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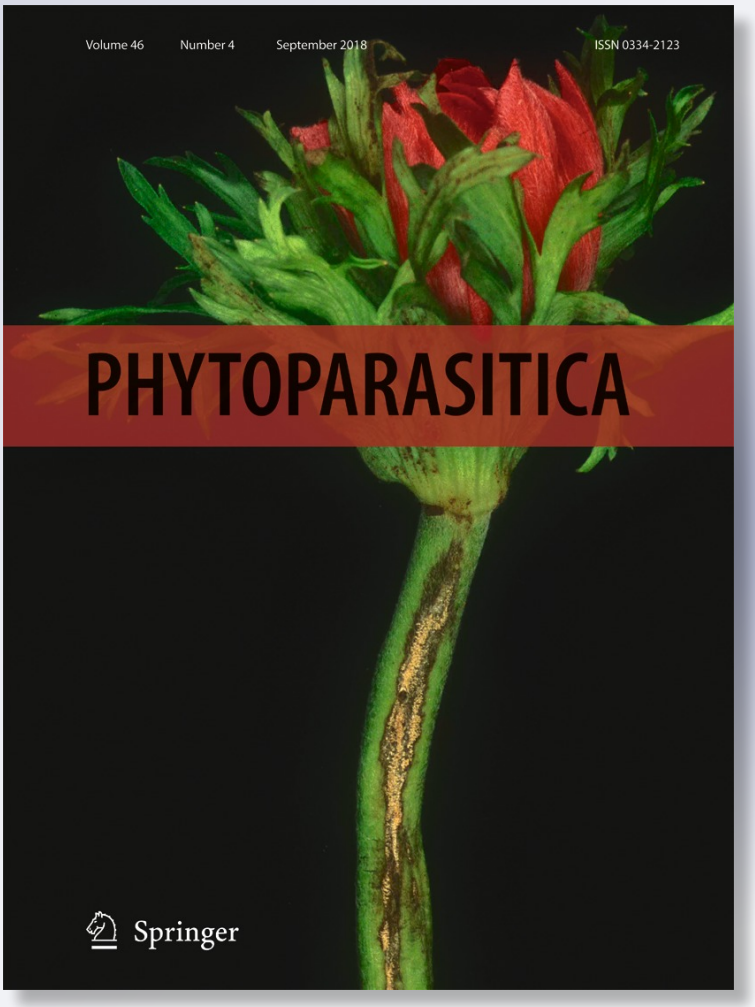

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Biochemical changes in the *Brassica juncea-fruticulosa* introgression lines after *Lipaphis erysimi* (Kaltenbach) infestation

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Abstract Insect damage leads to changes in biochemical profile of plants. Response of three *Brassica juncea-fruticulosa* introgression lines (already reported resistant to *Lipaphis erysimi*) in terms of changes in biochemical constituents after aphid infestation was studied along with *B. fruticulosa* (resistant parent), *B. juncea* var. PBR −210 (susceptible parent) and *B. rapa* ecotype brown *sarson* BSH-1 (susceptible check). These six genotypes were grown under aphid infested and uninfested conditions and were sampled at peak aphid infestation to analyze the biochemical changes caused by aphid feeding from top 10 cm central twig of plant. A significant reduction in glucosinolates content in aphid infested plants of three introgression lines (I_8, I_{79} and I_{82}) was observed while opposite was observed in *B. fruticulosa,* PBR-210 and BSH-1. Exactly opposite trend was observed for total phenols where aphid infestation resulted in significant increase in phenols content in the three introgression lines while a decrease was observed in *B. fruticulosa,* PBR-210 and BSH-1. A general trend of decline in flavonols, total sugars and free amino acids content was observed after aphid infestation in all the genotypes. Glucosinolates and total phenols served as biochemical bases of resistance in the three introgression lines since there was downregulation of glucosinolates and upregulation of total phenols as against opposite trend observed in BSH-1 and PBR-210.

Keywords Biochemical response · Mustard aphid · Rapeseed-mustard · Plant secondary metabolites

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Introduction

In nature, plants have evolved a plethora of defenses against biotic and abiotic stresses as, unlike animals, they cannot move to avoid any stress. In the pre-domestication era, there was strong selection pressure on plants to develop resistance and only resistant plants survived as a consequence of laws of adaptation. This is also true for members of the family Brassicaceae which have developed different defense strategies against insect herbivores. Insect herbivory leads to modulation of different defense chemicals (Schenk et al. 2000; Vos et al. 2005) to ward off insects and to ensure their survival. Glucosinolates are one such group of plant secondary metabolites (PSMs) exclusive to order Capparales that serve as chemical defense against many insects (Bridges et al. 2002; Hopkins et al. 2009; Rossiter et al. 2003) which yield further toxic compounds (cyanates, isothiocyanates, nitriles and oxazolidiethiones) upon hydrolysis with myrosinase (Bones et al. 1991; Jones and Vogt 2001; Kissen et al. 2009). Phenolic compounds are also known to play a crucial role in determining resistance of a plant to insect

attack. Majority of the phenolic compounds are toxic to herbivorous insects including aphids and impair their growth, development and fecundity (Dreyer and Campbell 1987). Sugars are known to enhance oxidative burst at early stages of injury, increasing the lignification of cell walls, stimulate the synthesis of flavonoids and induce certain PR proteins (Morkunas and Ratajczak 2014) while amino acids are precursors and constituents of many proteins (Corcuera 1993).

Mustard aphid, *Lipaphis erysimi* (Kaltenbach) is an economically important pest of oilseed *Brassica* in Indian subcontinent and many other countries. It is a limiting factor in realization of full yield potential of oilseed *Brassica* crops and the damage can range from as low as 9% to as high as 96% under different agro-climatic conditions, intensity of population development and crop growth stage (Singh and Sharma 2002; Kumar et al. 2011; Kumar and Sangha 2013). High fecundity, short generation period, parthenogenetic viviparity, telescoping of generations and atypical feeding mechanism exclusively on phloem tissue make it one of the most economically important pests (Goggin 2007). Being specialist insect of Brassica plants, this pest takes advantage of glucosinolates by their sequestration from host plant to protect it from predators. It has co-evolved to the extent that it can synthesize its own thioglucosidase endogenously (analogous to the plant myrosinase), which is separated spatially from sequestered glucosinolates in their flight muscles. When the aphid is crushed or fed upon by a predator, the enzyme leads to hydrolysis of sequestered glucosinolates (the concentration of glucosinolates in haemolymph is 15–20 times relative to that in the leaf tissue) to produce toxic products (Bridges et al. 2002; Rossiter et al. 2003). These crushed insects smell and taste badly and release volatiles alarming other insects in the colony. Further, these glucosinolates also serve as feeding stimulants not only for *L. erysimi* but for more than 25 insect species (Hopkins et al. 2009).

Though synthetic chemical insecticides are available for its management, but due to the ill effects associated with their use, the need for alternate pest management strategy is always sought after. Host plant resistance is considered as an important alternate pest management strategy that can form core of the Integrated Pest Management (IPM) module. Previous studies at this institute have identified three introgression lines with genomic introgression from wild *B. fruticulosa* to be resistant to *L. erysimi* (Palial 2017). These lines (I_8, I_{79} and I_{82}) were developed from selfing BC_1 generation of the cross, *B. juncea/ (B. fruticulosa/B. rapa)* and after extensive screening both under field and laboratory conditions, were found to be resistant to *L. erysimi* (Kumar et al. 2011; Atri et al. 2012). Infestation by *L. erysimi* is expected to result some biochemical changes in the resistant and susceptible genotypes. Therefore, the effect of aphid challenge on the biochemical changes in these genotypes was studied which will develop better understandings among researchers about the plant behavior against this insect and the mechanism of resistance.

Materials and methods

Plant materials

The plant material comprised three *B. juncea-B. fruticulosa* introgression lines along with one wild male parent, *Brassica fruticulosa* (resistant donor), female parent (*Brassica juncea* variety PBR-210, and *Brassica rapa* ecotype brown *sarson* var. BSH-1 (susceptible check). Introgression lines were developed from the selfing BC_1 generation of the cross, *B. juncea*/ (*B. fruticulosa*/*B. rapa*). The single pod descent method was followed for their development. The seeds of all the introgression lines, parents and checks were procured from Dr. SS Banga, ICAR National Professor, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The available introgression lines along with the resistant and susceptible parents and susceptible check (BSH-1) were used to study the changes in different biochemical constituents that occur after aphid infestation.

Raising of plant materials

The test genotypes (I_8, I_{79}, I_{82}, *B. fruticulosa, B. juncea* var. PBR-210 and *B. rapa* var. BSH-1) were sown in plots of size of 4 × 3 m following factorial randomized complete block design under insecticide protected and unprotected conditions. The genotypes served as first factor while infestation level (uninfested and infested) served as second factor. The infested set was subjected to *L. erysimi* attack @ 20 aphids plant^{-1} on 10 plants selected at random from each plot (Kumar et al. 2011), while the second set served as uninfested control. To avoid natural infestation, this set was sprayed with

thiamethoxam 25 WG @ 100 g ha^{-1}. Thiamethoxam is a systemic neonicotenoid insecticide recommended for the control of *L. erysimi* in this part of the country (Anonymous 2016). The experiment was laid out in the experimental area of Oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30.9°N and 75.85°E, 244 m above msl), India during 2016–17 crop season. The row to row and plant to plant spacing was maintained at 30 × 15 cm, respectively thus making a total of 270 plants per plot. All the recommended package of practices for raising a good crop was followed (Anonymous 2016). All the six genotypes were sown in three blocks. Each block consisted of 12 plots in sets of two (sub plots) per genotype. One sub plot of each genotype in each block was artificially infested with *L. erysimi* while the other served as uninfested control. Thus, in total, there were three blocks with 12 subplots in each block. Plots and blocks were separated by 1.5 and 3 m of open space, respectively. At the time of spray of insecticide, a 6 m high polythene sheet was placed on the sides of the plot to prevent pesticide drift to the adjacent unsprayed/infested plots.

After one week when aphid infestation was at its peak, the top inflorescence of the plants from each line were collected and brought to the Biochemistry Laboratory, Oilseed Section, Department of Plant Breeding and Genetics, PAU Ludhiana. Different biochemical constituents were determined using the known standard procedures i.e. glucosinolates (McGhee et al. 1965), total phenols (Swain and Hillis 1959), flavonols (Balbaa et al. 1974), total sugars (Dubois et al. 1956) and free amino acids (Spices 1957) details of which are discussed in the following sections. Aphid population was also recorded one week after release of aphids from top 10 cm central twig of these 10 plants selected at random from each plot on which aphids were released.

Biochemical analysis

Glucosinolates

Glucosinolates are sulphur containing secondary metabolites present in Brassicaceae. These are anti-nutritional factors. Na_2PdCl_4 forms complex with sulphur of glucosinolates giving red compound, read at 405 nm. Sinigrin (Sigma-Aldrich, India) is used as the standard and the estimation was done as per McGhee et al. (1965). Reagent was 0.2 mM Na_2PdCl_4 (Sigma-Aldrich, India). For the extraction, top inflorescences of *Brassica* genotypes from each replication were taken and placed at 100 °C in an oven to deactivate myrosinase enzyme. After that dried inflorescence twig was ground in pestle mortar and 0.3 g of crushed form was transferred to the test tubes. Then 300 μl (0.3 ml) 60% methanol (Merck, India) was added to deactivate the enzyme. The test tubes were kept in boiling water bath at 80 °C for 15 min. The extract was centrifuged in a tube and then 40 μl of the supernatant was taken in other test tubes in duplicates. After addition of 4 ml of 0.2 mM Na_2PdCl_4, it was kept at room temperature for 1 h and Optical Density (O. D.) reading (μ moles/g tissue) was recorded at 405 nm on the spectrophotometer.

Total phenols

The total phenols were determined as per Swain and Hillis (1959) using the top inflorescence twig from each replication of introgression lines. The top inflorescence twigs collected from the field were dried at 50–60 °C in an oven. Once dried, 40 mg of dried sample was placed in boiling test tubes and 5 ml of 80% methanol was added and then kept in water bath at 80 °C for 30 min. After that refluxary material was filtered with filter paper and volume was made to 10 ml by washing with 80% methanol. Reagents used in the estimation process were Folin phenol reagent (1:1) (Alpha Chemica, India) and saturated solution of Na_2CO_3 (17.5 g/50 ml of distilled water) [Sisco Research Laboratories (SRL) Private Limited, India]. Methanolic extract (0.5 ml) was taken in the test tubes in duplicates and was evaporated to dryness at 80 °C in water bath. The residue was dissolved in 6.5 ml of distilled water. To this, 0.5 ml of Folin phenol reagent was added and shaken thoroughly. After 5 min, 1 ml of saturated solution of Na_2CO_3 was added. After 1 h, the absorbance of blue colour was read in a spectrophotometer at 760 nm against the blank. The blank was prepared from water and reagent only. The concentration of the total phenols (mg/g of dry weight) was calculated from the standard curve prepared by using gallic acid (10–50 μg) (Thomas Baker Chemicals Private Limited, India).

Flavonols

The flavonols were determined as per Balbaa et al. (1974). 1 ml of methanolic extract was taken in the boiling test tubes and evaporated to dryness at 80 °C

in water bath. The residue left behind was dissolved in 10 ml of 0.1 M methanolic extract of aluminium chloride (Sigma Aldrich, India). After the 1 h incubation period, yellow colour develops, which was read at 420 nm against 0.1 M methanolic solution of $AlCl_3$ as blank. The concentration of flavonols (mg/g dry weight) was determined from the standard curve prepared by using rutin (40-200 μg) (S D Fine Chemicals Limited, India).

Total sugars

The phenol-sulphuric acid method is simple, rapid, sensitive, accurate and specific for carbohydrates and is widely applicable (Dubois et al. 1956). The hydroxymethyl furfural and furfural produced in this reaction, further reacts with phenol to yield coloured condensed product, which can be read at 490 nm. For the extraction, 200 mg of a sample was refluxed with 10 ml 80% ethanol (CSS Reagent, China) for 50 min. The filterate was collected and the residues again refluxed twice with 70% ethanol, keeping the tubes with water condensed in the boiling water bath for 20 min. After that, the supernatant was collected and pooled. Ethanol from pooled extract was removed at 50 °C in a flash evaporator under vacuum, and then the extract was diluted with water and the final volume was made 25 ml. The extract was used for the estimation of total soluble sugars. 0.1 ml of sugar extract was taken in the test tubes and, 1 ml of 5% phenol (SRL, India) and 0.9 ml of distilled water was added followed by addition of 5 ml of concentrated H_2SO_4 (HPLC Laboratory Reagents, India). The sulphuric acid was passed directly in the centre of the test tube to ensure proper mixing of the solution. The absorbance was measured at 490 nm after 30 min against blank. The blank was prepared from the water and reagent only. The concentration of the total sugars was calculated from the standard curve of standard glucose (10–100 μg) (Merck, Germany).

Free amino acids

The Ninhydrin is specifically reduced when heated in aqueous solution with free amino acids. The amino acid is oxidized to an aldehyde with one less carbon, with the release of carbon dioxide. The ninhydrin is reduced to a compound which can be condensed with ammonia and with more ninhydrin to form a blue coloured pigment (Spices 1957). For extraction of free amino acids, 200 mg of top inflorescence was taken from the field and the fresh sample was refluxed with 10 ml each of 80 and 70% ethanol on the boiling water bath for 15–20 min. The supernatants were pooled and then filtered through Whatman's filter paper no. 1 and then the filtrate was made from ethanol by evaporation on a flash evaporator. The aqueous extract was diluted to a volume of 25 ml with distilled water. 1 ml of aqueous extract was taken in the test tubes and then 5 ml of ninhydrin reagent (SRL, India) was added. The mixture was shaken and kept for 10–15 min in a boiling water bath. After cooling, the violet colour developed which was measured at the 570 nm. The blank was prepared with reagent only. The content of the free amino acids was calculated from the L-Glycine (5–25 μg) standard (SRL, India).

Statistical analysis

The results were expressed as mean ± SE. The data were subjected to two way analysis of variance to determine the difference between the genotypes and treatments using the statistical software SPSS version 20.0 (SPSS 2011). Further correlation analysis was also done to find out the relationship of different biochemical constituents with mustard aphid infestation.

Results

Glucosinolates contents

Significant variation in glucosinolates content was observed among the genotypes. The glucosinolates content in infested plants of all the three introgression lines (I_8, I_{79} and I_{82}) and resistant parent *B. fruticulosa* (33.3, 36.0, 36.4 and 36.2 μmoles/g dry weight of tissue, respectively) differed significantly from that of the BSH-1 (42.0) and PBR-210 (67.0) (Table 1). The three introgression lines (I_8, I_{79} and I_{82}) showed a decrease in glucosinolates content following aphid infestation (Fig. 1) ranging from 0.8 to 24.8%. Maximum reduction (−24.8%) was recorded in introgression line I_8. It was followed by I_{82} (−4.8% reduction) and I_{79} (−0.8% reduction). On the other hand, an increase in glucosinolates content was observed in *B. fruticulosa*, BSH-1 and PBR-210. The maximum increase (+66.0%) was observed in PBR-210 followed by BSH-1 (+10.2%) and *B. fruticulosa* (+6.4%). Previous studies have also reported an increase in glucosinolates content in aphid infested plants as observed in BSH-1 and PBR-210 in

Table 1 Total glucosinolates content (μmoles/g dry weight of tissue) in top 10 cm twig portion of different genotypes (Mean ± SE)

Genotypes	Uninfested	Infested
I_8	44.3 ± 1.4d	33.3 ± 0.4a
I_{79}	36.3 ± 0.1bc	36.0 ± 0.5b
I_{82}	38.3 ± 1.7c	36.4 ± 0.1b
B. fruticulosa	34.0 ± 0.4b	36.2 ± 1.1b
BSH-1	38.1 ± 0.8bc	42.0 ± 0.7c
PBR-210	27.4 ± 2.1a	67.0 ± 0.4d
LSD ($p \leq 0.05$)	A = 2.1 B = 1.2 AB = 3.0	

Means within a column followed by same alphabet are not significantly different at $p \leq 0.05$ (LSD test)

A: Genotypes, B: Infestation level

the present study. But the opposite trend in introgression lines warrants further detailed investigation in relation to plant resistance. For example, Gill and Bakhetia (1985) also reported increase in glucosinolates content in aphid infested plants. Similarly, increase in glucosinolates content in response to feeding by *M. persicae* and *B. brassicae* was also reported in *Arabidopsis thaliana* by Mewis et al. (2005). On the other hand, Ramdhari et al. (1994) and Singh et al. (2000) observed that genotypes rich in glucosinolates level harboured low aphid population. The reduction in glucosinolates content in the three introgression lines may be of adaptive advantage to them and may explain a part of their resistant response. In general, glucosinolates are considered as double-edged sword from plant's defense point of view. On one side, the glucosinolate-myrosinase system provides resistance against many generalist insect-pests (Rask et al. 2000), and on the other hand, it makes plants vulnerable to attack by specialist insects such as *L. erysimi* (Renwick 2002). During the course of evolution, these specialized insects have learned to use glucosinolates to their adaptive advantage which feed on these glucosinolate rich plants and are able to cope with glucosinolates and their breakdown products. Some specialist insects such as *L. erysimi* and *B. brassicae* have even learned to use glucosinolates to their own advantage by using these compounds as cues for host acceptance for feeding. Further, these insects sequester glucosinolates from plants and use them to protect themselves against predators. *L. erysimi* is known to synthesize its own glucosidase which is stored in insect flight muscles. When the aphid is attacked by a predator, the enzyme leads to hydrolysis of glucosinolates leading to production of more toxic products (Bridges et al. 2002; Rossiter et al. 2003). The reduction in glucosinolates content in the three introgression lines may explain part of their resistance response as it may make the plants less apparent for host finding and less preferred for feeding due to comparatively less glucosinolates content that serve as feeding stimulants. However, an increase in glucosinolates content was also observed in *B. fruticulosa* which needs further investigations to elucidate the actual basis of resistance in it.

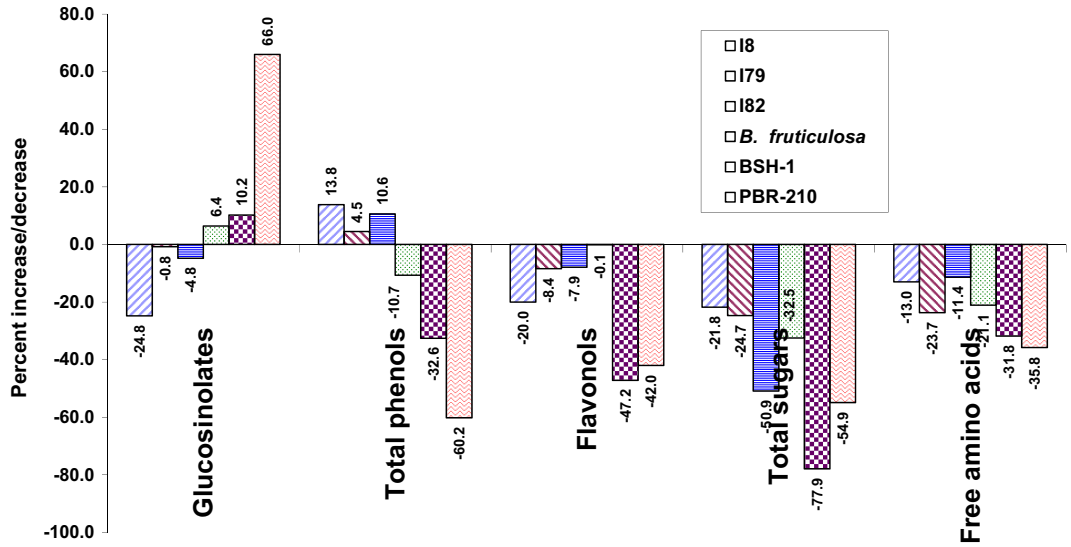

Fig. 1 Effect of *Lipaphis erysimi* infestation on concentration of different biochemical constituents in different genotypes

Total phenols

Significant variation in the concentration of total phenols was observed among the genotypes. The total phenols content of the infested plants in all the three introgression lines I_8, I_{79}, I_{82} and in resistant parent *B. fruticulosa* (14.8, 13.8, 14.6 and 10.0 mg/g dry weight of tissue, respectively) differed significantly as compared to the BSH-1 (7.0) and PBR-210 (5.6) (Table 2). It is interesting to note that aphid infestation resulted in significant increase in total phenols content in I_8, I_{79} and I_{82} (13.8, 4.5 and 10.6% increase, respectively) while opposite was observed for BSH-1, PBR-210 and *B. fruticulosa* (10.7, 32.6 and 60.2% decrease, respectively) (Fig. 1). Havlickova et al. (1998) reported induction of significantly higher amount of several cell wall bound phenolic acids and methoxyphenols in *Rhopalosiphum padi* infested wheat plants as compared to the uninfested plants. Increased aphid infestation also increases the activities of polyphenol oxidase and peroxidase enzymes while reduced the activities of superoxide dismutase, catalase, ascorbate peroxidase and oxidase enzymes (Khattab 2007). The polyphenol oxidase and polyphenol peroxidise are involved in defense mechanism, and these enzymes in the aphid saliva secretion are involved in the chemical stablization of the stylet sheath as well as in containing the plant defense by neutralising the phenolic compounds. These enzymes react with phenolics accumulated around the aphid stylet sheath and oxidise them to less toxic substances (Urbanska et al. 1998) so aphid resistant sorghum plants down-regulate the production of PPOs in response to *Sitobion graminum* feeding (Park et al. 2005).

Table 2 Total phenols content (mean ± SE) (mg/g dry weight of tissue) in the top 10 cm twig portion of different genotypes

Genotypes	Uninfested	Infested
I_8	13.0 ± 0.1b	14.8 ± 1.0c
I_{79}	13.2 ± 0.1b	13.8 ± 1.2c
I_{82}	13.2 ± 0.1b	14.6 ± 1.6c
B. fruticulosa	11.2 ± 0.2a	10.0 ± 0.3b
BSH-1	10.4 ± 0.2a	7.0 ± 0.7a
PBR-210	14.1 ± 0.5d	5.6 ± 0.4a
LSD ($p \leq 0.05$)	A = 1.5 B = 0.8 AB = 2.1	

Means within a column followed by same alphabet are not significantly different at $p \leq 0.05$ (LSD test)

A: Genotypes, B: Infestation level

Similar results were reported by Kumar et al. (2017) who reported the higher phenols content in the *E. sativa* var. T-27 (8.1 mg/ g) and *B. carinata* var. DLSC-2 (7.5 mg/ g) which harboured significantly lower aphid population (27.2 and 58.3 aphids/plants) as compared to the 4.1 mg/ g and 4.6 mg/ g phenols contents in BSH-1 and YST-151. Khattab (2007) observed a decrease in the level of total phenols content in the leaves infested by *B. brassicae* (8.12 mg/ g) as compared to healthy leaves (7.10 mg/g) in *Brassica olereacea* var. *capitata*. Eleftherianos et al. (2006) also reported almost similar related result that maize or barley infestation by *S. avenae* was followed by a decrease in levels of total phenols compared with the corresponding values in non-infested seedlings. Xu and Liang (2016) suggested that sucking insects may affect host plant metabolism and result in change in the concentration of foliar phenolics, and may indirectly affect the severity of infestations. The infested plants produced higher total phenolics content in leaves and the resistant cultivar maintained higher phenolics. Phenolics are known to play an antioxidative role in plant defense system as a backup to the primary ascorbate-dependent detoxification system (Sakihama et al. 2002). This is supported by observations such as rapid accumulation of phenolics in a significant proportion of the *Arabidopsis* cells undergoing a hypersensitive response (Soylu 2006). Increase in phenolics biosynthesis gene expression or enzyme activity is commonly associated with herbivory in many plants (Moran and Thompson 2001). Significantly high concentration of total phenols, *o*-dihydroxyphenols and total flavonoids was observed in *Pisum sativaum* plants infested with *Acyrthosiphon pisum* as compared to control ones (Golawska et al. 2008). However, infested and uninfested plants had similar phenolics profile.

Flavonols

Significant variation in flavonols content was observed among the genotypes. It was significantly high in the aphid infested plants of I_8, I_{79}, I_{82} and *B. fruticulosa* (9.6, 12.0, 13.9 and 10.1 mg/g dry weight, respectively) as compared to 4.8 and 5.8 mg/g dry weight in BSH 1 and PBR 210, respectively (Table 3). Aphid infestation resulted in a general decline in the flavonols content in all the genotypes which ranged from 0.1% decrease in *B. fruticulosa* to as high as 47.2% decrease in BSH-1 while it was 20.0, 8.4 and 7.9% in I_8, I_{79} and I_{82}, respectively (Fig. 1). Flavonls reduce the nutritive value

Table 3 Flavonols content (mean ± SE) (mg/g dry weight) in the top 10 cm twig portion of different genotypes

Genotypes	Uninfested	Infested
I_8	12.0 ± 1.4bc	9.6 ± 0.3b
I_{79}	13.1 ± 0.3 cd	12.0 ± 0.3c
I_{82}	15.1 ± 0.5d	13.9 ± 0.5d
B. fruticulosa	11.2 ± 0.1c	10.1 ± 0.5b
BSH-1	9.1 ± 0.4a	4.8 ± 0.4a
PBR-210	10.0 ± 0.6ab	5.8 ± 0.2a
LSD ($p \leq 0.05$)	A = 1.8 B = 0.7 AB = 1.7	

Means within a column followed by same alphabet are not significantly different at $p \leq 0.05$ (LSD test)

A: Genotypes, B: Infestation level

Table 4 Total sugars content (mean ± SE) (mg/g fresh weight of tissue) in the top 10 cm twig portion of different genotypes

Genotypes	Uninfested	Infested
I_8	97.1 ± 0.3ab	75.9 ± 1.1b
I_{79}	94.2 ± 21.7ab	70.9 ± 1.4b
I_{82}	77.1 ± 10.9a	37.8 ± 1.2a
B. fruticulosa	127.3 ± 16.5b	85.9 ± 11.2 b
BSH-1	183.5 ± 4.7c	40.6 ± 6.9a
PBR-210	71.6 ± 1.6a	32.3 ± 1.9a
LSD ($p \leq 0.05$)	A = 19.4 B = 11.2 AB = 27.5	

Means within a column followed by same alphabet are not significantly different at $p \leq 0.05$ (LSD test)

A: Genotypes, B: Infestation level

of the food and act as a feeding deterrents, digestibility reducer and toxins. Several insects are sensitive to flavonols or are deterred by flavonols in feeding tests (Thoison et al. 2004; Chen et al. 2004). Flavonols, isorhamnetin-3-sophoroside-7-glucoside- and kaempferol 3, 7-diglucoside found in *B. napus* were deterrent to *Mamestra configurata* (Walker), at levels above those found in vegetative tissues (Onyilagha et al. 2004). The phytosterols, strophanthidin and strophantidol, found in *Cheiranthus* and *Erysimum* species, exhibited feeding deterrent action against different species of flea beetles, *Phyllotreta undulata* (Kutschera), *P. tetrastigma* (Comolli) and *Phaedon cochleariae* (Fabricius), but no effect on *P. nemorum* (L.) (Nielsen 1978).

Total sugars

The concentration of total sugars differed significantly among the genotypes. The total sugars content in the aphid infested plants of I_8, I_{79} and *B. fruticulosa* (75.9, 70.9 and 85.9 mg/g fresh weight of tissue, respectively) was significantly higher than BSH-1 and PBR-210 (40.6 and 32.3 mg/g, respectively) (Table 4). Aphid infestation resulted in significant reduction in total sugars concentration in plants of all the genotypes possibly due to drainage of large amount of soluble sugars in phloem sap (Fig. 1). Khattab (2007) reported that the total soluble sugars in aphid (*B. brassicae*) infested cabbage leaves were 64.21 mg/ g in contrast to 124.74 mg/ g in the uninfested cabbage leaves. Kumar et al. (2017) reported the similar result that total sugars content in *B. juncea* (9.7–10.5 mg/g), *B. napus* (10.7 mg/ g), *B. carinata* (8.3 mg/ g) and *E. sativa* (7.9 mg/g) were lower than susceptible control BSH-1 (11.1 mg/g). Xu and Liang (2016) reported that the susceptible cultivar of wheat after grain aphid infestation resulted in significant reduction in sugars level as compared to the resistant cultivar that had higher sugar content during the duration of infestation. Similarly, Zhang and Liu (2011) have also reported that there was reduction in sugar content in the susceptible wheat cultivars after infestation of cereal aphid, *Macrosiphum avenae*.

Free amino acids

The mean level of free amino acids in aphid infested plants varied from 0.30 to 0.60 mg/ g dry weight and differed significantly among all the genotypes. It was significantly higher in I_8, I_{79}, I_{82} and *B. fruticulosa* (0.60, 0.58, 0.54 and 0.56 mg/g fresh weight of tissue) as compared to 0.30 and 0.34 mg/ g in BSH-1 and PBR-210 (Table 5). Aphid infestation resulted in general decline in the content of free amino acids in all the genotypes. There were 13.0, 23.7 and 11.4% reduction in free amino acids content in I_8, I_{79} and I_{82}, respectively as compared to 31.8 and 35.8% reduction in BSH-1 and PBR-210, respectively (Fig. 1). Similar related result was reported by Khattab (2007) that the free amino acids content in aphid (*B. brassicae*) infested cabbage leaves was 3.5 mg/ g in comparison to 5.01 mg/g in the uninfested control. Likewise, El-Khawas and El-Khawas (2008) reported that the infestation of aphid (*B. brassicae*) on the cabbage plants resulted in significant decline in free amino acids from 18.0 mg/g to

Table 5 Free amino acid content (mg/g fresh weight of tissue) in the top 10 cm twig portion of different genotypes

Genotypes	Uninfested	Infested
I_8	0.69 ± 0.03 cd	0.60 ± 0.01b
I_{79}	0.76 ± 0.02d	0.58 ± 0.03b
I_{82}	0.61 ± 0.03c	0.54 ± 0.03b
B. fruticulosa	0.71 ± 0.03d	0.56 ± 0.03b
BSH-1	0.44 ± 0.01b	0.30 ± 0.03a
PBR-210	0.53 ± 0.03a	0.34 ± 0.03a
LSD ($p \leq 0.05$)	A = 0.06 B = 0.03 AB = Non significant	

Means within a column followed by same alphabet are not significantly different at $p \leq 0.05$ (LSD test)

A: Genotypes, B: Infestation level

Table 6 Aphid population on different genotypes on uninfested and infested plants

Genotypes	Mean number of aphid/ plant (Means ± SE)	
	Uninfested	Infested
I_8	0.6 ± 0.2a	54.5 ± 2.6b
I_{79}	0.4 ± 0.0a	46.6 ± 3.1b
I_{82}	0.3 ± 0.0a	47.9 ± 2.6b
B. fruticulosa	0.1 ± 0.0a	11.2 ± 1.1a
BSH-1	0.4 ± 0.2a	202.0 ± 2.5d
PBR-210	0.3 ± 0.7a	180.2 ± 10.1c
LSD ($p \leq 0.05$)	A = 6.2 B = 3.6 AB = 8.8	

Means within a column followed by same alphabet are not significantly different at $p \leq 0.05$ (LSD test)

A: Genotypes, B: Infestation level

16.0 mg/g. The aphids affect the crop by feeding on the phloem sap. Free amino acids serve as the main source of carbon and nitrogen for insect growth, development, participate in protein biosynthesis and are used in various biogenetic pathways (Douglas 2006; van Emden and Harrington 2007). Thus, amino acids are an important component of otherwise nutritionally poor aphid diet.

Aphid population

Significant differences in the *L. erysimi* population were observed among all the genotypes. The aphid population was significantly low on all the genotypes except BSH-1 and PBR-210. The genotypes *B. fruticulosa,* I_8, I_{79} and I_{82} harboured significantly lower aphid population (11.2, 54.5, 46.6 and 47.9 aphids/plant, respectively) as compared to the BSH-1 (202.0) and PBR-210 (180.2) on the infested plants (Table 6). The significant and positive correlation of glucosinolates content was recorded with the aphid population both on the uninfested ($r = 0.56$) and infested plants ($r = 0.58$) (Fig. 2), whereas the negative and significant correlations of total phenols, flavonols and free amino acids was recorded with aphid population both on uninfested ($r = -0.81$, $r = -0.73$ and $r = -0.74$, respectively) and infested plants ($r = -0.83$, $r = -0.81$ and $r = -0.73$, respectively). Thus, glucosinolates favored the multiplication of aphid while the total phenols, flavonols and free amino acids had deleterious effect on the aphid population.

The continuous co-evolutionary arms race between plants and herbivorous insects has lead to development of many defenses and complex insect-plant interactions. While many defense related genes get activated after insect attack, herbivory sometimes leads to down regulation of production of many other defense related secondary plant metabolites. The reduction in the glucosinolates content after aphid herbivory may explain part of the resistance response of the plants as *L. erysimi*, being brassica specialist, uses glucosinolates as cues for feeding and further colonization (Renwick 2002; Hopkins et al. 2009). Increased glucosinolates content was also correlated with greater damage by *Psylliodes chrysocephala* on *B. napus* (Giamoustaris and Mithen 1995). *Pieris brassicae*, another brassica specialist, once thought to be strict foliovore, preferred *B. nigra* flowers which contain extremely high levels of glucosinolates (Smallegange et al. 2007). Thus, Hegedus and Erlandson (2012) have opined that selection of lines with lower glucosinolates levels may provide some measure of resistance to specialists as the three introgression lines in the present study. During their growth plants are exposed to a number of biotic and abiotic stresses which leads to gene expression and biochemical changes resulting in increased synthesis of a number of primary and secondary metabolites. In this process, a number of signal pathways get activated as *Brassica* defense responses, including salicylic acid (SA), jasmonic acid (JA), ethylene and abscisic acid pathways (Zhao et al. 2007). The mechanisms by which metabolites induce stress tolerance remain largely unclear. Undoubtedly, controlling these different defense systems, through a better understanding, may lead to development of more resistant plant varieties. This may, ultimately, result in incorporation of HPR in *Brassica*

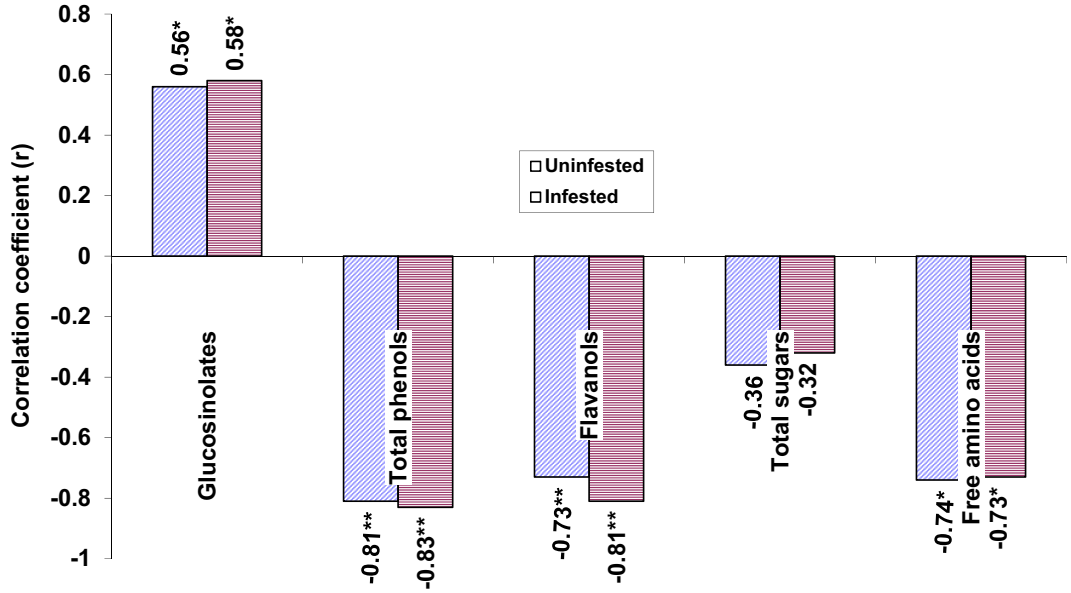

Fig. 2 Correlation of biochemical constituents with *Lipaphis erysimi* field population (**Significant at $p < 0.01$, *Significant at $p < 0.05$)

plants by using traditional (or introgressive) breeding techniques which can provide an alternative pest management strategy.

Conclusion

Brassica plants use a far more complex defense strategy than a common set of biosynthetic pathways. From the present study, it was evident that glucosinolates and total phenols served as biochemical bases of resistance in the three introgression lines and explained a part of the resistance response to *L. erysimi*, since there was down-regulation of glucosinolates and upregulation of total phenols as against opposite trend observed in BSH-1 and PBR-210. However, a general trend of decline in flavonols, total sugars and free amino acids content was observed after aphid infestation in all the genotypes including susceptible check. These plant secondary metabolites result in antibiosis mechanism in host plants. Aphids are known to induce many defense related compounds in many host plants. Dixon (1970) demonstrated that intensity of aphid feeding on sycamore trees in spring was negatively correlated with aphid performance on the same plants in autumn. Similarly, infestation of cotton plants by *Aphis gossypii* is known to reduce population growth of subsequent colonizing aphids by 30% compared to uninfested controls (Wool and Hales 1996). Thus, aphid infestation results in induction of plant resistance in resistant host plants. Since *Brassica* plants are important staple food, a proper understanding of the aphid-plant interactions and how aphid feeding affects biochemical composition of introgression lines will help in development of aphid resistant cultivars. These introgression lines can serve as important breeding tool for marker assisted transfer of aphid resistance gene(s) in the background of commercial mustard genotypes. This indigenous introgressive breeding strategy assumes special important in view of the environmental and ethical issues associated with alternate transgenic technology and the ill effects associated with use of synthetic insecticides.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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