seed formation, and low oil content. The economic threshold, speed of population increases, and severity of infestation are key to deciding on appropriate management practices. As the dynamics of insect-plant interactions and management options alter under a warming climate scenario, there is a need to have updated information on the extent of damage and yield loss and available insect pest control tools in the currently grown crop varieties to design an appropriate pest management strategy[13].

However, with appropriate management practices, this aphid can be controlled efficiently[14]. The aphids are currently being managed through insecticidal sprays, which have some limitations such as adverse effects on natural enemies, pollinators, and the environment. Moreover, the insecticides are lipophilic and may leave hazardous residues in the oil. Therefore, available pest management tactics must offer effective and economical pest control without negatively impacting the environment. Among various control methods, varietal resistance has gained priority in integrated pest management programmes. Extensive screening of *Brassica* germplasm has failed to identify any effective source of resistance to mustard aphids in India[15]. An artificial infestation screening technique under field conditions has also been developed to evaluate mustard germplasm for resistance against *L. erysimi*[16]. A thorough understanding of host-plant interactions – including metabolic fluxes involved in plant resistance and those used by aphids to bypass plant defences – is vital for developing aphid-resistant germplasm[17]. The host plant's defensive response to aphids can result from antixenosis, antibiosis, and tolerance. Various phenological, morphological, and biochemical traits influence the host selection process by mustard aphids. Characteristics such as colour, shape, size, thick cuticle, trichomes, surface waxes, pod size, seed number per pod, and leaf colour are morphological features of *B. juncea* that affect insect preference and confer phenotypic resistance to aphids[18].

Various biochemical constituents have also been reported to play a significant role in determining aphid herbivory. Biochemical constituents such as total antioxidants, tannins, and phenols have also been found to negatively affect the reproductive period, fecundity, and survival of mustard aphids in *B. juncea*[19]. Unlike many other chemical Defences, glucosinolates, a group of isothiocyanates found in *Brassicas*, are reported to play a key role in regulating the

infestation, establishment, and reproduction of aphids[20]. Besides the direct effects of these chemical compounds, some antioxidative enzymes, such as ascorbate oxidase, ascorbate peroxidase, and catalase, also contribute indirectly to plant defense against insects[21]. Additionally, in response to herbivore damage, the enzyme myrosinase interacts with its glucosinolate substrates to produce bioactive compounds[23]. Furthermore, plants challenged by insects have been reported to alter their biochemical composition, physical properties, and secondary metabolite biosynthesis. In this context, the present study aims to comprehensively elucidate the morpho-biochemical mechanisms governing host preference and resistance in *Brassica juncea* germplasm, linking trait-level variation to aphid performance and population dynamics, and bridging laboratory insights with field relevance to establish an empirical framework for breeding durable aphid-resistant cultivars and advancing sustainable integrated pest management strategies.

Materials and methods

The field experiments were conducted during the two consecutive *Rabi* seasons of 2023-24 and 2024-25 at the Student's Instructional Farm, Acharya Narendra Deva University of Agriculture and Technology (ANDUAT), located at Kumarganj, Ayodhya district, Uttar Pradesh, India. The university lies in the Middle Gangetic Plains of eastern Uttar Pradesh. It is geographically situated at approximately 26° 32' 8.16" N, 81° 50' 6.72" E, with an average altitude of about 113 meters above mean sea level. A total of 20 *B. juncea* germplasm, including one susceptible check (Giriraj), was evaluated under open field conditions and control conditions to assess their preference and resistance to the mustard aphid (*L. erysimi*) infestation. Seeds were obtained from the Department of Genetics and Plant Breeding, ANDUA&T, Kumarganj. Two to three seeds per hill were sown at a depth of 4–5 cm, maintaining a spacing of 30 × 15 cm (row × plant). After germination and seedling establishment, thinning was performed to retain one to two plants per hill. Each germplasm was grown in five rows, each 4 meters long. All recommended agronomic practices were followed, except for the application of insecticides.

Population build-up and host preference of L. erysimi on diverse B. juncea Germplasm

Natural infestation condition

The *B. juncea* germplasm was monitored daily to track the infestation and population of the mustard aphid, *L. erysimi*, reaching the economic threshold level (ETL). Five randomly selected plants of each test germplasm were tagged for observations, creating three replications in a completely randomized design. At 21 days after the *L. erysimi* population reached ETL, the number of aphids on the apical 10 cm of the main shoot of each selected plant of the test mustard germplasm was recorded and expressed as aphids per plant. Additionally, the aphid population index, aphid damage index, and aphid resistance index were calculated for all the cultivars as described by Dhillon *et al.* (2018)[16].

Artificial infestation condition

The multiplication rate and population build-up of *L. erysimi* on the test *B. juncea* germplasm were assessed under artificial infestation conditions following the method described by Dhillon *et al.* (2018)[16]. Five randomly selected plants of each germplasm were enclosed in a cylindrical twig cage (15 cm diameter × 60 cm height) mounted on three rings, one at the top, middle, and bottom. The muslin cloth used allowed easy penetration of air and light, enabling normal photosynthetic activity in the plant, which facilitates aphid growth and establishment. Plants from the middle two rows of each germplasm in each replication were tagged, and the third branch from the top was inoculated with approximately 20 mixed-stage aphids (nymphs and adults) at the bud initiation stage. Before inoculation, test plants were free from aphid infestation and other non-target insects. The experiment was conducted with three replications in a completely randomized block design. Observations of total aphids were recorded at 21 days after *L. erysimi* inoculation. The aphid multiplication rate on each cultivar was determined by dividing the total number of aphids on the inoculated branch by 20, and the daily multiplication rate per aphid was calculated by dividing the multiplication rate by 21.

Morphological Characterization of B. juncea Germplasm

Surface Wax Estimation

Surface wax content was determined according to the method of Ebercon *et al.* (1977)[31]. Fresh, healthy leaves were cleaned, air-dried, and immersed in 20 mL of chloroform for 30 seconds to 1 minute to dissolve the waxes. The chloroform extract was filtered and evaporated to dryness in a pre-weighed vial. The residue was weighed, and surface wax content was expressed as mg/cm² or mg/g fresh weight.

Stem Diameter Measurement

Stem diameter was measured using a Vernier calliper with 0.01 mm precision. Ten plants per germplasm were randomly selected. The apical 10 cm portion of the main shoot was identified, and the stem diameter was measured at a representative point within this section. The mean of three measurements was recorded for each germplasm.

Plant Height Measurement

Plant height was measured by using a measuring tape with centimetre precision. Ten plants per germplasm were randomly selected from the experimental plot. The height of each plant was recorded from the base of the plant at ground level to the tip of the apical meristem at physiological maturity. Three observations were taken for each plant, and the mean plant height was calculated and expressed in centimetres (cm) for each germplasm.

Main Shoot Length Measurement

The main shoot length was measured using a measuring tape with centimetre precision. Ten plants per germplasm were randomly selected from the experimental plot. The length of the main shoot was recorded from the base of the plant at ground level to the first branch arising from the main stem. Three observations were taken for each plant, and the mean main shoot length was calculated and expressed in centimetres (cm) for each germplasm.

Biochemical Characterization of B. juncea Germplasm

Sample Collection and Preparation

Healthy leaves from five randomly selected plants per germplasm were collected 48 hours after exposure to field conditions. Approximately 2 g of leaf tissue was frozen in liquid nitrogen and homogenised in 10 mL of phosphate buffer (50 mM, pH 7.8). The homogenate was centrifuged at 12,000–20,000 rpm for 20–30 minutes at 4°C. The supernatant was collected and stored at − 20°C for further biochemical analysis.

Total Sugar Estimation

Total sugar content was estimated using the phenol-sulphuric acid method (Dubois *et al.* 1956)[32] with glucose as the standard. 500 µL of plant extract was mixed with 0.5 mL of 5% phenol, followed by the addition of 2.5 mL of concentrated H_2SO_4. Absorbance was measured at 490 nm using a UV-spectrophotometer. Sugar content was calculated based on a glucose standard curve and expressed in mg/g of tissue.

Total Phenol Estimation

Total phenolic content was measured following the method of Singleton and Rossi (1965)[33], using gallic acid as the standard. Each sample consisted of 200 µL of plant extract and 300 µL of water, followed by the addition of 0.1 mL of Folin-Ciocalteu reagent. After 15 minutes, 2.5 mL of saturated sodium carbonate was added and incubated for 30 minutes. Absorbance was recorded at 760 nm, and values were expressed as mg/g of tissue.

Total Tannin Estimation

Tannin content was determined using the method of Amorim *et al.* (2008)[34]. 500 mg of plant tissue was extracted in 25 mL of 80% ethanol and filtered after 30 minutes. The final volume was adjusted with ethanol. Absorbance of 200 µL of each sample was measured at 760 nm, and tannin content was calculated using a gallic acid standard curve.

Total Glucosinolate Estimation

Glucosinolate content was estimated as per Mawlong *et al.* (2017)[35]. Dried leaf samples (0.3 g) were extracted with 300 mL of 80% methanol at 70°C for 5 minutes. After cooling and centrifugation at 15,000 rpm for 15 minutes, 100 µL of the supernatant was mixed with 4 mL of 0.2 mM sodium tetrachloropalladate and incubated for 1 hour. Absorbance was measured at 425 nm, and glucosinolate content was calculated using the formula:

$y = 1.40 + 118.86 \times A_{425}$ and expressed as µmol/g of tissue.

Total Chlorophyll Estimation

Chlorophyll content was estimated using the DMSO method (Nayek *et al.*, 2014)[36]. Fresh leaf tissue (50 mg) was incubated in 10 mL DMSO at 65°C for 1 hour. Absorbance was recorded at 645 and 663 nm, and total chlorophyll was calculated using the equation:

$$\text{Total chlorophyll} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times (V / 1000 \times W)$$

where V = volume of extract (mL), and W = weight of fresh tissue (g).

Enzymatic Assays

Ascorbate Peroxidase (APX) Activity

APX activity was determined using the method of Ali *et al.* (2005)[37]. The reaction mixture (1 mL) consisted of 100 mM Tris-acetate buffer (pH 7.0), 2 mM ascorbic acid, 2 mM H_2O_2, and 20 µL enzyme extract. Absorbance was recorded at 290 nm for 3 minutes. Activity was expressed as U/mg protein.

Phenylalanine Ammonia-Lyase (PAL) Activity

PAL activity was measured according to Fritz *et al.* (1976)[38]. The reaction mixture (1 mL) contained 0.1 mM Tris-acetate buffer (pH 8.5), 0.833 mM L-phenylalanine, and 50 µL enzyme extract. Absorbance was monitored at 290 nm over 10 minutes and expressed as U/mL of protein.

Tyrosine Ammonia-Lyase (TAL) Activity

TAL activity was estimated as per Thorpe and Beaudoin-Eagan (1985)[39]. The reaction mixture (1 mL) contained 0.1 mM Tris-acetate buffer (pH 8.5), 0.833 mM L-tyrosine, and 50 µL enzyme extract. Absorbance was recorded at 290 nm for 10 minutes, and activity was expressed as U/mL of protein.

Preference index

The preference index[40] by mustard aphid population was calculated at 21 days after inoculation by a 20 mixed-stage population, using the following formula:

$$C = 2A / (M + A)$$

where C = preference index.

A = consumption of the tested variety;

M = consumption of the susceptible check variety

Data interpretation was based on "C" values ranging from 0 to 2. In cases where C > 1, the tested variety was preferred over the check variety (GIRIRAJ); in cases where C = 1, the tested variety was neutral, similar to the check variety; and in cases where C < 1, the tested variety was less popular than the check variety (GIRIRAJ).

Statistical Analysis

The data on plant phenol-morphological traits, aphid preference, resistance indices, and multiplication rates were subjected to analysis of variance using a complete randomised block design, and the F-test was used to assess the significance of differences and to compare treatment means using the least significant differences at $P = 0.05$, using the statistical software SPSS version 27. Association of various phenol-morphological traits of wild crucifers with host preference, aphid resistance index and overall aphid multiplication rate of *L. erysimi* were also inferred by computing their correlation coefficients, and principal component analysis (PCA) was computed to assess the relationships between plant traits and aphid resistance using SPSS® version 27.0 software and R-Studio.

Results

Population build-up and resistance indices of L. erysimi diverse B. juncea Germplasm

Natural infestation

The mustard aphid infestation during the *Rabi* 2023–2024 season recorded significant results ($F_{19,38} = 68.049$, $P < 0.001$), and revealed that the total number of mustard aphids (*L. erysimi*) found on the top 10 cm of the central shoot of various *B. juncea* germplasm ranged from about 21.67 to 782.88 aphids per 10 cm of the top shoot, with none of the mustard germplasm found free from mustard aphid infestation (Table 2). The germplasm TARAMIRA recorded the highest aphid population (782.88 nos.), indicating high susceptibility to mustard aphid infestation, followed by LACMA-SP523N5 (272.29 nos.), SHIVANI (186.38 nos.), and BRIJRAJ (194.17 nos.), which also supported relatively high aphid populations, suggesting they are more prone to mustard aphid infestation. Conversely, germplasm such as PM-25 (21.67 nos.) and KRANTI (22.88 nos.) showed fewer aphids and demonstrated better resistance to mustard aphid attacks. Additionally, the study measured different indices aphid population index, damage index, and resistance index that varied considerably across the germplasm.

Table 1
Details of *Brassica* germplasm used for their performance against mustard aphid

Sr. No.	Name of Germplasm	Maturity	Yield Potential	Oil Content	Notable Characteristics
1	BRIJRAJ	Medium	High	High	Bold seeds, stable yield
2	PDL-1	Early	Moderate	Medium	Suitable for rainfed conditions
3	RH-725	Medium	High	High	Good adaptability
4	LACMA-SP523N5	Medium	Moderate	Medium	Diverse germplasm line
5	GIRIRAJ*	Medium	High	High	Susceptible to aphid infestation
6	PUSA TARAK	Early	Moderate	Medium	Suitable for late sowing
7	PM-29	Medium	High	High	Good oil quality
8	PM-22	Early	Moderate	Medium	Early flowering germplasm
9	EC-399301	Medium	Low–Moderate	Medium	Exotic germplasm, breeding resource
10	SHIVANI	Medium	Moderate–High	Medium	Stable performance
11	KRANTI	Medium	Moderate	Medium	Widely adapted variety
12	PM-25	Medium	High	High	Bold-seeded germplasm
13	JM-3	Early	Moderate	Medium	Suitable for multiple cropping
14	CS-56	Medium	Moderate	High	Stress-tolerant germplasm
15	MASC-1	Medium	High	Medium	Profuse branching
16	RH-406	Medium	High	High	Disease-resistant line
17	TARAMERA	Early	Low–Moderate	Medium	Low input requirement
18	RADHIKA	Medium	High	High	Good oil quality
19	CS-60	Early-Medium	Moderate	Medium	Adapted to marginal soils
20	PUSA BAHAR	Medium	High	High	Bold seeds, high yield

*Susceptible check

Table 2
Population build-up and resistance indices of *L. erysimi* on different *Brassica* germplasm under natural conditions during *Rabi* 2023-24 and 2024-25

Germplasm	Aphids/10 cm top shoot portion		API		ADI		ARI	
	2023-24	2024-25	2023-24	2024-25	2023-24	2024-25	2023-24	2024-25
BRIJRAJ	194.17 ± 42.57^{cde}	115.54 ± 21.03^{cdef}	3.80 ± 0.42^{h}	3.10 ± 0.21^{de}	2.10 ± 0.18^{ef}	2.80 ± 0.20^{i}	3.05 ± 0.10^{ef}	2.95 ± 0.30^{a}
PDL-1	30.75 ± 4.10^{ab}	41.96 ± 8.13^{ab}	2.00 ± 0.15^{a}	2.10 ± 0.14^{a}	1.00 ± 0.2.6^{a}	1.20 ± 0.32^{bc}	1.52 ± 0.20^{a}	1.65 ± 0.10^{b}
RH-725	162.63 ± 31.05^{cd}	181.08 ± 34.37^{f}	3.50 ± 0.20^{fg}	3.70 ± 0.30^{f}	2.11 ± 0.3.5^{e}	3.40 ± 0.23^{k}	2.76 ± 0.30^{de}	3.55 ± 0.20^{c}
LACMA-SP523N5	272.29 ± 57.15^{e}	170.21 ± 34.05^{ef}	4.10 ± 0.35^{i}	3.30 ± 0.34^{e}	3.10 ± 0.30^{i}	3.10 ± 0.20^{j}	3.64 ± 0.27^{h}	3.20 ± 0.21^{d}
GIRIRAJ	127.91 ± 30.50^{abcd}	157.33 ± 29.16^{def}	3.30 ± 0.27^{b}	3.30 ± 0.26^{e}	1.80 ± 0.20^{b}	3.70 ± 0.20^{l}	2.56 ± 0.10^{b}	3.50 ± 0.30^{e}
PUSA TARAK	135.79 ± 28.75^{cd}	125.71 ± 24.50^{cdef}	3.20 ± 0.12^{cde}	3.10 ± 0.23^{de}	2.00 ± 0.10^{e}	3.00 ± 0.10^{j}	2.61 ± 0.10^{d}	3.05 ± 0.21^{f}
PM-29	146.54 ± 31.22^{cd}	102.63 ± 19.00^{bcde}	3.40 ± 0.34^{ef}	3.00 ± 0.21^{d}	2.10 ± 0.25^{ef}	2.40 ± 0.30^{g}	2.75 ± 0.33^{de}	2.70 ± 0.20^{g}
PM-22	140.21 ± 28.06^{cd}	115.1 ± 20.00^{cdef}	3.40 ± 0.34^{ef}	3.00 ± 0.14^{d}	2.00 ± 0.23^{e}	2.60 ± 0.14^{h}	2.68 ± 0.27^{d}	2.80 ± 0.33^{h}
EC-399301	84.71 ± 16.13^{abc}	118.63 ± 21.07^{cdef}	2.70 ± 0.21^{b}	3.10 ± 0.10^{de}	1.58 ± 0.12^{b}	2.60 ± 0.20^{h}	2.14 ± 0.32^{b}	2.85 ± 0.20^{i}
SHIVANI	186.38 ± 39.98^{cde}	93.33 ± 16.29^{bcd}	3.80 ± 0.30^{h}	2.60 ± 0.30^{c}	2.40 ± 0.29^{g}	1.70 ± 0.20^{d}	3.10 ± 0.14^{fg}	2.95 ± 0.30^{j}
KRANTI	22.88 ± 3.76^{a}	20.67 ± 3.33^{a}	2.00 ± 0.20^{a}	2.00 ± 0.22^{a}	1.00 ± 0.22^{a}	1.00 ± 0.10^{a}	1.46 ± 0.16^{a}	1.50 ± 0.16^{k}
PM-25	21.67 ± 3.30^{a}	20.08 ± 2.94^{a}	2.00 ± 0.14^{a}	2.00 ± 0.11^{a}	1.00 ± 0.14^{a}	1.00 ± 0.30^{a}	1.55 ± 0.25^{a}	1.50 ± 0.20^{l}
JM-3	133.38 ± 29.27^{bcd}	118.88 ± 25.33^{cdef}	3.20 ± 0.16^{cde}	3.10 ± 0.33^{de}	2.24 ± 0.36^{f}	1.20 ± 0.30^{bc}	2.70 ± 0.22^{d}	2.15 ± 0.22^{m}
CS-56	166.04 ± 36.78^{cd}	121.33 ± 28.69^{cdef}	3.70 ± 0.24^{gh}	3.20 ± 0.30^{de}	2.56 ± 0.34^{g}	1.00 ± 0.10^{a}	3.13 ± 0.30^{fg}	2.10 ± 0.24^{a}
MASC-1	96.54 ± 21.06^{abcd}	95.00 ± 21.22^{bcd}	2.60 ± 0.22^{b}	2.40 ± 0.12^{bc}	2.04 ± 0.23^{e}	1.80 ± 0.20^{e}	2.34 ± 0.17^{c}	2.10 ± 0.31^{b}
RH-406	103.25 ± 22.87^{abcd}	114.71 ± 27.10^{cdef}	3.00 ± 0.23^{c}	3.20 ± 0.24^{de}	1.62 ± 0.20^{c}	2.00 ± 0.20^{f}	2.27 ± 0.25^{c}	2.60 ± 0.30^{c}
TARAMERA	782.88 ± 126.32^{f}	444.42 ± 85.37^{g}	4.80 ± 0.42^{j}	4.30 ± 0.45^{g}	4.69 ± 0.36^{j}	3.40 ± 0.30^{k}	4.76 ± 0.14^{i}	3.85 ± 0.40^{d}
RADHIKA	113.50 ± 26.57^{abcd}	85.63 ± 17.27^{abc}	3.10 ± 0.31^{cd}	2.60 ± 0.27^{ab}	2.20 ± 0.23^{f}	1.60 ± 0.20^{cd}	2.65 ± 0.23^{d}	1.75 ± 0.22^{e}
CS-60	177.79 ± 43.57^{cde}	87.96 ± 19.04^{abc}	3.70 ± 0.12^{gh}	2.20 ± 0.38^{ab}	2.82 ± 0.29^{h}	1.10 ± 0.10^{ab}	3.30 ± 0.20^{g}	1.65 ± 0.33^{f}
PUSA BAHAR	96.20 ± 18.76^{bcd}	74.04 ± 15.62^{abc}	2.70 ± 0.40^{def}	2.10 ± 0.20^{a}	1.78 ± 0.14^{d}	1.00 ± 0.20^{a}	2.22 ± 0.26^{d}	1.55 ± 0.20^{g}
F- value	68.049	45.580	273.063	202.142	55.012	761.036	450.284	1019.053
LSD (P = 0.05)	78.635	53.675	0.706	0.841	0.987	0.651	0.759	0.658
P- value (0.05)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

API = Aphid Population Index, ADI= Aphid damage Index, ARI = Aphid Resistance Index

The total aphid count, along with indices such as the aphid population index ($F_{19,38}$ = 0.706, P < 0.001), damage index ($F_{19,38}$ = 0.987, P < 0.001), and resistance index ($F_{19,38}$ = 0.7059, P < 0.001), ranged from 21.67 to 782.88, 2.00 to 4.80, 1.00 to 3.40, and 1.46 to 3.64, respectively (Table 2). Meanwhile, TARAMIRA, RH-725, LACMA-SP523N5, CS-56, and SHIVANI showed higher values, indicating greater vulnerability. Conversely, germplasm such as PM-25, KRANTI, and PDL-1 had lower scores, reflecting stronger resistance against mustard aphid infestation.

Based on the resistance scale, we grouped the germplasm into five categories: resistant, moderately resistant, tolerant, susceptible, and highly susceptible, depending on the number of mustard aphids found on each plant. None of the germplasm fell into the resistant category, with aphid counts between 0.00

and 1.00. Germplasm in the 1.10-2.00 range, such as PM-25 (1.55), PDL-1 (1.52) and KRANTI (1.46) were considered moderately resistant. Those with 2.10–2.50 aphids, including MASC-1 (2.34), RH-406 (2.27), PUSA BAHAR (2.22) and EC-399301 (2.14) were classified as tolerant. The susceptible group includes plants like GIRIRAJ (2.56), PUSA TARAK (2.61), and PM-22 (2.68), which had aphid counts between 2.60 and 3.50. Finally, the two germplasm LACMA-SP523N5 (3.64) and TARAMERA (4.76) supported the highest number of aphids in the 3.60 to 5.00 range, making them highly susceptible.

Similarly, during the *Rabi* season of 2024–2025 ($F_{19,38}$ = 0.45.580, P < 0.001) ranged from as low as 20.08 to as high as 444.42 aphids per 10 cm of the top shoot. This variation emphasizes major differences in how susceptible or resistant these germplasms are (Table 2). Notably, the germplasm TARAMIRA experienced the highest average aphid population with 444.42 aphids per 10 cm of the top shoot, indicating very high susceptibility, followed by RH-725 (181.08 nos.) and LACMA-SP523N5 (170.21 nos.). Conversely, the lowest populations were observed in PM-25, with an average of 20.08 nos., followed by KRANTI (20.67 nos.) both maintaining minimal aphid counts throughout the observation period even during peak infestation periods demonstrating their strong resistance.

Results from different samples varied significantly in terms of the total aphid count and associated indices. The aphid population index ($F_{19,38}$ = 0.841, P < 0.001), damage index ($F_{19,38}$ = 0.651, P < 0.001), and resistance index ($F_{19,38}$ = 0.658, P < 0.001), all showed statistically significant differences. The observed ranges were 20.08-444.42 for total aphid count, 2.00-4.30 for aphid population index, 1.00-3.40 for damage index, and 1.50–3.85 for resistance index (Table 2). Major variation among these indices was evident, with TARAMERA, RH-725, and LACMA-SP523N5 showing notably higher aphid populations. In contrast, PM-25, KRANTI, and PDL-1 exhibited considerably lower aphid populations, indicating superior resistance. Notably, the aphid population index, damage index, and resistance index were markedly higher in TARAMERA, RH-725, GIRIRAJ, and LACMA-SP523N5, whereas these indices were significantly lower in PM-25, KRANTI, PUSA BAHAR, CS-60, PDL-1, RADHIKA, and SHIVANI. These findings suggest that the latter germplasm demonstrates some degree of tolerance against *L. erysimi*.

Artificial infestation

There were significant differences in total aphid population after 21 days of inoculation, aphid multiplication rate and daily aphid multiplication rate of *L. erysimi* on the test *B. juncea* germplasm, which varied between 74.22 to 1044.67 ($F_{19,38}$ = 641.632, P < 0.001), 3.71 and 52.23 ($F_{19,38}$ =787.449, P < 0.001), and 0.18 and 2.49 ($F_{19,38}$ =1415.270, P < 0.001), respectively, during the *Rabi* 2023-24 cropping season (Table 3). The total aphid population, aphid multiplication rate and daily aphid multiplication rate were significantly higher on TARAMERA (1044.67, 52.23 and 2.49, respectively), followed by PM-29 (1009.78, 50.49 and 2.40, respectively), LACMA-SP523N5 (936.67, 46.83 and 2.23, respectively) and RH-406 (833.67, 41.68 and 1.98, respectively), while lower aphid populations were recorded on PM-25 (74.22, 3.71 and 0.18, respectively), followed by KRANTI (78.22, 3.91 and 0.19, respectively) and PDL-1 (89.22, 4.46 and 0.21, respectively) (Tables 3).

Table 3
Population build-up and resistance indices of *L. erysimi* on different Brassica germplasm under artificial infestation conditions during *rabi* 2023–2025

Germplasm	Aphid population (21 days after initial release)		Overall multiplication rate		Daily Aphid Multiplication (rate/adult)	
	2023-24	2024-25	2023-24	2024-25	2023-24	2024-25
BRIJRAJ	755.89 ± 6.54^{f}	742.56 ± 17.23^{fg}	37.79 ± 0.85^{f}	37.13 ± 0.12^{f}	1.80 ± 0.04^{f}	1.77 ± 0.01^{f}
PDL-1	89.22 ± 2.55^{a}	94.20 ± 2.87^{a}	4.46 ± 0.10^{a}	4.71 ± 0.07^{a}	0.21 ± 0.00^{a}	0.22 ± 0.00^{a}
RH-725	755.33 ± 5.73^{f}	715.67 ± 8.19^{f}	37.77 ± 0.53^{f}	35.78 ± 0.48^{f}	1.80 ± 0.01^{f}	1.70 ± 0.03^{f}
LACMA-SP523N5	936.67 ± 13.40^{h}	897.78 ± 16.64^{i}	46.83 ± 0.39^{h}	44.89 ± 0.98^{h}	2.23 ± 0.04^{h}	2.14 ± 0.02^{h}
GIRIRAJ	647.67 ± 8.40^{e}	616.89 ± 18.70^{e}	32.38 ± 0.74^{e}	30.84 ± 0.22^{e}	1.54 ± 0.02^{e}	1.47 ± 0.02^{e}
PUSA TARAK	467.00 ± 9.15^{c}	437.33 ± 5.69^{c}	23.35 ± 0.36^{c}	21.87 ± 0.51^{c}	1.11 ± 0.03^{c}	1.04 ± 0.00^{c}
PM-29	1009.78 ± 25.01^{i}	920.33 ± 15.71^{i}	50.49 ± 0.55^{i}	46.02 ± 0.60^{h}	2.40 ± 0.05^{i}	2.19 ± 0.02^{h}
PM-22	557.22 ± 7.79^{d}	530.44 ± 6.80^{d}	27.86 ± 0.17^{d}	26.52 ± 0.06^{d}	1.33 ± 0.02^{d}	1.26 ± 0.00^{d}
EC-399301	244.67 ± 2.22^{b}	231.33 ± 2.69^{b}	12.23 ± 0.20^{b}	11.57 ± 0.04^{b}	0.58 ± 0.01^{b}	0.55 ± 0.01^{b}
SHIVANI	820.78 ± 4.57^{g}	786.00 ± 12.92^{gh}	41.04 ± 0.06^{g}	39.30 ± 0.55^{g}	1.95 ± 0.04^{g}	1.87 ± 0.01^{g}
KRANTI	78.22 ± 1.94^{a}	74.56 ± 2.10^{a}	3.91 ± 0.02^{a}	3.73 ± 0.05^{a}	0.19 ± 0.00^{a}	0.18 ± 0.00^{a}
PM-25	74.22 ± 1.51^{a}	81.56 ± 2.82^{a}	3.71 ± 0.07^{a}	4.08 ± 0.01^{a}	0.18 ± 0.00^{a}	0.19 ± 0.00^{a}
JM-3	636.22 ± 8.40^{e}	606.67 ± 16.92^{e}	31.81 ± 0.81^{e}	30.33 ± 0.33^{e}	1.51 ± 0.02^{e}	1.44 ± 0.03^{e}
CS-56	759.89 ± 7.42^{f}	721.44 ± 8.12^{f}	37.99 ± 0.03^{f}	36.07 ± 0.86^{f}	1.81 ± 0.02^{f}	1.72 ± 0.03^{f}
MASC-1	239.56 ± 2.72^{b}	235.67 ± 3.36^{b}	11.98 ± 0.01^{b}	11.78 ± 0.29^{b}	0.57 ± 0.01^{b}	0.56 ± 0.01^{b}
RH-406	833.67 ± 5.82^{g}	798.00 ± 13.77^{h}	41.68 ± 0.52^{g}	39.90 ± 0.31^{g}	1.98 ± 0.04^{g}	1.90 ± 0.01^{g}
TARAMERA	1044.67 ± 23.79^{i}	995.44 ± 21.46^{j}	52.23 ± 0.65^{i}	49.77 ± 0.49^{i}	2.49 ± 0.02^{i}	2.37 ± 0.01^{i}
RADHIKA	767.00 ± 12.60^{f}	723.78 ± 11.32^{f}	38.35 ± 0.06^{f}	36.19 ± 0.30^{f}	1.83 ± 0.04^{f}	1.72 ± 0.02^{f}
CS-60	235.89 ± 2.95^{b}	228.78 ± 3.22^{b}	11.79 ± 0.23^{b}	11.44 ± 0.03^{b}	0.56 ± 0.01^{b}	0.54 ± 0.00^{b}
PUSA BAHAR	245.11 ± 4.24^{b}	227.22 ± 2.58^{b}	12.26 ± 0.28^{b}	11.36 ± 0.23^{b}	0.58 ± 0.00^{b}	0.54 ± 0.01^{b}
F- value	641.632	709.489	1415.270	1287.045	787.499	1862.719
LSD (P = 0.05)	36.600	32.784	1.027	1.014	0.061	0.043
P- value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

There was substantial variation in aphid abundance and associated indices across the sampled populations (Table 4). Significant differences were observed for the total aphid count ($F_{19,38}$ = 0.180, P < 0.001), aphid population index ($F_{19,38}$ =0.342, P < 0.001), damage index ($F_{19,38}$ = 0.165, P < 0.001), and resistance index ($F_{19,38}$ =0.165, P < 0.001). Total aphid counts ranged from 74.22 to 1044.67. The aphid population index, aphid damage index, and aphid resistance index varied from 2.60 to 5.00, 1.00 to 4.80, and 1.80 to 4.90, respectively. Total aphid counts exhibited a wide range, extending from 74.22 in the resistant genotype PM-25 to 1044.67 in the highly susceptible entry TARAMERA. Consistently, the indices mirrored these population trends; germplasm such as PM-25, Kranti, and PDL-1 maintained the lowest index scores, while TARAMERA, PM-29, and LACMA-SP523N5 recorded the maximum values for API, ADI, and ARI, indicating their high susceptibility under artificial screening conditions.

Table 4
Population build-up and resistance indices of *L. erysimi* on different *Brassica* germplasm under artificial conditions during *Rabi* 2023-24 and 2024-25

Germplasm	Number of aphids		API		ADI		ARI	
	2023-24	2024-25	2023-24	2024-25	2023-24	2024-25	2023-24	2024-25
BRIJRAJ	755.89 ± 6.54^{f}	742.56 ± 17.23^{fg}	5.00 ± 0.02^{c}	5.00 ± 0.12^{c}	3.40 ± 0.03^{d}	3.00 ± 0.04^{f}	4.20 ± 0.04^{ef}	4.00 ± 0.02^{e}
PDL-1	89.22 ± 2.55^{a}	94.20 ± 2.87^{a}	2.80 ± 0.06^{a}	2.90 ± 0.05^{a}	1.00 ± 0.00^{a}	1.00 ± 0.01^{a}	1.90 ± 0.03^{a}	2.00 ± 0.04^{a}
RH-725	755.33 ± 5.73^{f}	715.67 ± 8.19^{f}	5.00 ± 0.04^{c}	5.00 ± 0.10^{c}	3.00 ± 0.06^{c}	3.00 ± 0.01^{f}	4.00 ± 0.00^{e}	4.00 ± 0.03^{e}
LACMA-SP523N5	936.67 ± 13.40^{h}	897.78 ± 16.64^{i}	5.00 ± 0.03^{c}	5.00 ± 0.11^{c}	3.50 ± 0.01^{cd}	3.00 ± 0.04^{f}	4.30 ± 0.08^{f}	4.00 ± 0.06^{e}
GIRIRAJ	647.67 ± 8.40^{e}	616.89 ± 18.70^{e}	5.00 ± 0.08^{c}	5.00 ± 0.04^{c}	3.00 ± 0.04^{c}	2.00 ± 0.01^{d}	4.00 ± 0.08^{e}	3.50 ± 0.02^{d}
PUSA TARAK	467.00 ± 9.15^{c}	437.33 ± 5.69^{c}	4.80 ± 0.04^{c}	4.60 ± 0.11^{c}	2.00 ± 0.02^{b}	1.40 ± 0.01^{b}	3.40 ± 0.01^{c}	3.00 ± 0.02^{c}
PM-29	1009.78 ± 25.01^{i}	920.33 ± 15.71^{i}	5.00 ± 0.10^{c}	5.00 ± 0.02^{c}	4.80 ± 0.11^{e}	4.60 ± 0.08^{h}	4.90 ± 0.11^{g}	4.80 ± 0.11^{g}
PM-22	557.22 ± 7.79^{d}	530.44 ± 6.80^{d}	5.00 ± 0.06^{c}	5.00 ± 0.11^{c}	2.40 ± 0.03^{b}	3.00 ± 0.08^{f}	3.70 ± 0.02^{d}	4.00 ± 0.09^{e}
EC-399301	244.67 ± 2.22^{b}	231.33 ± 2.69^{b}	3.90 ± 0.02^{b}	3.80 ± 0.00^{b}	2.00 ± 0.02^{b}	1.60 ± 0.00^{c}	3.00 ± 0.07^{b}	2.70 ± 0.04^{b}
SHIVANI	820.78 ± 4.57^{g}	786.00 ± 12.92^{gh}	5.00 ± 0.04^{c}	5.00 ± 0.11^{c}	2.00 ± 0.04^{b}	2.00 ± 0.03^{d}	3.50 ± 0.06^{cd}	3.50 ± 0.06^{d}
KRANTI	78.22 ± 1.94^{a}	74.56 ± 2.10^{a}	2.70 ± 0.00^{a}	2.60 ± 0.04^{a}	1.20 ± 0.01^{a}	1.00 ± 0.02^{a}	2.00 ± 0.05^{a}	1.80 ± 0.01^{a}
PM-25	74.22 ± 1.51^{a}	81.56 ± 2.82^{a}	2.60 ± 0.02^{a}	2.70 ± 0.03^{a}	1.00 ± 0.02^{a}	1.00 ± 0.00^{a}	1.80 ± 0.01^{a}	1.90 ± 0.01^{a}
JM-3	636.22 ± 8.40^{e}	606.67 ± 16.92^{e}	5.00 ± 0.09^{c}	5.00 ± 0.04^{c}	2.00 ± 0.04^{b}	1.00 ± 0.02^{a}	3.50 ± 0.09^{cd}	3.00 ± 0.05^{c}
CS-56	759.89 ± 7.42^{f}	721.44 ± 8.12^{f}	5.00 ± 0.02^{c}	5.00 ± 0.04^{c}	2.00 ± 0.01^{c}	1.00 ± 0.01^{a}	3.50 ± 0.04^{cd}	3.00 ± 0.02^{c}
MASC-1	239.56 ± 2.72^{b}	235.67 ± 3.36^{b}	3.90 ± 0.03^{b}	3.90 ± 0.10^{b}	2.20 ± 0.03^{b}	2.00 ± 0.01^{d}	3.10 ± 0.02^{b}	3.00 ± 0.01^{c}
RH-406	833.67 ± 5.82^{g}	798.00 ± 13.77^{h}	5.00 ± 0.11^{c}	5.00 ± 0.11^{c}	2.00 ± 0.00^{b}	2.00 ± 0.04^{d}	3.50 ± 0.09^{cd}	3.50 ± 0.02^{d}
TARAMERA	1044.67 ± 23.79^{i}	995.44 ± 21.46^{j}	5.00 ± 0.03^{c}	5.00 ± 0.05^{c}	4.50 ± 0.07^{e}	4.00 ± 0.06^{g}	4.80 ± 0.06^{g}	4.50 ± 0.07^{f}
RADHIKA	767.00 ± 12.60^{f}	723.78 ± 11.32^{f}	5.00 ± 0.13^{c}	5.00 ± 0.11^{c}	3.00 ± 0.03^{c}	2.80 ± 0.02^{e}	4.00 ± 0.07^{e}	3.90 ± 0.04^{e}
CS-60	235.89 ± 2.95^{b}	228.78 ± 3.22^{b}	3.90 ± 0.05^{b}	3.80 ± 0.03^{b}	3.00 ± 0.01^{c}	2.00 ± 0.04^{d}	3.50 ± 0.03^{cd}	2.90 ± 0.07^{bc}
PUSA BAHAR	245.11 ± 4.24^{b}	227.22 ± 2.58^{b}	3.90 ± 0.08^{b}	3.80 ± 0.04^{b}	2.20 ± 0.03^{b}	2.00 ± 0.05^{d}	3.10 ± 0.04^{b}	2.90 ± 0.03^{bc}
F- value	641.632	709.489	189.467	121.785	78.108	765.466	214.384	274.912
LSD (P = 0.05)	36.600	32.784	0.180	0.220	0.342	0.104	0.165	0.148
P- value (0.05)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

API = Aphid Population Index, ADI = Aphid damage Index, ARI = Aphid Resistance Index

Aphid population and index parameters differ significantly across sample populations (Table 4). A highly significant variation was seen for total aphid count ($F_{19,38}$ =0.180, P < 0.001), aphid population index ($F_{19,38}$ =0.342, P < 0.001), damage index ($F_{19,38}$ =0.165, P < 0.001) and resistance index ($F_{19,38}$ =0.165, P < 0.001), showing a huge variation across all tested germplasm when grown in the same experimental condition. Total aphid count varied from 74.22 to 1044.67, aphid population index, aphid damage index and aphid resistance index from 2.60 to 5.00, 1.00 to 4.80 and 1.80 to 4.90, respectively. The total aphid counts ranged from 74.22 in the resistant genotype PM-25 to 1044.67 in the highly susceptible entry TARAMERA, showing variability in the infested plant population between the germplasm. The variation of aphid population index, aphid damage index and aphid resistance index from 2.60 to 5.00, 1.00 to 4.80 and 1.80 to 4.90, respectively, in all tested germplasm. Similarly, the highest counts of API, ADI, and ARI were recorded highest in the highly susceptible entries TARAMERA, PM-29, and LACMA-SP523N5, while they were recorded highest in the resistant entries PM-25, Kranti, and PDL-1.

Similarly, during *Rabi* season 2024-25 the total aphid population after 21 days of inoculation, along with the aphid multiplication rate and daily aphid multiplication rate of *L. erysimi*, also varied significantly across the test *B. juncea* germplasm. The total number of aphids, aphid multiplication rate, and daily aphid multiplication rate under artificial infestation conditions ranged from 74.56 to 995.44 ($F_{19,38}$ =709.489, P < 0.001), 3.73 to 49.77 ($F_{19,38}$ =1287.045, P < 0.001), and 0.18 to 2.37 ($F_{19,38}$ =1862.719, P < 0.001), respectively (Table 3). The total aphid population, aphid multiplication rate, and daily aphid multiplication rate were significantly higher on germplasm TARAMERA (995.44, 49.77, and 2.37, respectively), followed by PM-29 (920.33, 46.02, and 2.19, respectively); LACMA-SP523N5 (897.78, 44.89, and 2.14, respectively); and RH-406 (798.00, 39.90, and 1.90, respectively). Conversely, germplasm PM-25 recorded the lowest numbers of aphid population, aphid multiplication rate, and daily aphid multiplication rate (81.56, 4.08, and 0.19, respectively), followed by KRANTI (78.22, 3.73, and 0.18, respectively) and PDL-1 (94.20, 4.71, and 0.22, respectively).

Significant variation was observed in total aphid counts and associated indices across the sampled populations (Table 3). Statistical analysis revealed significant differences for the aphid population index ($F_{19,38}$ =0.220, P < 0.001), damage index ($F_{19,38}$ =0.104, P < 0.001), and resistance index ($F_{19,38}$ =0.148, P < 0.001). The observed ranges were as follows: total aphid count (74.56–995.44), aphid population index (2.60–5.00), damage index (1.00–4.60), and resistance index (1.80–4.80).

The results demonstrated significant variation in aphid infestation parameters, total aphid population, aphid multiplication rate, and daily aphid multiplication rate across different *B. juncea* germplasm during 2023-24 and 2024-25. Susceptible germplasm such as TARAMERA, PM-29, LACMA-SP523N5 and RH- 406 recorded higher aphid populations, likely due to favourable morphological or biochemical traits. In contrast, PM-25, KRANTI, PDL-1 and CS-60 showed lower aphid populations, suggesting resistance possibly due to higher phenolics or glucosinolates.

Aphid infestation parameters, total aphid population, aphid multiplication rate and daily aphid multiplication rate differed significantly for each germplasm for *B. Juncea* during the 2023-24 and 2024-25 seasons, indicating varying responses for the tested entries under similar environmental and experimental conditions. The susceptible germplasm, namely TARAMERA, PM-29, LACMA-SP523N5 and RH-406 exhibited high aphid population and high multiplication rates for aphids, as it indicates higher colonization for fast reproduction. It has been found that TARAMERA, PM-29, LACMA-SP523N5 and RH-406 showed high populations on each occasion and maintained their consistency throughout 2 years in terms of aphid multiplication rate as indicated by the same amount of daily aphid multiplication rate. On the other hand, low population and moderate multiplication rates for aphids on PM-25, KRANTI, PDL-1, and CS-60 germplasm, indicating slower multiplication for aphids, are also reported for these germplasm as well. Low aphid population on each occasion was consistent with the reduction in multiplication rate, thus it may suggest the consistent trend of population build-up for the two years.

Association of various morpho-physicochemical traits of diverse B. juncea Germplasm with aphid population dynamics, damage and resistance indices

Plant morphological characterization of B. juncea Germplasm

Stem diameter

Stem diameter among the germplasm ($F_{19,38}$ =1.943, P < 0.001) ranged between 2.11 and 3.13 mm (Table 5). The maximum stem thickness was observed in TARAMERA (3.13 mm), followed by SHIVANI (3.06 mm), PUSA TARAK (3.00 mm), RH-725 (2.94 mm) and PM-29 (2.90 mm). Thicker stems may act as a physical barrier against aphid infestation. The minimum stem diameter was recorded in RADHIKA (2.11 mm), followed by JM-3 (2.39 mm) and CS-60 (2.53 mm).

Table 5
Evaluation of mustard germplasm based on aphid resistance-related plant morphological parameters

Germplasm	Stem Diameter (mm)	Surface Wax (%)	Plant Height (cm)	Main shoot length (cm)
BRIJRAJ	2.81 ± 0.19^{ab}	3.36 ± 0.05^{ab}	155.90 ± 3.10^{ef}	84.00 ± 1.55^{cdefg}
PDL-1	2.68 ± 0.19^{ab}	4.28 ± 0.05^{def}	255.33 ± 3.37^{k}	78.40 ± 1.63^{cd}
RH-725	2.94 ± 0.15^{ab}	3.62 ± 0.05^{bc}	241.00 ± 2.70^{k}	86.00 ± 1.49^{defg}
LACMA-SP523N5	2.56 ± 0.20^{ab}	3.68 ± 0.05^{c}	110.60 ± 3.40^{bc}	91.00 ± 1.59^{fgh}
GIRIRAJ	2.56 ± 0.17^{ab}	3.82 ± 0.04^{fg}	210.40 ± 3.76^{j}	83.50 ± 1.80^{cdefg}
PUSA TARAK	3.00 ± 0.00^{ab}	3.32 ± 0.05^{h}	175.00 ± 2.55^{ghi}	96.00 ± 1.83^{h}
PM-29	2.90 ± 0.08^{ab}	3.56 ± 0.04^{abc}	178.73 ± 3.61^{hi}	77.00 ± 1.49^{bc}
PM-22	2.56 ± 0.18^{ab}	3.52 ± 0.06^{hi}	165.30 ± 2.29^{fgh}	82.20 ± 1.69^{cde}
EC-399301	2.75 ± 0.18^{ab}	3.20 ± 0.07^{g}	161.00 ± 3.46^{fg}	46.60 ± 1.72^{a}
SHIVANI	3.06 ± 0.15^{ab}	3.26 ± 0.04^{a}	98.80 ± 3.11^{ab}	69.00 ± 1.49^{b}
KRANTI	2.62 ± 0.08^{b}	4.82 ± 0.06^{i}	257.00 ± 3.48^{k}	83.00 ± 1.70^{cdef}
PM-25	2.89 ± 0.17^{ab}	5.11 ± 0.06^{j}	188.00 ± 2.63^{i}	81.00 ± 1.69^{cde}
JM-3	2.39 ± 0.13^{a}	3.43 ± 0.04^{efg}	126.80 ± 3.05^{cd}	83.00 ± 1.55^{cdef}
CS-56	2.89 ± 0.17^{ab}	3.70 ± 0.04^{d}	186.00 ± 3.46^{i}	89.00 ± 1.76^{efgh}
MASC-1	2.61 ± 0.17^{ab}	4.63 ± 0.10^{hi}	116.00 ± 2.46^{c}	92.00 ± 1.49^{gh}
RH-406	2.78 ± 0.14^{ab}	4.27 ± 0.09^{de}	173.00 ± 3.31^{ghi}	80.00 ± 1.55^{cd}
TARAMERA	3.13 ± 0.22^{ab}	3.10 ± 0.04^{de}	91.40 ± 3.35^{a}	68.50 ± 1.76^{b}
RADHIKA	2.11 ± 0.17^{ab}	3.91 ± 0.08^{h}	134.60 ± 3.29^{d}	78.00 ± 1.70^{cd}
CS-60	2.53 ± 0.08^{ab}	3.62 ± 0.07^{h}	181.00 ± 3.08^{hi}	86.00 ± 1.55^{defg}
PUSA BAHAR	2.61 ± 0.15^{ab}	4.12 ± 0.03^{d}	142.00 ± 2.91^{de}	91.00 ± 1.55^{fgh}
F- value	1.943	294.619	234.910	43.440
LSD (P = 0.05)	0.452	0.035	21.061	3.888
P- value (0.05)	< 0.001	< 0.001	< 0.001	< 0.001

Surface wax content

The surface wax content varied significantly ($F_{19,38}$ =294.619, P < 0.001) among the germplasm, ranging from 3.10% to 5.11% (Table 5). The highest wax deposition was found in PM-25 (5.11%), followed by KRANTI (4.82%), MASC-1 (4.63%) and PDL-1 (4.28%), which may hinder aphid adhesion and feeding. However, the lowest surface wax content was observed in TARAMERA (3.10%), followed by EC-399301 (3.20%) and SHIVANI (3.26%).

Plant height

Plant height among the germplasm ($F_{19,38}$ =234.910, P < 0.001) ranged between 91.40 and 257.00 cm (Table 5). The minimum Plant height was observed in TARAMERA (91.40 cm) followed by SHIVANI (98.80 cm), LACMA-SP523N5 (110.60 cm), MASC-1 (116.00 cm) and JM-3 (126.80 cm). Plant height may act as a physical barrier against aphid infestation. The maximum plant height was recorded in KRANTI (257.00 cm), followed by PDL-1 (255.33 cm) and RH-725 (241.00 cm).

Main shoot length

The main shoot length varied significantly ($F_{19,38}$ =43.440, P < 0.001), ranging between 46.60 cm and 96.00 cm (Table 5). The minimum main shoot length was observed in EC-399301 (46.60 cm), followed by TARAMERA (68.50 cm), SHIVANI (69.00 cm), PM-29 (77.00 cm) and RADHIKA (78.00 cm). The main shoot length in this experiment does not act as a physical barrier against aphid infestation. The maximum plant height was recorded in PUSA TARAK (96.00 cm), followed by MASC-1 (92.00 cm), PUSA BAHAR (91.00 cm) and LACMA-SP523N5 (91.00 cm).

Biochemical characterization of B. juncea germplasm

Total chlorophyll content in leaves

The total chlorophyll content among different *B. juncea* germplasm varied significantly ($F_{19,38}$ =102.127, $P < 0.001$), ranging from 3.72 to 9.57 mg/g in the leaves of various Brassica germplasm (Table 6). The highest chlorophyll content was observed in PUSA TARAK (9.57 mg/g), followed by RH-725 (9.32 mg/g), PM-22 (8.79 mg/g), PDL-1 (8.53 mg/g), GIRIRAJ (7.93 mg/g), and PM-25 (7.92 mg/g), indicating better photosynthetic capacity. In contrast, the lowest values were recorded in BRIJRAJ (3.72 mg/g), followed by CS-60 (4.19 mg/g), RH-406 (4.65 mg/g), JM-3 (4.57 mg/g), CS-56 (4.73 mg/g), and TARAMERA (4.90 mg/g), compared to another *B. juncea* germplasm.

Table 6
Evaluation of mustard germplasm based on aphid resistance-related biochemical parameters

Germplasm	Total Chlorophylls	Total Sugar	Total Phenols	Peroxidase Activity	Tannin Estimation	Total Glucosinolates	PAL	TAL
BRIJRAJ	3.72 ± 0.1^{a}	8.60 ± 0.1^{j}	2.82 ± 0.1^{c}	1076.03 ± 0.9^{cde}	2.37 ± 0.1^{h}	37.89 ± 0.8^{c}	1782.73 ± 13.8^{jk}	1419.53 ± 17.6^{k}
PDL-1	8.53 ± 0.1^{jk}	3.41 ± 0.1^{i}	4.22 ± 0.1^{fg}	1035.90 ± 1.7^{bcd}	3.10 ± 0.1^{l}	82.40 ± 1.6^{gh}	1276.17 ± 9.5^{e}	1366.50 ± 20.5^{jk}
RH-725	9.32 ± 0.1^{kl}	14.20 ± 0.1^{cde}	3.12 ± 0.1^{d}	777.17 ± 0.9^{a}	1.54 ± 0.1^{f}	24.21 ± 0.4^{a}	1042.47 ± 19.5^{c}	1221.70 ± 12.1^{c}
LACMA-SP523N5	5.40 ± 0.1^{cde}	12.12 ± 0.1^{h}	3.12 ± 0.1^{c}	1017.43 ± 1.2^{bc}	1.10 ± 0.1^{e}	26.67 ± 0.2^{a}	807.43 ± 9.0^{b}	1050.77 ± 9.6^{gh}
GIRIRAJ	7.93 ± 0.3^{ij}	10.56 ± 0.1^{m}	1.54 ± 0.1^{a}	986.17 ± 1.9^{b}	0.43 ± 0.1^{bc}	56.00 ± 1.1^{e}	1154.30 ± 20.3^{d}	774.70 ± 12.1^{i}
PUSA TARAK	9.57 ± 0.1^{l}	11.10 ± 0.1^{k}	2.35 ± 0.1^{b}	1120.43 ± 3.1^{e}	0.15 ± 0.1^{a}	27.80 ± 0.9^{b}	1160.07 ± 2.2^{d}	559.17 ± 7.4^{a}
PM-29	7.25 ± 0.3^{hi}	8.67 ± 0.2^{q}	2.81 ± 0.1^{c}	1123.47 ± 1.6^{e}	0.50 ± 0.1^{bcd}	43.92 ± 1.1^{d}	1274.63 ± 5.8^{e}	666.43 ± 7.9^{b}
PM-22	8.79 ± 0.3^{jkl}	9.79 ± 0.1^{p}	2.77 ± 0.1^{c}	1075.80 ± 1.7^{cde}	0.14 ± 0.1^{a}	38.40 ± 1.0^{cd}	1460.80 ± 17.7^{fg}	778.53 ± 7.7^{c}
EC-399301	5.40 ± 0.2^{cde}	6.70 ± 0.1^{o}	2.52 ± 0.1^{e}	1107.80 ± 4.5^{de}	0.16 ± 0.1^{a}	51.27 ± 1.0^{e}	1679.73 ± 19.3^{hi}	884.63 ± 6.0^{de}
SHIVANI	5.74 ± 0.2^{def}	4.65 ± 0.1^{n}	4.90 ± 0.1^{h}	1075.03 ± 0.8^{cde}	1.68 ± 0.1^{bcd}	63.67 ± 0.8^{f}	1888.03 ± 4.1^{l}	994.10 ± 10.5^{fg}
KRANTI	6.29 ± 0.1^{efg}	1.40 ± 0.1^{e}	6.44 ± 0.1^{j}	1399.53 ± 59.3^{g}	3.57 ± 0.1^{h}	103.60 ± 2.1^{jk}	1998.80 ± 39.7^{m}	1619.80 ± 14.6^{l}
PM-25	7.92 ± 0.1^{ij}	1.66 ± 0.1^{e}	5.16 ± 0.1^{h}	1125.13 ± 2.0^{e}	2.77 ± 0.1^{j}	108.17 ± 2.0^{kl}	551.57 ± 11.5^{a}	508.83 ± 21.2^{a}
JM-3	4.57 ± 0.1^{abc}	7.85 ± 0.1^{f}	2.82 ± 0.1^{ef}	1082.63 ± 0.9^{cde}	0.30 ± 0.1^{b}	13.70 ± 0.2^{a}	1757.37 ± 32.4^{ij}	1294.03 ± 20.0^{ij}
CS-56	4.73 ± 0.1^{bc}	6.68 ± 0.1^{de}	3.14 ± 0.1^{i}	1117.13 ± 1.8^{e}	1.70 ± 0.1^{ij}	36.10 ± 1.4^{ij}	1650.80 ± 27.1^{h}	922.60 ± 10.6^{ef}
MASC-1	6.87 ± 0.1^{gh}	5.86 ± 0.1^{ab}	4.30 ± 0.1^{d}	1210.53 ± 1.2^{f}	2.13 ± 0.1^{g}	86.00 ± 1.6^{h}	1867.90 ± 32.4^{kl}	797.13 ± 54.3^{cd}
RH-406	4.65 ± 0.3^{abc}	9.24 ± 0.1^{l}	4.87 ± 0.1^{h}	1089.97 ± 4.0^{de}	0.65 ± 0.1^{d}	34.20 ± 1.5^{jkl}	1529.73 ± 16.3^{g}	1096.40 ± 6.3^{h}
TARAMERA	4.90 ± 0.1^{bcd}	17.62 ± 0.1^{bcd}	2.21 ± 0.1^{g}	1107.04 ± 4.7^{de}	3.10 ± 0.1	38.36 ± 1.6^{i}	1407.73 ± 13.8^{f}	1210.44 ± 4.3^{i}
RADHIKA	5.69 ± 0.4^{de}	6.20 ± 0.1^{g}	4.20 ± 0.1^{d}	1037.13 ± 3.7^{bcd}	1.75 ± 0.1^{cd}	92.42 ± 1.6^{l}	1231.03 ± 13.2^{de}	1322.40 ± 6.7^{j}
CS-60	4.19 ± 0.2^{ab}	5.47 ± 0.1^{bc}	4.78 ± 0.1^{h}	1067.77 ± 2.1^{cde}	2.14 ± 0.1^{k}	81.00 ± 1.5^{gh}	1042.37 ± 4.4^{c}	1441.63 ± 6.0^{k}
PUSA BAHAR	6.68 ± 0.1^{fgh}	4.14 ± 0.1^{a}	3.85 ± 0.1^{c}	1120.00 ± 4.5^{e}	1.52 ± 0.1^{i}	75.65 ± 0.8^{g}	974.53 ± 7.1^{c}	1540.27 ± 5.6^{l}
F- value	102.127	4481.426	681.814	68.361	1839.229	696.172	430.809	381.318
LSD (P = 0.05)	0.513	0.222	0.137	38.715	0.107	3.646	54.238	48.564
P- value (0.05)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

PAL Phenylalanine ammonia lyase activity, *TAL* Tyrosine ammonia lyase activity.

Total sugar content in leaves

The total sugar content of different germplasm was observed ($F_{19,38}$ =4481.426, P < 0.001) to range from 1.40 to 17.62 mg/g in the leaves of various *Brassica* germplasm (Table 6). The highest sugar accumulation was recorded in TARAMERA (17.62 mg/g), followed by RH-725 (14.20 mg/g), LACMA-SP523N5 (12.12 mg/g), PUSA TARAK (11.10 mg/g), and GIRIRAJ (10.56 mg/g). The lowest sugar content was found in KRANTI (1.40 mg/g), followed by PM-25 (1.66 mg/g), PDL-1 (3.41 mg/g), PUSA BAHAR (4.14 mg/g), SHIVANI (4.65 mg/g), RADHIKA (6.20 mg/g), and CS-56 (6.68 mg/g).

Total phenols content in leaves

The data on the total phenols content showed significant variation ($F_{19,38}$ =681.814, P < 0.001), observed to be in the range from 1.54 to 6.44 mg/g in the leaves of different Brassica germplasm (Table 6). The amount of total phenols was observed to be maximum in KRANTI (6.44 mg/g) and PM-25 (5.16 mg/g), followed by SHIVANI (4.90 mg/g), CS-60 (4.78 mg/g) and RADHIKA (4.20 mg/g), which are known to enhance insect resistance. While the minimum total phenol content was found in GIRIRAJ (1.5 mg/g), followed by TARAMERA (2.21 mg/g), PUSA TARAK (2.35 mg/g), EC-399301 (2.52 mg/g) and JM-3 (2.82 mg/g) as compared to another *B. juncea* germplasm.

Peroxidase activity in leaves

The data of Peroxidase activity among the germplasm ($F_{19,38}$ =68.361, P < 0.001) ranged between 777.17 and 1399.53 U/mg in the leaves of different *Brassica* germplasm (Table 6). The maximum peroxidase activity was found in KRANTI (1399.53 U/mg), followed by MASC-1 (1210.53 U/mg). While the lowest peroxidase activity was observed in RH-725 (777.17 U/mg) and GIRIRAJ (986.17 U/mg).

Tannin content in leaves

Tannin quantity varied from 0.14 to 3.57 ($F_{19,38}$ =1839.229, P < 0.001) mg/g across Brassica germplasm in leaves (Table 6). The highest tannin content levels were recorded in KRANTI (3.57 mg/g), followed by PDL-1 (3.10 mg/g), TARAMERA (3.10 mg/g), and PM-25 (2.77 mg/g). The lowest tannin concentration was reported in LACMA-SP523N5 (0.10 mg/g) and PM-22 (0.14 mg/g), followed by PUSA TARAK (0.15 mg/g) and EC-399301 (0.16 mg/g).

Total glucosinolate content in leaves

The total glucosinolate content data ($F_{19,38}$ =696.171, *0.001)* ranged from 13.70 to 108.17 µmol/g in the leaves of various *Brassica* germplasm (Table 6). The highest glucosinolate levels were found in PM-25 (108.17 µmol/g), followed by KRANTI (103.60 µmol/g). RADHIKA (92.42 µmol/g), MASC-1 (86.00 µmol/g), and PDL-1 (82.40 µmol/g), suggesting potential aphid resistance. Conversely, the lowest total glucosinolate content was observed in JM-3 (13.70 µmol/g), with lower levels also seen in RH-725 (24.21 µmol/g), LACMA-SP523N5 (26.67 µmol/g), and PUSA TARAK (27.80 µmol/g).

Total Phenylalanine ammonia-lyase (PAL) activity in leaves

PAL activity among the *B. juncea* germplasm ranged ($F_{19,38}$ =430.809, P < 0.001) from 551.57 to 1998.80 u/ml in the leaves of different *Brassica* germplasm (Table 6). The highest PAL activity was recorded in KRANTI (1998.80 u/ml), followed by SHIVANI (1888.03 u/ml), MASC-1 (1867.90 u/ml), JM-3 (1757.37 u/ml) and BRIJRAJ (1782.73 u/ml), indicating stronger phenolic biosynthesis pathways and potential resistance. The lowest activity was observed in PM-25 (551.57 u/ml), LACMA-SP523N5 (807.43 u/ml) and PUSA BAHAR (974.53 u/ml).

Total Tyrosine ammonia-lyase (TAL) activity in leaves

TAL activity in the leaves of different germplasm ($F_{19,38}$ =381.318, P < 0.001) varied from 508.83 to 1619.80 u/ml in the leaves of different *Brassica* germplasm (Table 6). The highest TAL activity was observed in KRANTI (1619.80 u/ml), followed by PUSA BAHAR (1540.27 u/ml), CS-60 (1441.63 u/ml), BRIJRAJ (1419.53 u/ml), PDL-1 (1366.50 u/ml), and RADHIKA (1322.40 u/ml), suggesting efficient biosynthesis of defense metabolites. The lowest TAL activity was found in PM-25 (508.83 u/ml), followed by PUSA TARAK (559.17 u/ml), PM-29 (666.43 u/ml) and GIRIRAJ (774.70 u/ml).

Correlation between Aphid Population and Biochemical and Morphological Parameters

The correlation matrix presented in Table 7 gave us a clear picture of the relationships among different indices of aphid infestation, biochemical characteristics and morphological features. Aphid population (AP) showed a strong, positive and highly significant correlation with aphid population index (API) (r = 0.847***; P < 0.001) and aphid resistance index (ARI) (r = 0.766***; P < 0.001). These findings indicated a high consistency among all aphid infestation-related indices. The aphid damage index (ADI) was also positively correlated and highly significant with AP (r = 0.648**; P < 0.01) and demonstrated the direct proportion of aphid population and severity of damage. In case of biochemical parameters, total sugar content (TS) was observed to be in a highly significant positive relation with aphid population (r = 0.870***; P < 0.001), which may be due to enhanced nutrient suitability of the plant. Whereas total phenols (TP) (r = -0.580**; P < 0 .01) showed a negative significant association which may be interpreted as its role in inhibiting aphid colonies, and total glucosinolates (TG) also showed a negative correlation (r = -0.523*; P < 0.05) with aphid population, which reflects the potential defensive secondary metabolite. Total chlorophyll content (TC) showed a weak and non-significant negative association (r = -0.170) with aphid population, indicating low impact on the aphid population. Tannin content (TN) showed a negligible non-significant correlation (r = -0.016), and there may be a little direct role in resistance. Enzymatic activities like ascorbate peroxidase (AP) (r = -0.090), phenylalanine ammonia lyase (PAL) (r = -0.059) and tyrosine ammonia lyase (TAL) (r = -0.202) were also showing non-significant negative associations, and their contribution to the defence mechanism may not be constitutive. Morphological parameters: stem diameter (SD) was positively but non-significantly correlated with aphid population (r = 0.377), and surface wax (SW) was

found to have a significant negative correlation with aphid population (r = -0.570**; *P* < 0.01), which may serve as a physical barrier against aphid feeding. Plant height (PH) (r = 0.142) and main shoot length (MSL) (r = -0.192) showed a non-significant relation with aphid population.

Table 7
Correlation matrix among biochemical, morphological and resistance traits in mustard germplasm under natural infestation con

Parameters	AP	API	ADI	ARI	TC	TS	TP	TN	TG	PA	PAL	TAL	SD
AP	1.000	0.847***	0.648**	0.766***	-0.170	0.870***	-0.580**	-0.016	-0.523*	-0.285	-0.020	-0.017	0.377
API		1.000	0.792***	0.922***	-0.086	0.930***	-0.738***	-0.354	-0.833***	-0.433	0.049	-0.205	0.424
ADI			1.000	0.967***	0.252	0.850***	-0.736***	-0.379	-0.593**	-0.464*	-0.143	-0.321	0.305
ARI				1.000	0.124	0.929***	-0.776***	-0.389	-0.725***	-0.476*	-0.071	-0.290	0.371
TC					1.000	0.047	-0.134	-0.157	0.090	-0.232	-0.381	-0.449*	0.153
TS						1.000	-0.720***	-0.329	-0.757***	-0.521*	-0.137	-0.158	0.300
TP							1.000	0.579**	0.703***	0.470*	0.120	0.349	-0.135
TN								1.000	0.618**	0.313	0.052	0.481*	0.125
TG									1.000	0.454*	-0.094	0.161	-0.272
PA										1.000	0.464*	0.062	-0.036
PAL											1.000	0.221	0.037
TAL												1.000	-0.346
SD													1.000
SW													
PH													
MSL													

****, **, * Correlation coefficients significant at P = 0.001, 0.01, 0.05 level, respectively*

AP Aphid population, *API* Aphid population index, *ADI* Aphid damage index, *ARI* Aphid resistance Index, *TCC* Total Chlorophyll Content, *TS* Total Sugar Estima *TN* Tannin Estimation, *TG* Total glucosinolates, *PA* Ascorbate peroxidase activity, *PAL* Phenylalanine ammonia lyase activity, *TAL* Tyrosine ammonia lyase ac Surface wax, *PH* Plant Height, *MSL* Main Shoot Length

Using PCA, it was possible to identify the correlation between the various morpho-biochemical characters and parameters of aphid infestation of mustard germplasm for *L. erysimi* preference. Four principal components (PCs) were derived, and four components (PC1, PC2, PC3, and PC4) had eigenvalues greater than 1.0, together explaining most of the total variation, as shown in Tables 8 & 9. Figure 1 displays a wide range of morphological and biochemical parameters. The first principal component (PC1) explained the greatest variance and accounted for 45.893% of the total variation with an eigenvalue of 7.343. This component was dominated by parameters of aphid infestation, viz., aphid population (AP), aphid population index (API), aphid damage index (ADI) and aphid resistance index (ARI), as well as important biochemical characters: total sugar (TS), total phenols (TP), total glucosinolates (TG) and ascorbate peroxidase activity (PA) and morphological trait surface wax (SW). The co-occurrence of both infestation indices and biochemical characters indicates a combined axis of aphid pressure and plant biochemical defense in PC1, thus suggesting that both these features play a vital role in germplasm selection by the aphids. The second principal component (PC2) explained 14.426% of the total variation with eigenvalue 2.308 and a cumulative variation of 60.319%. This component was dominated by enzymatic traits like phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and total chlorophyll content (TC). All these characters occur together, which suggests that PC2 is primarily a physiological and enzymatic response axis that might be linked with induced defence and the metabolic state of the plant. The third component (PC3) was 9.876% with an eigenvalue of 1.580 and a cumulative variation of 70.194%. This component was strongly correlated with morphological characters, stem diameter (SD) and plant height (PH), which represent the structural or growth-related features influencing aphid colonisation and development indirectly. The fourth principal component (PC4) explained 8.527% of total variation with eigenvalue 1.364 and a cumulative variation of 78.722%. This component was dominated by tannin content (TN) and main shoot length (MSL), indicating a secondary defence and growth interaction axis where both biochemical defence components and growth features contribute equally (Tables 8 & 9).

Table 8
Principal components (PCs) with Eigen values and variances in germplasm preference by *L. erysimi* in mustard

PCs	Variables	Eigen value	% Variance	Cumulative % of Variance
1	AP, API, ADI, ARI, TS, TP, TG, PA, SW	7.343	45.893	45.893
2	PAL, TAL, TC	2.308	14.426	60.319
3	SD, PH	1.580	9.876	70.194
4	TN, MSL	1.364	8.527	78.722

AP Aphid population, *API* Aphid population index, *ADI* Aphid damage index, *ARI* Aphid resistance Index, *TCC* Total Chlorophyll Content, *TS* Total Sugar Estimation, *TP* Total Phenols Estimation, *TN* Tannin Estimation, *TG* Total glucosinolates, *PA* Ascorbate peroxidase activity, *PAL* Phenylalanine ammonia lyase activity, *TAL* Tyrosine ammonia lyase activity, *SD* Stem Diameter, *SW* Surface wax, *PH* Plant Height, *MSL* Main Shoot Length

Cluster analysis of *B. juncea* germplasm revealed distinct groupings based on plant defense responses to *L. erysimi* infestation. The dendrogram (Fig. 2) grouped the germplasm into three major clusters (C1, C2, C3 and C4), indicating varying levels of similarity in defence-related variability, such as aphid population buildup, biochemical constituents, or resistance traits. Cluster C1 (green branch), with the largest number of germplasm (GIRIRAJ, RH-725, LACMA-SP523N5, PM-22, PM-29, PUSA TARAK, BRIJRAJ, SHIVANI, RH-406 and EC-399301), pointed to a broad group with homogeneous responses, susceptible as seen in diverse *B. juncea* screenings. Cluster C2 (Orange branch) comprised PM-25, KRANTI and PDL-1, likely sharing possibly Moderately resistant defence mechanisms against the aphid. Cluster C3 (blue branch) included germplasm PUSA BAHAR, MASC-1, RADHIKA, CS-60, JM-3 and CS-56, suggesting these exhibited closely similar defense profiles, potentially tolerant mechanisms against the aphid. The Cluster C4 branch, represented by the blue branch, included the germplasm TARAMERA, likely sharing high susceptibility to aphids.

Host preference by L. erysimi diverse B. juncea Germplasm

The preference index for infestation showed significant differences between the susceptible check variety BRIJRAJ and the other test germplasm. Across both seasons (2023-24 and 2024-25), the varieties LACMA-SP523N5 (1.18 and 1.19), PM-29 (1.22 and 1.22), and TARAMERA (1.13 and 1.23) were classified as most preferred (Fig. 3). In contrast, the remaining germplasm PDL-1 (0.24 and 0.26), KARNTI (0.22 and 0.22), PM-25 (0.21 and 0.23), CS-60 (0.53 and 0.54), and PUSA BAHAR (0.55 and 0.54) were categorised as non-preferred by *L. erysimi*. The index of preference for infestation showed remarkable disparity among the susceptible check variety BRIJRAJ and the other test germplasm. During both the years (2023-24 and 2024-25) the varieties LACMA-SP523N5 (1.18 and 1.19), PM-29 (1.22 and 1.22), and TARAMERA (1.13 and 1.23) were highly preferred (Fig. 3). The values of the index of preference over these two years were almost identical, which signifies these germplasm had exhibited almost similar infestation patterns irrespective of seasons. The moderately high value in the above entries signified that these entries were found highly susceptible to a high rate of infestation.

The other germplasm, like PDL-1 (0.24 and 0.26), KARNTI (0.22 and 0.22), PM-25 (0.21 and 0.23), CS-60 (0.53 and 0.54) and PUSA BAHAR (0.55 and 0.54) were found to be non-preferred by *L. erysimi*. These varieties exhibited a comparatively lesser index of preference in both years, which shows they were consistently found to be non-preferred and less infested by aphid. Also, the smaller difference between the two years proved that there is a stable trend of lesser infestation on these germplasm. Overall, the difference in the germplasm shows a very clear distinction of infestation preference level on different entries.

Discussion

The formation of antagonistic metabolites in plants through morphological, chemical, and biological alterations is the most crucial barrier against insect attack. Since these morphological and biochemical molecules participate in the plant's defense mechanisms against insect herbivory, most people view them as protective substances[42, 43]. In the present study, we investigated morpho-biochemical determinants of host preferences and population dynamics in *L. erysimi* across diverse *brassica juncea* germplasm and revealed significant variation in aphid infestation parameters, including total aphid population, aphid multiplication rate, and daily multiplication rate among different *B. juncea* germplasm under both natural and artificial conditions during 2023-24 and 2024-25. The higher aphid population observed in susceptible germplasm such as TARAMERA and LACMA-SP523N5 may be attributed to favourable morphological and biochemical traits that enhance host suitability, whereas resistant germplasm like KRANTI, PM-25, and PDL-1 exhibited lower infestation levels, suggesting the involvement of inherent defense mechanisms. These findings are in agreement with earlier report[22] which also documented lower aphid infestation levels in PM-25 and KRANTI, thereby supporting the consistent expression of resistance in these genotypes across different studies and environmental conditions. Further support the concept that resistance in *Brassica* is a complex trait governed by an interplay of morphological, anatomical, and biochemical factors[41]. Morphological traits such as stem diameter exhibited noticeable variation among the germplasm, with thicker stems recorded in TARAMERA, SHIVANI, and PUSA TARAK. Although primarily structural, increased stem thickness may provide mechanical resistance by restricting stylet penetration and delaying aphid access to phloem tissues. This is consistent with previous findings indicating that structural barriers can influence aphid feeding efficiency[22]. Similarly, surface wax content emerged as an important defensive trait, with higher wax deposition observed in KRANTI and PDL-1, which corresponded with reduced aphid infestation. Epicuticular wax acts as a physical barrier that interferes with aphid landing, probing, and feeding behaviour, thereby reducing host acceptance. These results corroborate earlier studies[23, 41] highlighting the protective role of surface wax in *B. species*. Plant architectural traits also contributed variably to aphid resistance.

Increased plant height was associated with reduced aphid colonisation, possibly due to physical hindrance and altered host accessibility, thereby influencing insect behaviour. This observation aligns with recent findings[20] emphasising the role of plant architecture in pest dynamics. However, despite significant variation, main shoot length did not exhibit a direct association with aphid resistance, suggesting that elongation alone does not confer structural defense.

Similar observations have been reported previously, indicating that resistance is governed by a combination of multiple traits rather than individual morphological parameters[22, 20]. Biochemical constituents played a more decisive role in determining host plant resistance. Total chlorophyll content varied significantly among germplasm, with higher levels recorded in relatively resistant germplasm such as PUSA TARAK and RH-725. Although chlorophyll does not directly influence aphid resistance, increased photosynthetic efficiency may enhance plant vigour and indirectly strengthen defense signalling pathways. Comparable trends have been reported[4, 18], who suggested a moderate association between chlorophyll content and delayed aphid colonisation. In contrast, total sugar content exhibited a strong positive association with aphid population, with higher concentrations recorded in susceptible germplasm such as TARAMIRA, RH-725, and LACMA-SP523N5. Elevated sugar levels enhance the nutritional quality and palatability of phloem sap, thereby promoting aphid feeding and reproduction. This relationship is well supported by earlier studies[18, 23, 41], confirming that sugar-rich germplasm is more prone to aphid infestation.

Secondary metabolites, particularly phenols, tannins, and glucosinolates, played a critical role in imparting resistance[20, 41]. Higher phenolic content in resistant germplasm such as KRANTI and PM-25 suggests their role as antifeedants, disrupting aphid digestion and reducing host acceptance. The significant negative correlation between phenols and aphid population observed in this study is in agreement with previous findings by[24– 26]. Similarly, glucosinolates were found in higher concentrations in resistant germplasm, where their hydrolysis products, particularly isothiocyanates, exert toxic effects on aphids. These findings align with earlier reports[18, 27, 28, 41] emphasizing their role as key biochemical markers of resistance. However, the dual role of glucosinolates in attracting specialist pests such as *L. erysimi*, as suggested by[29] highlights the complexity of plant-insect interactions. Enzymatic defense mechanisms further supported resistance responses. Elevated peroxidase activity observed in resistant germplasm indicates enhanced oxidative defense through lignification and detoxification of reactive oxygen species during aphid attack. These findings are consistent with earlier reports[22,30], which similarly demonstrated that increased peroxidase activity is closely associated with reduced aphid colonization and improved resistance expression in Brassica genotypes.

Similarly, increased activities of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) in resistant germplasm suggest activation of the phenylpropanoid pathway, leading to the synthesis of defensive secondary metabolites. These results are in close agreement with reports by[22,24,31] confirming the importance of these enzymes in plant defense regulation. Correlation analysis of the data revealed that the mean aphid population and associated indices have a strong relationship with a few biochemical and morphological characteristics of the *B. juncea* germplasm. The strong and positive association among aphid population, aphid population index, aphid damage index, and aphid resistance index proves the efficacy of these parameters for screening the germplasm against aphid infestation. The parameters proved to be suitable for measuring the resistance of the variety through the index approach and visual scoring, which was also suggested by[22]. With respect to biochemical traits, there is a significant and positive correlation between aphid population and total sugar content. It is inferred that plants rich in total sugar would serve better in terms of aphid reproduction rate because the high concentration of sugars increases the feeding attractiveness of the plant and hence its palatability of the phloem sap of *L. erysimi*. These results corroborate the findings[31], who reported a significant positive correlation between total sugar content and aphid population. Significant negative correlation between aphid population and total phenol and total glucosinolates strongly supported the role of the two secondary metabolites in deterring aphid infestation. Glucosinolates are considered a major allelochemical in Brassicaceae that when hydrolyzed produce toxic isothiocyanates and prevent aphids from settling and growing on them, a finding reported[24]. Positive and significant correlation among phenol, tannin and glucosinolates shows the interaction and interdependence among these characters to give a unified chemical defense system in *B. juncea* germplasm. In the case of morphology, there is a significant and negative correlation between surface wax content and aphid population. It indicates that a higher content of surface wax in plants would impede the mobility, feeding and settling of aphids and thus provide resistance to the variety. A similar finding has been reported earlier, where epicuticular wax could impede probing of the aphids and their stylet penetration[23]. No significant correlation between the aphid population and other morphological traits like plant height, stem diameter and main shoot length may be attributed to the lack of structural immunity against the insect pest.

The analysis of the main component provided further evidence to support the above findings. The first principal component (PC1), with the largest variation, consists of aphid infestation characters, sugars, phenols, glucosinolates (biochemical characters) and surface wax (morphological character). This shows that both the nutritional value of the plant and biochemical traits play an important role in affecting the aphid infestation, suggesting them to be the main factors affecting preference and resistance. The second principal component (PC2) consists of biochemical characters such as phenylalanine ammonia lyase, tyrosine ammonia lyase, and chlorophyll content and thus shows the role of these factors in contributing towards the resistance. The rest of the components consist of morphological characters and biochemical characters with lesser contributions and are therefore considered to be of relatively minor importance in resistance development. In sum, correlation and principal component analysis clearly indicate that resistance to *the Brassica* aphid is a complicated trait, involving both biochemical constituents, like sugars, phenols and glucosinolates, with secondary importance from some morphological features like surface wax. All these characters, in agreement with the previous findings, have reported that the biochemical and morphological traits play a crucial role in imparting resistance to aphids[18]. Cluster analysis complemented the above findings by grouping germplasm into distinct categories based on their defense profiles. Germplasm in Cluster C2 exhibited relatively higher resistance, likely due to favourable biochemical traits such as higher phenols and glucosinolates and lower sugar content. Cluster C3 represented intermediate resistance, possibly governed by specific enzymatic responses, while Cluster C1 comprised susceptible germplasm and Cluster C4 highly susceptible, characterised by lower defensive metabolites and higher nutritional suitability for aphids.

The aforementioned results were supported by cluster analysis in grouping germplasm under different categories according to their defence mechanisms and relative suitability for aphids to infest. In Cluster C1, we grouped susceptible germplasm having higher levels of aphid infestation and the highest rate of population development. Thus, this exhibited lower levels of defense mechanisms and higher suitability for aphids to infest in this category. In Cluster C2, we grouped the relatively higher resistant entries where aphid infestation and population were lower and their correlation with the best biochemical variables, i.e., highest phenolics and glucosinalates and lowest sugars, which are considered as non-preferential factors for aphid feed and multiplication. C3 had

moderate levels of aphid infestation and resistance, which shows a balanced interaction of the host defense system and aphid adaptation may be due to certain enzyme actions where multiplication of aphids is not as great as compared to other classes. Cluster C4 are highly susceptible entries which showed the highest level of aphid infestation and very highest rate of population multiplication and related indices, suggesting the very lowest level of defensive mechanism of this class and higher values of nutritional parameters for aphid feeding, survival and reproduction which makes them clearly separable from other classes.

Conclusion

This study thoroughly examined the biophysical and biochemical mechanisms that contribute to resistance in *Brassica juncea* germplasm against the mustard aphid (*L erysimi).* Notable genotypic variation was detected over two successive *Rabi* seasons, thereby validating the presence of stable and heritable resistance and preference characteristics. The germplasm varieties PM-25, KRANTI, and PDL-1 demonstrated consistently lower populations of aphids, reduced damage, and lower resistance indices under natural and control condition. In contrast, TARAMIRA and LACMA-SP523N5 were classified as highly susceptible to aphid infestation. The research unequivocally illustrated that resistance is a trait influenced by multiple factors, which are governed by the interplay of morphological and biochemical elements. The increased deposition of surface wax and advantageous stem traits played a significant role in physical deterrence. Concurrently, heightened concentrations of total phenols, glucosinolates, and tannins, along with enhanced activities of defense-related enzymes such as peroxidase, phenylalanine ammonia-lyase (PAL), and tyrosine ammonia-lyase (TAL), were closely linked to a decrease in aphid infestation. In contrast, a positive correlation was observed between elevated total sugar content and aphid population, indicating that enhanced nutritional suitability may facilitate pest proliferation. The correlation and PCA analyses provided additional validation for the robust association observed between defensive metabolites and parameters related to aphid resistance. Cluster analysis complemented the above findings by grouping germplasm into distinct categories based on their defense profiles. Notwithstanding these significant findings, it is essential to recognize certain limitations. The research was performed in a specific field setting at one geographical site, potentially constraining the generalizability of the findings to a wider environmental context. The biochemical analyses relied on bulk tissue measurements and did not investigate the molecular or gene expression-level mechanisms that contribute to resistance. Furthermore, the impact of environmental factors, including variations in microclimate and interactions with natural enemies, was not explicitly measured. The results offer significant insights for breeding initiatives focused on the development of aphid-resistant mustard cultivars. The incorporation of essential biochemical markers alongside morphological characteristics into selection methodologies has the potential to enhance host plant resistance. This approach may decrease dependence on chemical insecticides, thus fostering sustainable and environmentally friendly pest management practices within rapeseed-mustard production systems.

Declarations

Competing interests

The authors declare no competing interests.

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Author Contribution

HM: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing-Original draft; KRS: Conceptualization, Methodology, Investigation, Resources, Writing-review and editing, Validation, Supervision; SKS: Investigation, Writing-review and editing, Validation; UC, SVSR: Writing-review and editing, Validation; SKS and AP: Software, Visualization, Formal analysis. All authors reviewed the manuscript.

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Data Availability

All data generated or analyzed during this study are included in this published article.

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Figures

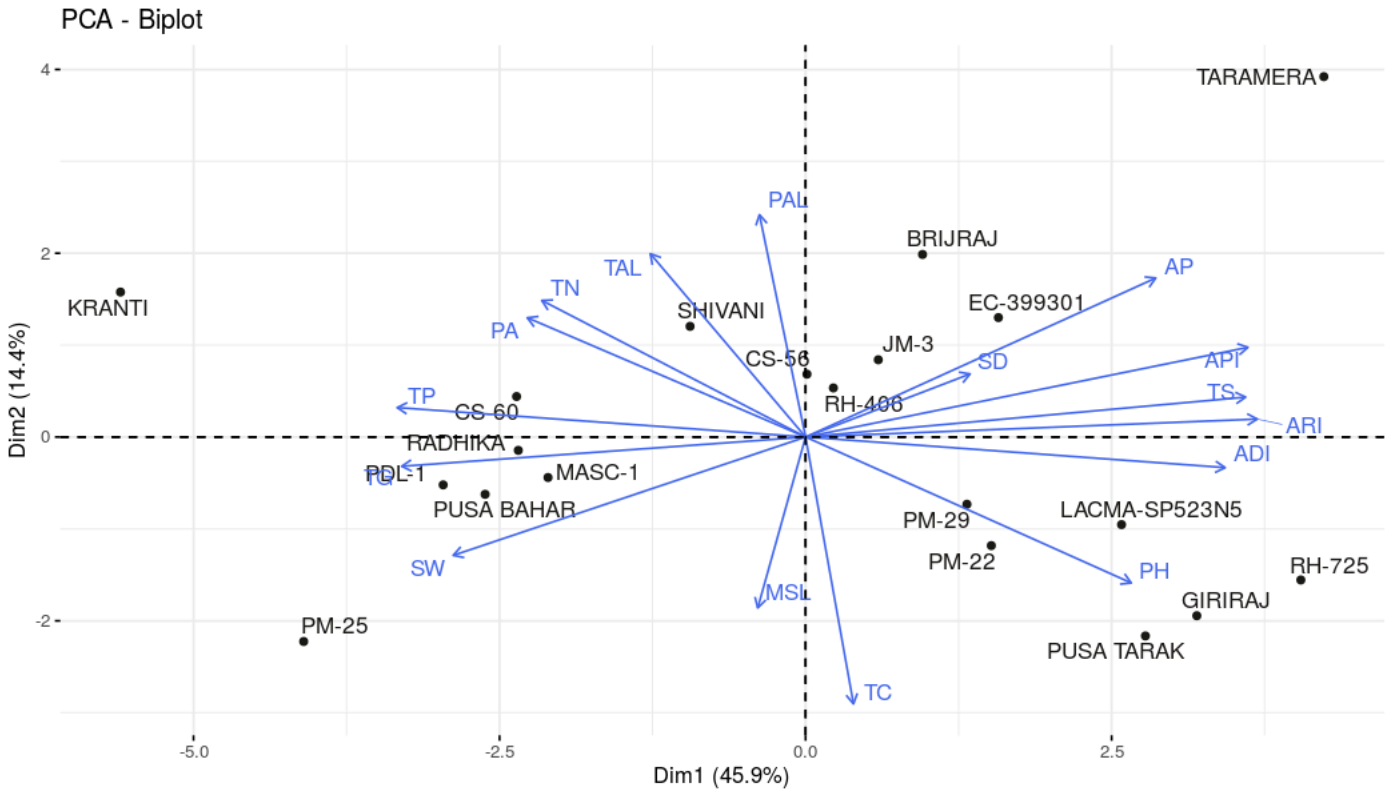


Figure 1

Principal component analysis (PCA) bi-plot of various morpho-biochemical constituents of mustard germplasm biochemical traits in the mustard germplasm preference by *L. erysimi* in mustard: a grouping of the variables into two principal components. *AP* Aphid population, *API* Aphid population index, *ADI* Aphid damage index, *ARI* Aphid resistance Index, *TCC* Total Chlorophyll Content, *TS* Total Sugar Estimation, *TP* Total Phenols Estimation, *TN* Tannin Estimation, *TG* Total glucosinolates, *PA* Ascorbate peroxidase activity, *PAL* Phenylalanine ammonia lyase activity, *TAL*Tyrosine ammonia lyase activity, *SD* Stem Diameter, *SW* Surface wax, *PH* Plant Height, *MSL* Main Shoot Length.

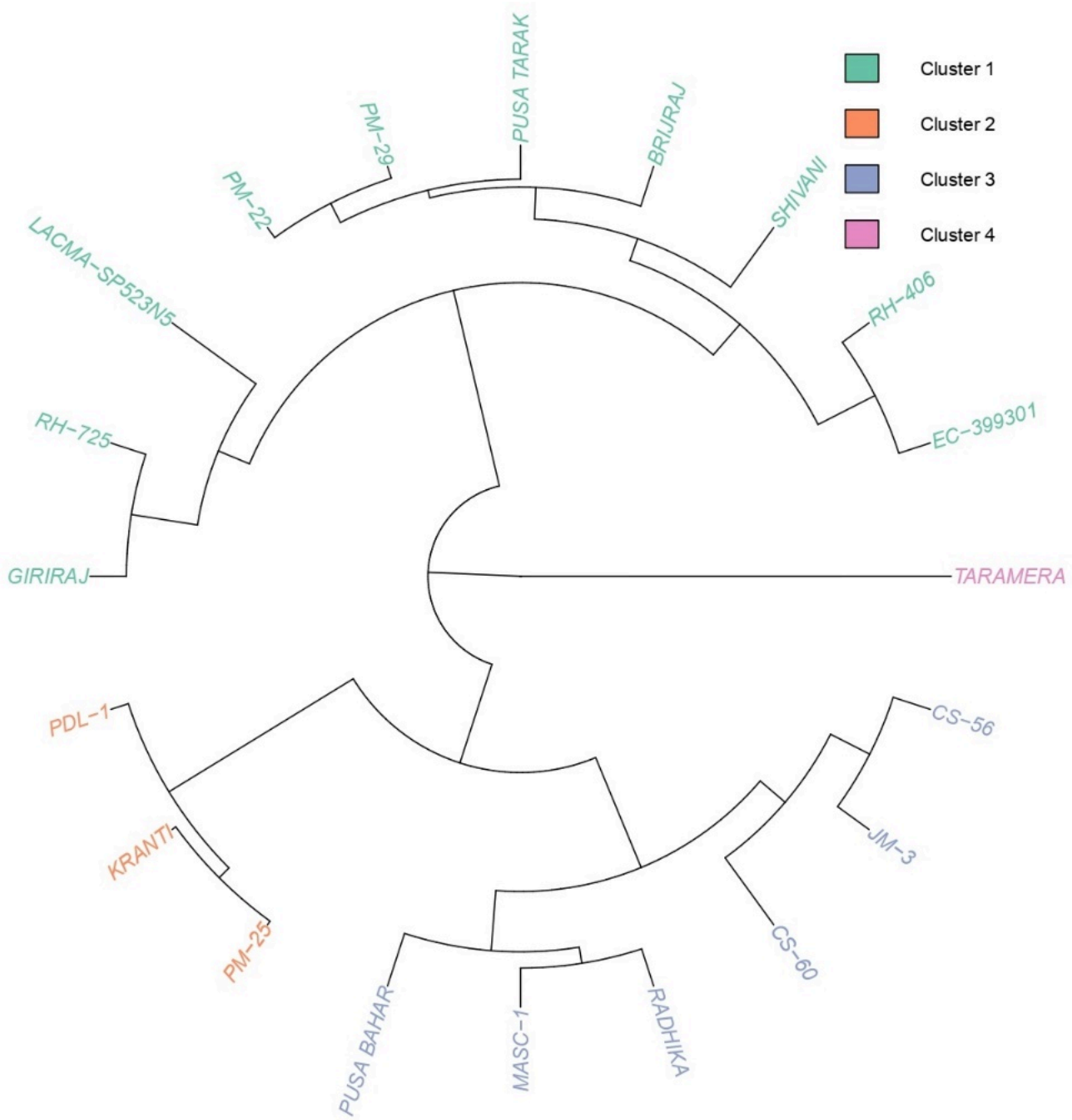


Figure 2

Radial cluster dendrogram of *B. juncea* germplasm based on variability in plant defense in response to *L. erysimi*. The dendrogram illustrates the grouping of *B. juncea* germplasm s into four distinct clusters. Each cluster represents a set of germplasm with similar characteristics in terms of their defense.

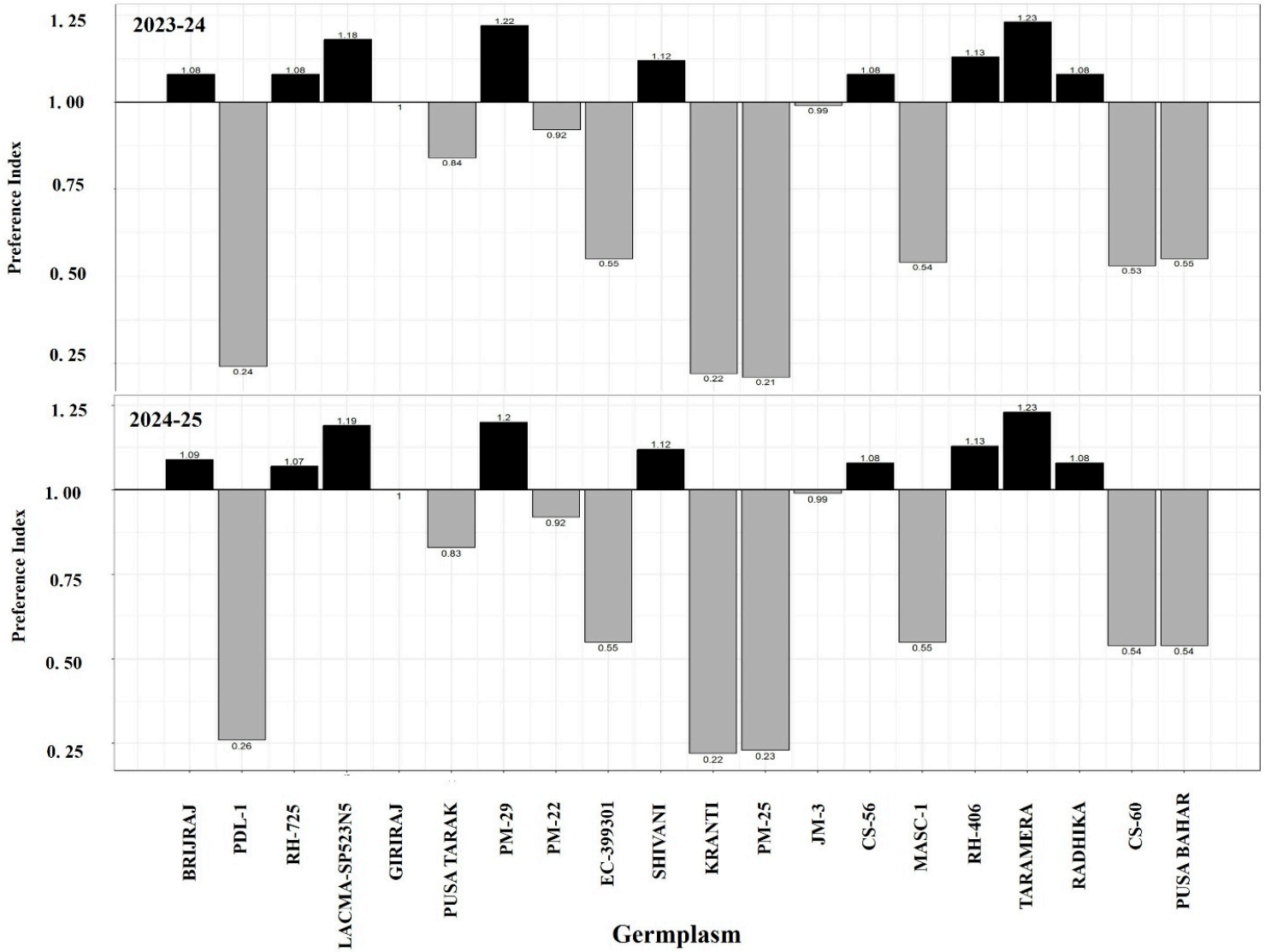


Figure 3

Preference index of feeding by *L. erysimi* on different mustard germplasm during *Rabi* 2023-24 and 2024-25