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Induced immune defense in *Brassica juncea* (L.) Czern. against *Lipaphis erysimi* (Kalt.) *via* altered photosynthetic and enzymatic levels

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Deviations in the biochemical composition and the formation of secondary metabolites have all been linked to the metabolic fluxes that underlie plant resistance. The current investigation was undertaken to know the induced defence against *Lipaphis erysimi* (Kalt.) *via* altered metabolic flux in *Brassica juncea* (L.) Czern. Pusa Mustard 30, RH 749, NRCHB 101, DRMR 150-35, RH 0406, Pusa Mustard 27 and Pusa Vijay had higher levels constitutive photosynthetic pigments, activity of constitutive enzymes and total glucosinolates, except in a few cases. However, due to infestation by the *L. erysimi*, the levels of photosynthetic pigments and total glucosinolates significantly decreased, while the activity of antioxidative enzymes and myrosinase increased. However, decrease in the photosynthetic pigments was significantly higher in NRCHB 101, RLC 3, DRMR 150-35, Chattisgarh Sarson and Pusa Double Zero Mustard 31, except in a few cases. Further, RH 725, Pusa Mustard 26, DRMR 150-35, NRCHB 101 and RH 0406 displayed significant enhancement in the antioxidative enzymes, except in a few cases. Total glucosinolate content reduced significantly in Pusa Mustard 26, DRMRIJ 16-38, NRCHB 101, DRMR 150-35 and RH 749, while the activity of myrosinase enhance notably in Pusa Vijay, Pusa Double Zero Mustard 31, Pusa Mustard 25 and NRCHB 101. Present study displayed phytochemicals defence against *L. erysimi* through shift in the levels of various pigments, enzymes and glucosinolate content. Cultivars RH 725, Pusa Mustard 26, DRMR 150-35, NRCHB 101 and RH 0406 showed significant variation the induced levels of various phytochemicals and enzymes, thus cane be used in *Brassica* breeding programme.

Keywords: Chlorophyll, Carotenoids, Enzymatic defence, Glucosinolate-myrosinase, Mustard aphid, Photosynthetic pigments

In India, mustard (*Brassica juncea*: Cruciferae) is grown in 8.06 million ha with 11.75 million tonnes of production and average productivity of 1458 kg/ha[1]. The yield and productivity of rapeseed-mustard are highly variable due to various biotic and abiotic stresses experienced across crop-growing agro-ecologies of India. Among the insect pests, mustard aphid, *Lipaphis erysimi* (Kalt.) (Aphididae: Hemiptera) is the major yield reducing factor in rapeseed-mustard causing awful yield losses depending upon the climatic conditions, intensity of population build up and crop growth stage[2,3]. However, with appropriate management practices, this aphid can be controlled by 10.2 to 61.1%[2]. The integrated pest management programme has prioritised varietal resistance among the many control strategies[4]. It is crucial that the existing pest management tactics offer efficient and cost-effective pest management short of having any negative environmental effects. The insect resistant plants have the unique advantage of providing inherent insect control in the crop, and could be the best alternative for the management of aphids. To gain detailed knowledge on the sources of insect resistance is the first stage in the development of insect resistant cultivars[5,6]. To develop genotypes resistant to aphids, it is crucial to have a complete understanding of host-plant interactions, including the metabolic fluxes that underlie plant resistance and those that aphids use to avoid plant defence[6-8].

Metabolic fluxes underlying plant resistance have been attributed to alteration in the biochemical composition, presence of physical properties, and biosynthesis of secondary metabolites[6,7,9,10]. Various biochemical constituents have been reported to play significant role in deciding the herbivory by aphids[6-11]. Glucosinolates, a group of isothiocyanates present in the *Brassicas* have been reported to play key role in regulating infestation, establishment and reproduction of aphids[6-8,12-14]. In order to hydrolyse the parent glucosinolates into physiologically active derivatives,

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the glucosinolate defence system also needs the assistance of another enzyme, myrosinase. Further, in plant defense against herbivores, reactive oxygen species (ROS) play a major role and act as secondary messenger for signaling various defense reaction pathways in plants[15,16]. They promote beneficial oxidation to generate energy and kill microbial invaders. But in excess, they cause pigment co-oxidation, lipid peroxidation, membrane destruction, protein denaturation, and DNA mutation[17]. Hence, plant itself develop important ROS scavenging mechanism[18], where antioxidative enzymes are the most important machineries in the scavenging system of ROS. Induced resistance in host plants is regulated by various antioxidative defense enzyme *viz*., ascorbate oxidase (AO), ascorbate peroxidase (APX), catalase, Phenylalanine Ammonia Lyase (PAL) and Tyrosine Ammonia Lyase (TAL) also play indirect task in host defence against insects[6,19,20]. Qualitative or quantitative alteration in enhanced activity of oxidative enzymes in response to herbivore attack is general phenomenon[21], and play major role in plant defense against insect pests[22]. Apart from defence molecules and antioxidative enzymes, plant pigments like carotenoids and chlorophylls are also recognised as active compounds that have important role in plant defence[23]. Further, the levels of these pigments under stress conditions alter according to mode of feeding by the herbivores and could influence the inherent defence capacity of the host plant.

Studies about the defence role of the antioxidative enzymes, glucosinolate-myrosinase activity and photosynthetic pigments in *B. juncea* cultivars against mustard aphids are scarce. Henceforth, the current investigation mainly focuses on the shift in the activity of same regulating enzymes and photosynthetic pigments in response to mustard aphid, *L. erysimi* damage in diverse *B. juncea* cultivars.

Material and Methods

Plant material

Twenty-three diverse *B. juncea* cultivars released for different agro-ecological zones of India, and are in seed chain and in great demand by the farmers were selected for the present studies. The chosen cultivars were differing greatly in physico-chemical properties. These were grown in 5 row plots of 5 m length, with 30 × 15 cm spacing in experimental plots of the Division of Entomology, Indian Agricultural Research Institute, New Delhi during the 2021-22 cropping season. All recommended agronomic practices (fertigation @ 80N:40P:40K as basal dose, thinning at 20 days after sowing, weeding and irrigation at 35 days after germination), except insecticidal application were followed to raise the crop. Each treatment plot was separated by proper distance and aphid inoculation was uniform across all the test cultivars for induced biochemical estimation. The 20 mixed aphids were inoculated at the time of siliquae formation and samples were collected after 48h for biochemical estimation. Ten randomly selected plants of each test cultivar were tagged for biochemical studies.

Estimation of different constitutive and induced levels of antioxidative enzymes, total glucosinolates, myrosinase and photosynthetic pigments in test *B. juncea* cultivars

After 48 h of exposure, the siliquae of aforesaid *B. juncea* cultivars from three plants each were collected from *L. erysimi* infested (to estimate induced biochemicals) and those plants free from aphid infestation (to estimate constitutive biochemicals). From the aphid infested samples, the aphids were removed with the help of camel hair brush before processing for biochemical analysis. The samples were collected in polythene zip bags separately and brought to the laboratory for the estimation of various enzymes (AO, APX, catalase, PAL and TAL). The fresh siliquae tissues were used for yjr estimation of total glucosinolates, myrosinase and photosynthetic pigments. Total glucosinolate content was estimated using standard method by Kraling *et al.*[24], and values obtained were expressed in µmol/g of tissue. Total glucosinolates content was calculated by putting the OD of each sample taken at 425 nm in the formula $y = 1.40 + 118.86 \times A_{425}$. For enzyme analysis (except myrosinase), two-gram tissues from siliquae of aforesaid test *B. juncea* cultivars were crushed in liquid nitrogen separately and added with 10 mL of 50 mM phosphate buffer (*p*H 7.8). The slurry was transferred to centrifuge tubes and centrifuged at 12000 rpm for 20 min at 4°C. The supernatant was collected and stored in 2.5 ml micro-centrifuge tubes at –20°C in the deep freezer to estimate aforesaid enzymes. Further, the activity of AO, APX, catalase, PAL, TAL and myrosinase were estimated as per the standard protocols[25-30]. The activity of AO, APX, catalase, PAL and TAL were recorded at 265, 290, 240, 290 and 290 nm, respectively, and expressed as U/mL of enzyme extract. The myrosinase activity was determined based on measurements of decomposition of sinigrin by following the decrease in absorbance of reaction mixture at 230 nm[30]. The chlorophyll-a, b and total, and total carotenoids were estimated using Dimethyl-Sulphoxide (DMSO) method [31]. There were three replications for each test cultivar and the biochemical constituent in a completely randomised design.

Statistical analysis

Data on different enzymes, total glucosinolates, and photosynthetic pigments were all subjected to one-way analysis of variance (ANOVA). Using the statistical programme SPSS version 16.0, the significance of differences in the test cultivars was examined using the F-test, and the treatment means were compared using the least significant differences at $P = 0.05$.

Results

Different photosynthetic pigments in siliquae of diverse *B. juncea* cultivars

Chlorophyll-A content in siliquae

The chlorophyll A content varied significantly in healthy ($F_{22, 46} = 82.51$; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22, 46} = 47.39$; $P < 0.001$) of *B. juncea* cultivars, and ranged from 2.7 to 6.0 and 1.2 to 4.4 µg/mL of tissue, respectively (Table 1). The constitutive chlorophyll A content was notably maximum in the siliquae of Pusa Mustard 30 and RH 749, while lower in the siliquae of Pusa Vijay and Pusa Mustard 26 as compared to other *B. juncea* cultivars (Table 1). However, the chlorophyll A content reduced considerably in the *L. erysimi* damaged siliquae of *B. juncea* cultivars. The per cent reduction in the chlorophyll A ($F_{22, 46} = 22.36$; $P < 0.001$) content was notably higher in the siliquae of NRCHB 101, Pusa Mustard 26, Chattisgarh Sarson, RLC 3 and Pusa Tarak, while lower in the siliquae of DRMRIJ 16-38, Radhika, Pusa Mustard 32, Pusa Mustard 28 and RH 725 (Suppl Fig. S1). [*All supplementary data are available only online along with the respective paper at the journal website* (*http://ijeb.res.in*) *as well as NOPR repository at http://nopr.res.in*].

Chlorophyll-B content in siliquae

The significant differences in chlorophyll B content were observed in healthy ($F_{22, 46} = 6.68$; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22, 46} = 19.20$; $P < 0.001$) of *B. juncea* cultivars. The chlorophyll B content in the healthy and damaged siliquae of *B. juncea* ranged from 1.3 to 4.9 and 0.6 to 3.5 µg/mL of tissue, respectively (Table 1). The constitutive chlorophyll B content was significantly highest in the

Table 1 — The constitutive and induced levels of various photosynthetic pigments in the siliquae of diverse *Brassica juncea* cultivars

Cultivars	Chlorophyll A Constitutive (mg/g)	Chlorophyll A Induced (mg/g)	Chlorophyll B Constitutive (µg/ml)	Chlorophyll B Induced (µg/ml)	Total chlorophyll Constitutive (µg/ml)	Total chlorophyll Induced (µg/ml)	Total carotenoids Constitutive (µg/ml)	Total carotenoids Induced (µg/ml)
RH 0761	3.4 ± 0.1	2.6 ± 0.1	3.5 ± 0.4	2.5 ± 0.4	6.9 ± 0.3	5.1 ± 0.4	1.1 ± 0.1	0.5 ± 0.1
RH 30	4.2 ± 0.0	2.6 ± 0.1	1.6 ± 0.0	0.8 ± 0.2	5.8 ± 0.0	3.5 ± 0.1	1.7 ± 0.0	1.4 ± 0.0
RLC 3	4.8 ± 0.1	2.3 ± 0.1	4.2 ± 0.1	1.2 ± 0.1	9.0 ± 0.0	3.5 ± 0.1	1.5 ± 0.2	0.6 ± 0.0
DRMIJ 31	4.4 ± 0.1	2.6 ± 0.2	1.7 ± 0.0	1.3 ± 0.2	6.1 ± 0.1	3.9 ± 0.1	1.6 ± 0.0	1.4 ± 0.1
DRMR 1165-40	5.1 ± 0.1	2.9 ± 0.0	1.3 ± 0.2	1.1 ± 0.1	6.3 ± 0.3	4.0 ± 0.0	2.2 ± 0.0	1.3 ± 0.0
NRCHB 101	5.4 ± 0.2	1.8 ± 0.1	3.7 ± 0.8	1.5 ± 0.1	9.1 ± 0.7	3.2 ± 0.1	2.1 ± 0.3	0.8 ± 0.0
Radhika	3.9 ± 0.1	3.4 ± 0.0	1.6 ± 0.0	1.0 ± 0.1	5.5 ± 0.1	4.4 ± 0.2	2.0 ± 0.0	1.3 ± 0.1
DRMR 150-35	4.6 ± 0.0	2.5 ± 0.1	4.5 ± 0.2	1.3 ± 0.1	9.0 ± 0.2	3.8 ± 0.1	2.2 ± 0.3	1.0 ± 0.1
Pusa Mustard 28	4.3 ± 0.0	3.5 ± 0.1	1.5 ± 0.0	1.1 ± 0.1	5.7 ± 0.0	4.5 ± 0.1	1.7 ± 0.0	1.2 ± 0.1
Pusa Tarak	4.8 ± 0.1	2.5 ± 0.1	1.5 ± 0.0	1.1 ± 0.2	6.4 ± 0.1	3.6 ± 0.3	2.1 ± 0.0	1.1 ± 0.0
Chattisgarh Sarson	3.2 ± 0.0	1.4 ± 0.4	3.9 ± 0.0	2.1 ± 0.3	7.1 ± 0.1	3.5 ± 0.1	1.0 ± 0.0	0.8 ± 0.0
RH 725	3.4 ± 0.0	2.7 ± 0.0	4.0 ± 0.0	2.2 ± 0.1	7.4 ± 0.1	4.9 ± 0.1	0.9 ± 0.1	0.2 ± 0.0
RH 0406	3.4 ± 0.3	2.3 ± 0.1	4.9 ± 1.6	2.2 ± 0.1	8.3 ± 1.4	4.5 ± 0.1	0.8 ± 0.4	0.2 ± 0.0
Pusa Vijay	2.7 ± 0.2	1.9 ± 0.0	4.8 ± 0.8	2.2 ± 0.2	7.5 ± 0.7	4.1 ± 0.2	1.0 ± 0.2	0.4 ± 0.1
RH 749	5.7 ± 0.0	3.8 ± 0.0	1.7 ± 0.0	1.3 ± 0.0	7.5 ± 0.0	5.0 ± 0.0	1.6 ± 0.1	1.1 ± 0.0
DRMRIJ 16-38	3.3 ± 0.1	2.9 ± 0.0	3.4 ± 0.8	1.3 ± 0.0	6.6 ± 0.7	4.2 ± 0.1	1.4 ± 0.2	1.0 ± 0.0
Pusa Mustard 32	4.0 ± 0.1	3.4 ± 0.1	4.1 ± 0.0	1.5 ± 0.0	8.0 ± 0.1	4.8 ± 0.1	1.5 ± 0.0	1.1 ± 0.0
Pusa Double Zero Mustard 31	4.2 ± 0.0	2.3 ± 0.1	1.4 ± 0.0	0.6 ± 0.1	5.6 ± 0.0	2.9 ± 0.1	1.6 ± 0.0	1.3 ± 0.0
Pusa Mustard 25	4.9 ± 0.0	3.7 ± 0.0	1.8 ± 0.0	0.9 ± 0.1	6.7 ± 0.0	4.6 ± 0.1	1.9 ± 0.0	1.3 ± 0.0
Pusa Mustard 26	2.7 ± 0.0	1.2 ± 0.0	4.2 ± 0.0	3.5 ± 0.0	6.9 ± 0.1	4.7 ± 0.0	1.2 ± 0.0	0.5 ± 0.0
Pusa Mustard 30	6.0 ± 0.1	4.4 ± 0.1	2.1 ± 0.1	1.6 ± 0.0	8.2 ± 0.1	6.0 ± 0.1	2.0 ± 0.0	1.4 ± 0.0
Pusa Mustard 27	3.5 ± 0.2	2.5 ± 0.0	3.2 ± 0.8	1.3 ± 0.1	6.7 ± 0.7	3.8 ± 0.1	1.7 ± 0.2	1.1 ± 0.0
RVM 1	4.1 ± 0.1	2.2 ± 0.1	4.0 ± 0.0	2.3 ± 0.1	8.1 ± 0.1	4.5 ± 0.1	1.0 ± 0.0	0.7 ± 0.0
F-probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (P = 0.05)	0.29	0.32	1.42	0.44	1.19	0.39	0.47	0.13

siliquae of RH 0406, Pusa Vijay, DRMR 150-35, Pusa Mustard 26, RLC 3, Pusa Mustard 32, RH 725, RVM 1, Chattisgarh Sarson, NRCHB 101 and RH 0761, while lower in the siliquae of DRMR 1165-40, Pusa Double Zero Mustard 31, Pusa Mustard 28, Pusa Tarak, Radhika, RH 30, DRMIJ 31, RH 749 and Pusa Mustard 25 as compared to other *B. juncea* cultivars (Table 1). The chlorophyll B content reduced significantly due to *L. erysimi* infestation in siliquae of *B. juncea* cultivars. Further, significantly higher chlorophyll B ($F_{22,\ 46}$ = 2.68; $P < 0.001$) content change in the siliquae of DRMR 150-35, RLC 3, Pusa Mustard 32, Pusa Double Zero Mustard 31 and NRCHB 101, while lower in the siliquae of DRMR 1165-40, Pusa Mustard 26, DRMIJ 31, Pusa Mustard 30 and Pusa Tarak was observed (Suppl Fig. S2).

Total chlorophyll content in siliquae

The total chlorophyll content in healthy ($F_{22,\ 46}$ = 7.14; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22,\ 46}$ = 27.45; $P < 0.001$) of *B. juncea* cultivars varied significantly, and ranged from 5.5 to 9.1 and 2.9 to 6.0 µg/mL of tissue, respectively (Table 1). The constitutive total chlorophyll content was notably higher in the siliquae of NRCHB 101, DRMR 150-35 and RLC 3, while lower in the siliquae of Radhika as compared to other *B. juncea* cultivars (Table 1). The total chlorophyll content reduced remarkably due to *L. erysimi* damage in the siliquae of *B. juncea* cultivars. However, the per cent decline in the total chlorophyll ($F_{22,\ 46}$ =9.19; $P < 0.001$) content was notably higher in the siliquae of NRCHB 101, RLC 3, DRMR 150-35, Chattisgarh Sarson and Pusa Double Zero Mustard 31, while lower in the siliquae of Pusa Mustard 25, Pusa Mustard 30, RH 0761, Pusa Mustard 28 and Radhika (Suppl Fig. S3).

Total carotenoids content in siliquae

There were significant variations in total carotenoid content both constitutively ($F_{22,\ 46}$ = 6.86; $P < 0.001$) and inductively ($F_{22,\ 46}$ = 73.14; $P < 0.001$) in the siliquae of *B. juncea* cultivars varied significantly, and ranged from 0.8 to 2.2 and 0.2 to 1.4 µg/mL of tissue, respectively (Table 1). The total carotenoid content was constitutively higher in the siliquae of DRMR 150-35, DRMR 1165-40, NRCHB 101, Pusa Tarak, Radhika, Pusa Mustard 30 and Pusa Mustard 25, while lower in the siliquae of RH 0406, RH 725, RVM 1, Chattisgarh Sarson, Pusa Vijay, RH 0761 and Pusa Mustard 26 as compared to other *B. juncea* cultivars (Table 1). However, total carotenoid content reduced significantly owing to *L. erysimi* infestation in siliquae of *B. juncea* cultivars. The per cent reduction in the total carotenoids ($F_{22,\ 46}$ =6.71; $P<0.001$) was significantly more in the siliquae of RH 725, RLC 3, NRCHB 101, RH 0406 and Pusa Mustard 26, while lower in the siliquae of DRMIJ 31, Pusa Double Zero Mustard 31, RH 30, Chattisgarh Sarson and DRMRIJ 16-38 as compared to other cultivars (Suppl Fig. S4).

Antioxidative enzyme activity in the siliquae of test *B. juncea* cultivars

Ascorbate oxidase activity

The ascorbate oxidase activity in healthy ($F_{22,\ 46}$ = 23.99; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22,46}$ = 34.82; $P < 0.001$) of *B. juncea* cultivars varied significantly. The activity of AO in the healthy and damaged siliquae of *B. juncea* ranged from 180.90 to 445.80 and 266.02 to 784.68 U/mL of tissue, respectively (Table 2). The ascorbate oxidase activity was significantly higher in the siliquae of DRMR 150-35 and RLC 3, while lower in the siliquae of DRMR 1165-40 and RH 0406 as compared to other *B. juncea* cultivars (Table 2). However, NRCHB 101, Pusa Mustard 25, DRMR 150-35, RH 749 and RLC 3 showed highest upsurge in the AO activity, while Pusa Mustard 32, Pusa Vijay, RH 30 and RH 0761 showed lowest rise in the activity of AO (Suppl Fig. S5).

Ascorbate peroxidase activity

There were significant differences in the activity of APX in healthy ($F_{22,\ 46}$ = 2.91; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22,\ 46}$ = 8.66; $P<0.001$) of *B. juncea* cultivars, and ranged from 953.5 to 1280.3 and 1330.5 to 2191.3 U/mL of tissue, respectively (Table 2). The activity of APX was significantly more in the siliquae of Radhika, Pusa Mustard 28, DRMR 150-35, DRMIJ 31, NRCHB 101, Pusa Double Zero Mustard 31, DRMR 1165-40 and DRMRIJ 16-38, while lower in the siliquae of RLC 3, RH 749, RH 0406 and Pusa Vijay (Table 2). Further, the activity of APX was significantly enhanced in the siliquae of RLC 3, DRMR 150-35, Pusa Mustard 25, RH 725 and Pusa Mustard 32, while lower in the siliquae of Pusa Mustard 30, RH 0761, DRMRIJ 16-38 and DRMR 1165-40 as compared to other cultivars (Suppl Fig. S6).

Catalase activity

The catalase activity was varied significantly in healthy ($F_{22,\ 46}$ = 41.68; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22,\ 46}$ = 52.34; $P < 0.001$) of *B. juncea* cultivars, and ranged from 204.4 to 537.9 and 295.6 to 839.9 U/mL of tissue, respectively (Table 2). The constitutive catalase activity was notably more in the siliquae of RLC 3, Pusa Mustard 25 and Pusa Mustard 30, while lower in the siliquae of Pusa Vijay and RH 30 as compared to other *B. juncea* cultivars (Table 2). However, the enhancement in the catalase activity was notably higher in the siliquae of RH 725, Pusa Mustard 25, Pusa Mustard 30, DRMIJ 31 and Chattisgarh Sarson, while lower in the siliquae of NRCHB 101, Pusa Mustard 27, RH 749, RH 30 and Pusa Mustard 26 (Suppl Fig. S7).

Phenylalanine ammonia lyase activity

The PAL activity varied significantly both constitutively ($F_{22, 46} = 33.48$; $P < 0.001$) and

Table 2 — The activity of various antioxidative enzymes in the siliquae of diverse *Brassica juncea* cultivars

Cultivars	Ascorbate oxidase		Ascorbate peroxidase		Catalase		Phenylalanine ammonia lyase		Tyrosine ammonia lyase	
	Constitutive (U/mL)	Induced (U/mL)	Constitutive (U/mL)	Induced (U/mL)	Constitutive (U/mL)	Induced (U/mL)	Constitutive (U/mL)	Induced (U/mL)	Constitutive (U/mL)	Induced (U/mL)
RH 0761	352.2 ±20.1	497.1 ±25.5	1110.5 ±72.2	1429.2 ±44.8	359.2 ±19.2	541.4 ±11.1	1459.2 ±88.1	1857.5 ±131.7	1500.7 ±98.9	1902.1 ±86.8
RH 30	346.7 ±8.0	450.7 ±22.6	1053.5 ±73.3	1501.0 ±87.3	204.4 ±8.5	295.6 ±9.0	1451.5 ±84.5	2029.2 ±27.8	1472.1 ±82.2	1833.8 ±124.1
RLC 3	416.2 ±21.5	696.5 ±12.9	1005.4 ±70.4	1775.3 ±78.1	537.9 ±13.0	879.7 ±20.4	1780.0 ±78.3	2870.3 ±84.2	1766.0 ±70.2	2866.4 ±143.7
DRMIJ 31	318.6 ±8.6	458.1 ±20.8	1178.5 ±15.6	1666.5 ±82.0	272.5 ±7.9	470.6 ±31.8	1307.2 ±76.1	1953.6 ±86.0	1360.8 ±24.5	1688.1 ±49.5
DRMR 1165-40	226.9 ±10.6	328.6 ±18.2	1125.6 ±34.1	1533.1 ±86.3	266.5 ±7.1	429.3 ±18.5	873.9 ±37.6	1082.3 ±15.9	954.2 ±67.3	1350.5 ±72.8
NRCHB 101	234.3 ±3.7	429.9 ±25.4	1173.1 ±43.8	1982.6 ±36.6	446.4 ±25.3	541.1 ±6.2	1900.5 ±95.3	3236.9 ±139.4	1981.6 ±47.8	3483.9 ±224.2
Radhika	300.9 ±14.7	443.9 ±9.0	1280.3 ±86.6	1875.7 ±85.2	255.9 ±12.5	417.2 ±28.6	876.6 ±47.3	1342.6 ±70.5	829.1 ±32.9	1196.7 ±54.4
DRMR 150-35	445.8 ±12.9	784.7 ±39.0	1260.6 ±71.0	2191.3 ±60.1	421.7 ±22.3	672.4 ±25.5	1881.5 ±121.1	3218.4 ±39.6	1810.7 ±64.2	3132.8 ±59.3
Pusa Mustard 28	252.7 ±7.1	401.3 ±9.1	1269.5 ±82.3	1820.6 ±95.6	257.3 ±12.2	432.3 ±9.4	988.4 ±40.2	1539.9 ±91.1	1066.6 ±43.9	1291.4 ±23.2
Pusa Tarak	282.2 ±5.9	423.2 ±9.4	1039.2 ±17.7	1674.4 ±104.6	336.7 ±7.6	492.2 ±7.7	989.0 ±17.8	1236.4 ±81.3	890.0 ±26.5	1166.8 ±24.8
Chattisgarh Sarson	262.9 ±4.7	401.0 ±27.5	1032.1 ±33.7	1562.6 ±29.6	266.6 ±11.5	455.0 ±13.6	1035.2 ±54.4	1576.9 ±38.8	1109.7 ±44.1	1589.4 ±24.8
RH 725	329.2 ±20.3	479.4 ±22.5	1046.3 ±69.8	1782.5 ±67.5	266.1 ±7.4	501.0 ±25.8	1360.2 ±47.0	2465.7 ±170.3	1390.7 ±44.1	2438.5 ±166.1
RH 0406	180.9 ±8.7	266.0 ±14.2	964.2 ±21.4	1459.1 ±62.8	303.7 ±11.4	477.4 ±24.4	642.7 ±9.4	1095.9 ±49.8	754.6 ±13.6	1128.5 ±15.0
Pusa Vijay	286.0 ±3.7	365.6 ±14.2	953.5 ±29.3	1371.8 ±41.5	213.2 ±2.7	312.9 ±11.4	805.2 ±32.8	1038.8 ±63.9	519.0 ±18.9	702.7 ±30.6
RH 749	256.9 ±8.5	438.5 ±12.0	976.7 ±28.2	1552.5 ±20.6	383.9 ±13.3	534.5 ±14.7	1012.4 ±69.0	1560.9 ±88.6	1081.8 ±18.9	1858.1 ±113.4
DRMRIJ 16-38	272.6 ±5.6	427.1 ±19.2	1123.1 ±26.0	1503.0 ±102.4	317.9 ±6.6	464.9 ±8.1	1082.9 ±43.5	1367.7 ±77.0	1163.2 ±63.8	1521.4 ±62.6
Pusa Mustard 32	280.9 ±17.9	344.4 ±4.4	1119.6 ±74.7	1898.8 ±28.7	335.6 ±16.5	509.6 ±30.1	1117.9 ±24.9	1651.1 ±41.4	1187.6 ±17.4	1871.6 ±43.4
Pusa Double Zero Mustard 31	230.6 ±7.9	371.0 ±8.8	1126.7 ±75.7	1633.3 ±51.8	324.4 ±14.4	514.7 ±28.3	1083.7 ±22.0	1527.8 ±16.6	968.7 ±29.3	1347.2 ±59.9
Pusa Mustard 25	382.7 ±24.8	682.1 ±26.1	1024.9 ±38.3	1758.6 ±68.2	506.7 ±12.9	893.9 ±18.2	1610.0 ±53.3	2405.7 ±100.2	1628.8 ±33.9	2031.5 ±35.6
Pusa Mustard 26	392.8 ±23.0	628.2 ±39.5	1016.0 ±51.9	1703.3 ±59.6	400.5 ±17.8	581.9 ±30.8	1761.9 ±87.5	3149.9 ±119.2	1671.2 ±86.2	2977.8 ±50.7
Pusa Mustard 30	308.5 ±19.6	447.8 ±18.6	1030.3 ±25.3	1330.5 ±62.9	490.5 ±18.6	844.5 ±15.6	1258.3 ±37.5	2013.9 ±137.2	1309.6 ±88.0	1882.3 ±81.0
Pusa Mustard 27	291.4 ±7.7	430.5 ±26.5	1112.0 ±60.0	1663.1 ±86.6	442.6 ±14.2	608.3 ±27.9	1176.8 ±75.7	1914.7 ±88.8	1225.6 ±76.5	1657.2 ±113.7
RVM 1	232.4 ±5.4	378.1 ±16.5	1104.9 ±55.4	1523.8 ±83.6	376.8 ±25.0	560.9 ±34.5	887.4 ±46.6	1120.2 ±38.2	974.0 ±32.7	1358.1 ±95.7
F-Probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (P = 0.05)	38.7	60.57	157.62	201.38	41.7	61.55	178.68	251.91	157.5	261.84

inductively ($F_{22, 46} = 63.56$; $P < 0.001$) in the siliquae of *B. juncea* cultivars. The PAL activity in the healthy and damaged siliquae of *B. juncea* ranged from 642.7 to 1900.5 and 1038.84 to 3236.93 U/mL of tissue, respectively (Table 2). The constitutive PAL activity was significantly more in the siliquae of NRCHB 101, DRMR 150-35 and RLC 3, while lower in the siliquae of Pusa Vijay and RH 0406 as compared to other *B. juncea* cultivars (Table 2). Further, RH 725, Pusa Mustard 26, DRMR 150-35, NRCHB 101 and RH 0406 displayed highest upsurge in the PAL activity, while DRMR 1165-40, Pusa Tarak, DRMRIJ 16-38, RVM 1 and Pusa Vijay exhibited lowest surge in the activity of PAL (Suppl Fig. S8).

Tyrosine ammonia lyase activity

The tyrosine ammonia lyase activity was significantly differed in healthy ($F_{22, 46} = 44.9$; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22, 46} = 59.62$; $P < 0.001$) of *B. juncea* cultivars, and ranged from 519.0 to 1981.6 and 702.73 to 3483.93 U/mL of tissue, respectively (Table 2). The TAL activity was notably higher in the siliquae of NRCHB 101, DRMR 150-35, RLC 3, Pusa Mustard 26, Pusa Mustard 25, RH 0761 and RH 30, while lower in the siliquae of Pusa Vijay in relation to other *B. juncea* cultivars (Table 2). However, the TAL activity was increased significantly, which was highest in the siliquae of Pusa Mustard 26, NRCHB 101, RH 725, DRMR 150-35 and RH 749, while lower in the siliquae of Pusa Mustard 28, DRMIJ 31, Pusa Mustard 25, RH 30 and RH 0761 (Suppl Fig. S9).

Total glucosinolates and activity of myrosinase in the diverse *B. juncea* cultivars

Total glucosinolate content in the siliquae

The significant variations in the total glucosinolate content were noticed in healthy ($F_{22, 46} = 53.83$; $P<0.001$) and *L. erysimi* damaged siliquae ($F_{22, 46} = 49.95$; $P < 0.001$) of *B. juncea* cultivars, which ranged from 15.9 to 180.1 and 9.8 to 89.8 μ mol/g of tissue, respectively (Table 3). Total constitutive glucosinolate content was notably higher in the siliquae of NRCHB 101, while lower in the siliquae of Pusa Double Zero Mustard 31 and RLC 3 (Table 3). However, diminution of total glucosinolates owing to *L. erysimi* infestation was significantly higher in Pusa Mustard 26, DRMRIJ 16-38, NRCHB 101, DRMR 150-35 and RH 749, while lower in the siliquae of DRMIJ 31, Chattisgarh Sarson, Pusa Mustard 27, RVM 1 and RH 0406 in relation to other cultivars (Suppl Fig. S10).

Table 3 — Total glucosinolate content and myrosinase activity in the siliquae of diverse *Brassica juncea* cultivars

Cultivars	Total glucosinolate content		Myrosinase activity	
	Constitutive (μmol/g)	Induced (μmol/g)	Constitutive (U/ml)	Induced (U/ml)
RH 0761	107.0±2.2	66.8±3.8	3.0±0.1	3.6±0.1
RH 30	68.9±1.5	51.0±3.2	4.0±0.2	5.3±0.1
RLC 3	18.5±1.2	9.8±0.6	7.2±0.2	9.4±0.3
DRMIJ 31	102.0±5.0	80.4±3.5	4.8±0.2	6.7±0.4
DRMR 1165-40	102.3±6.4	67.8±1.9	5.2±0.1	7.7±0.3
NRCHB 101	180.1±11.7	83.6±1.5	6.7±0.4	11.2±0.8
Radhika	98.4±4.8	64.3±1.0	3.5±0.1	5.0±0.3
DRMR 150-35	122.3±2.3	58.9±3.4	6.4±0.1	10.8±0.2
Pusa Mustard 28	130.0±2.0	68.0±1.4	3.6±0.2	5.1±0.2
Pusa Tarak	100.1±6.8	74.8±5.1	3.6±0.1	5.8±0.3
Chattisgarh Sarson	112.3±7.8	87.0±2.6	5.8±0.3	7.3±0.1
RH 725	122.1±6.4	74.0±1.7	5.4±0.1	7.9±0.1
RH 0406	119.2±4.3	89.8±5.1	6.3±0.4	9.7±0.7
Pusa Vijay	89.6±2.9	47.1±2.8	4.2±0.1	7.7±0.3
RH 749	147.9±2.9	73.0±4.5	3.0±0.0	4.4±0.1
DRMRIJ 16-38	111.6±1.6	48.2±2.4	4.5±0.3	5.9±0.2
Pusa Mustard 32	60.1±2.5	34.7±0.6	5.5±0.4	7.6±0.4
Pusa Double Zero Mustard 31	15.9±0.8	10.2±0.1	4.4±0.3	7.8±0.2
Pusa Mustard 25	112.4±1.3	73.7±2.5	3.2±0.2	5.6±0.4
Pusa Mustard 26	103.4±4.8	40.2±1.2	6.5±0.4	10.3±0.5
Pusa Mustard 30	67.8±2.0	49.2±3.4	4.5±0.2	5.5±0.4
Pusa Mustard 27	113.5±7.6	87.0±5.9	5.5±0.2	6.8±0.2
RVM 1	104.5±6.6	78.7±5.1	4.6±0.1	7.0±0.4
F-probability	<0.001	<0.001	<0.001	<0.001
LSD (P = 0.05)	14.16	9.08	0.7	0.98

Myrosinase activity in siliquae

The significant difference in the myrosinase activity was noticed in healthy ($F_{22, 46} = 26.1$; $P < 0.001$) and *L. erysimi* damaged siliquae ($F_{22, 46} = 36.57$; $P < 0.001$) of *B. juncea* cultivars. The myrosinase activity in the healthy and damaged siliquae of *B. juncea* ranged from 3.0 to 7.2 and 3.6 to 11.2 U/mL of tissue, respectively (Table 3). The myrosinase activity was constitutively higher in the siliquae of RLC 3, NRCHB 101 and Pusa Mustard 26, while lower in the siliquae of RH 749, RH 0761, Pusa Mustard 25, Radhika, Pusa Tarak and Pusa Mustard 28 as compared to other *B. juncea* cultivars (Table 3). However, the enhancement in the myrosinase activity due to *L. erysimi* infestation was significantly enhanced in the siliquae of Pusa Vijay, Pusa Double Zero Mustard 31, Pusa Mustard 25, NRCHB 101 and DRMR 150-35, while lower in the siliquae of RH 0761, Pusa Mustard 30, Pusa Mustard 27, Chattisgarh Sarson and RLC 3 as compared to other cultivars (Suppl Fig. S11).

Discussion

The chlorophyll content is a crucial factor in determining the compatibility of plants and herbivores. As a plant develops, its chlorophyll levels change[32], and they also change in response to a range of challenges[33,34]. The present studies reported significant variations in the constitutive levels of photosynthetic pigments *viz*., chlorophyll A, chlorophyll B, total chlorophyll and total carotenoids, which further subsided due to *L. erysimi* infestation. Similarly, it is also reported diminution in the amount of different photosynthetic pigments in *B. juncea* genotypes due to *L. erysimi*, which resulted in detrimental effect on the population build-up of *L. erysimi*[6,23]. The decrease in the quantity of various photosynthetic pigments implying significant role in the host defence against *L. erysimi*. It is also stated that the stresses damage plant tissues resulting in release of chlorophyll from the thylakoid membranes[35]. Under such situation, the chlorophylls need to be degraded quickly to avoid cellular damage by their photodynamic action[36], otherwise the host plant will display susceptible reaction, indicating significant role of chlorophyll in plant defence and plant–insect interactions.

Plants in response to herbivory realise the significant shift in oxidative status due to increased production of ROS, the major regulatory signalling molecules in plants[37]. This increased ROS activates the antioxidative enzymes further increase the levels of primary compounds and secondary metabolites to induce resistance against insect damage[38]. It is eminent that the nutritional quality of the host plant can influence the life cycle characteristics of herbivorous insects by limiting growth, raising mortality, and lowering fertility[39]. Chemical properties such as secondary metabolites and nutrient balance, photosynthetic pigments, and various enzymes such as AO, APX, catalase, PAL, TAL, and myrosinase vary from one host plant to another and consequently influence population of the herbivore insects [6,7,20,23,40]. As a normal occurrence[21] and a key component of plant defence against insect pests[22], the qualitative or quantitative alteration in activity of different enzymes in response to herbivore attack is a common phenomenon. The current investigation unveiled significant variation in the activity of AO, APX and catalase in siliquae of test *B. juncea* cultivars. The siliquae of DRMR 150-35, RLC 3, NRCHB 101 and Pusa Mustard 30 had greater activities of antioxidant enzymes. Similarly, greater activities of antioxidant enzymes such as AO, APX and catalase were reported in resistant genotypes signifying that these antioxidant enzymes also engage in defending the plants from biotic stresses[6,7,20]. PAL is the entry-point enzyme into the phenylpropanoid pathway accountable for the synthesis of plant phenylpropanoids or phenolics, many of which portray a vital role in plant defence under stress conditions[41]. The present study recorded the higher activity of PAL and TAL in siliquae of RLC 3, Pusa Mustard 26, NRCHB 101, DRMR 150-35, RH 30, RH 725 and DRMIJ 31 suggesting higher activity of these enzymes may impact the reproduction and survival of *L. erysimi* negatively. Earlier studies have also reported increased PAL and TAL activity in host plants, while reduced infestation by insect pests[6,20]. It is also reported that the increased level of antioxidative and defence related enzymes in *B. juncea* cultivars owing to stress has the potential to reduce the mustard aphid population[6,42].

Glucosinolates myrosinase is the compounds characteristic of cruciferae and a few other plant families[43]. These substances and their breakdown products have been demonstrated to stimulate plant approach, feeding, and reproduction in some insects specialised for feeding on crucifers, whereas other herbivores are often adversely affected by them[44,45]. These affect the probing behaviour and nutritional uptake by aphid, *L. erysimi*[46,47], consequently hamper the development and population build-up of aphids[48]. Present studies found the greater total glucosinolates in NRCHB 101, RH 0406 and DRMR 150-35. High levels of glucosinolates, total phenols, ortho-hydroxy phenols are associated with low infestation of aphids[49]. Similarly, a negative association between the total glucosinolates and mustard aphid population was also testified[50,6,7]. The current study showed significant reduction in the content of total glucosinolates across the test cultivars due to *L. erysimi* infestation. Similarly, a significant reduction in glucosinolates content in aphid-infested plants of three introgression lines of *B. juncea* (I8, I79, and I82) was observed[50]. Further, myrosinase enzyme is engaged in the breakdown of glucosinolates into other by-products, which have also been demonstrated to stimulate plant approach, feeding and reproduction in several insects specialised for feeding on crucifers[44,45]. In the current study, myrosinase content was significantly more in the siliquae of DRMR 150-35, RLC 3, NRCHB 101 and Pusa Mustard 26. Further, enhancement in the myrosinase activity may linked to the detrimental effects on the fecundity and survival of *L. erysimi*[6].

Conclusion

Nutritive and defensive status of *B. juncea* cultivars determine the preference and biological performance of *L. erysimi*. Direct defence involving biochemical synergy of *B. juncea* cultivars, and indirect induced defence involving up and down-regulation of various phytochemicals were observed to obstruct *L. erysimi* growth and development. Current investigation showed significant variation in the enzymatic antioxidant and glucosinolate mediated defence systems, and photosynthetic pigments across the test cultivars. However, cultivars RLC 3, Pusa Mustard 26, NRCHB 101, DRMR 150-35, RH 30, RH 725 and DRMIJ 31 showed remarkable shift in the antioxidative enzymes, glucosinolate- myrosinase content and photosynthetic pigments due to *L. erysimi* infestation, indicating their potential impact on the development and survival of mustard aphid. Thus, these cultivars can be exploited to breed aphid tolerant Indian mustard.

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Conflict of interest

Authors declare no competing interests.

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