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ORIGINAL PAPER

Antixenosis and antibiosis mechanisms of resistance to turnip aphid, *Lipaphis erysimi* (Kaltenbach) in *Brassica juncea-fruticulosa* introgression lines

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Abstract

Lipaphis erysimi is a key pest of rapeseed-mustard in Indian subcontinent. Although chemical control is the basis of its management, the unsustainability of this approach has accelerated global research efforts to find alternate solutions. Host plant resistance is one among these. A set of introgression lines were developed using *Brassica fruticulosa* previously found to be resistant to *L. erysimi*. Rigorous screening over the years led to the identification of 3 introgression lines (I_8, I_{79}, and I_{82}) for field resistance to aphids. We evaluated these introgression lines under field and laboratory conditions along with *B. fruticulosa* (resistant parent), *B. juncea* var. PBR-210 (susceptible parent) to elucidate the mechanism of resistance. Significantly a smaller number of aphids settled on circular leaf discs of *B. fruticulosa*, I_8 and I_{82} compared to that on PBR-210 after 24 and 48 h of release. A similar trend was observed in free choice field experiment with significantly less aphid colonization on *B. fruticulosa*, I_8, I_{79} and I_{82} compared to PBR-210 indicating lower aphid preference for these genotypes. Further, no choice experiments revealed significant negative effects of these genotypes on aphid demographic parameters (nymphal survival, development period, fecundity and longevity). Tolerance may not be a mechanism of resistance as aphid population failed to develop on these genotypes. Thus, resistance in these introgression lines may be attributed to a synergistic combination of antixenosis and antibiosis mechanisms.

Keywords Antibiosis · Antixenosis · Host-plant resistance · Introgression · Mustard aphid · Crop wild relatives

Key message

- Wild *B. fruticulosa* and selected *B. juncea* lines carrying genomic fragments from *B. fruticulosa* showed consistent resistance responses to turnip aphid under both laboratory and field conditions.
- Our results show the involvement of antixenosis and antibiosis mechanisms to explain *B. fruticulosa*-based resistance.
- Given the lack of genetic resistance in cultivated *Brassica* germplasm, these introgression lines can serve as an important pre-breeding germplasm for developing aphid-resistant cultivars in *B. juncea*.

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Introduction

Rapeseed-mustard is the third most important group of oilseed crops in the world after soybean and palm oil. These were cultivated on an area of 36.5 million hectares during 2018–19 with production and productivity of 72.3 million tons and 1980 kg/ha, respectively (USDA 2020). India is the third most important producer of rapeseed-mustard, accounting for 19.8 per cent of global acreage and 9.8 per cent of total production (USDA 2020). Mustard (*Brassica juncea*) is the predominant winter oilseed crop in India. Average productivity (1511 kg/ha) of mustard in India is much lower than the world average of 1980 kg/ha for rapeseed-mustard crops (SOPA 2020). Many factors are responsible

for low productivity. These include yield losses caused by insect pests such as the phloem-feeding aphids, which are of worldwide distribution. Aphids parasitize plants by manipulating their defensive responses (Giordanengo et al. 2010; Jaouannet et al. 2014; Kumar 2019). They feed through sieve elements and, while feeding, inject elicitors and transmit viruses including cabbage black ringspot and mosaic diseases of cauliflower, radish, turnip (Blackman and Eastop 1984). Continuous feeding by nymphs and adults inhibits plant growth, resulting in reduced productivity and oil content (Bakhetia 1983; Malik and Anand 1984). The turnip aphid, *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae), is a major pest of rapeseed-mustard crops in the Indian subcontinent. It is reported to cause yield losses up to 90% depending upon the severity of infestation and crop growth stage (Ahuja et al. 2010; Kular and Kumar 2011). Besides direct damage, turnip aphid is a vector of 13 plant viruses (Kennedy et al. 1962; Adhab and Schoelz 2015). The pest has high fecundity and population growth rate (Goggin 2007) due to quick generation turnover. Nymphs attain sexual maturity in less than 10 days (Goggin 2007) and a large number of nymphs and adults can cover the central surface of shoots, flowers and pods. *L. erysimi* also exhibits parthenogenetic viviparity, which not only obviates sexual reproduction but also eliminates the egg stage from life cycle. All these factors help *L. erysimi* to multiply at a faster rate. At present, the only effective and easily available strategy against this pest is the application of systemic insecticides, including the controversial neonicotinoids (El-Wakeil et al. 2013; Stapel et al. 2000). However, this mode of pest control is ecologically unsustainable because recommended insecticides are hazardous to honey bees and other friendly insects such as ladybug beetles, *Chrysoperla* spp. (Chrysopidae: Neuroptera). Further, there is an associated risk of insecticide resistance in the pest and its resurgence (Zhang et al. 2014a,b).

Plant resistance to insects can result from antixenosis, antibiosis and tolerance (Smith 2005). Reports also suggest that antixenosis and antibiosis modalities of resistance can occur simultaneously in the same host-plant (Smith 2005; Sharma 2008). Effects of various plant species or cultivars on the fitness and fecundity of aphids have been studied in many aphid-plant systems (Alvarez et al. 2006; Le Roux et al. 2008; Sun et al. 2018). These studies revealed the existence of varied defensive response to deter aphid feeding in different layers of plant tissues (Kuhlmann et al. 2013). The responses of aphids to stimuli necessary for host-plant discrimination can reflect the aphid feeding preference (Pettersson et al. 2007; Canassa et al. 2021). There is no source of resistance to aphids in the primary gene pool of crop brassicas. However, *B. rapa* and *B. juncea* are considered better hosts than *B. napus, B. nigra* and *B. carinata* (Rana 2005). Host plant resistance is an excellent option as it is effective, environment friendly and can be easily combined with prevailing integrated pest management (IPM) strategies. Moreover, this mode of pest control is self-perpetuating with the seed and may have little or no impact on non-target organisms. Unfortunately, aphid-resistant cultivars are yet to be developed due to the absence of any source of resistance in the primary gene pool of crop brassicas. A wild relative (*Brassica fruticulosa*) of crop *Brassica* possesses resistance to *Brevicoryne brassicae* (L.) (Cole 1994) and *L. erysimi* (Kumar et al. 2011). *Brassica* breeders from our group have been able to produce *B. juncea-B. fruticulosa* introgression lines (ILs) (Chandra et al. 2004; Kumar et al. 2011). Many of these ILs demonstrated field resistance to mustard aphid infestation (Atri et al. 2012) as these harboured smaller populations of *L. erysimi* compared to commercial checks. Multiple cycles of inbreeding and selection (2009–10 to 2016–17) under artificial infestation conditions have led to the identification of 3 ILs with high resistance to *L. erysimi.* Molecular-cytogenetic analysis of these lines has revealed large chromosome translocations from *B. fruticulosa* in the terminal regions of chromosomes A05, B02, B03 and B04 of the *B. juncea-B. fruticulosa* introgression lines (Agrawal et al. 2021). The aim of the present research was to develop a thorough understanding of the mechanism(s) of aphid resistance in these ILs. Such information is critical for the future deployment of the introgressed gene(s) for aphid resistance in superior agronomic bases.

Materials and methods

Experimental area

The studies were conducted in the Plant Protection Laboratory, Entomology Screen House and Oilseeds Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30.9°N and 75.85°E, 244 m above msl), India. The locale has sandy loam soil type. It has sub-tropical to semi-arid climate with both summer and winter seasons. Crop season (October/November to April) is characterized by cold winters with temperature extremes of ≤ 1 °C and ≥ 30 °C during December-January and March–April, respectively, and humidity range of 30.0 to 90.0% along with few rain showers.

Plant materials and insect culture

Plant materials comprised three ILs (I_8, I_{79} and I_{82}), which showed resistant reaction after field screening (Palial 2017), along with one wild genome donor parent, *Brassica fruticulosa* (resistant donor), female parent (*Brassica juncea* cv. PBR-210), and *Brassica rapa* ecotype brown sarson var. BSH-1 (susceptible check). *L. erysimi* nymphs and adults

were collected from naturally infested early flowering *B. rapa* plants in the field and were released on the susceptible host-plant (BSH-1) for multiplication in the screen house. Fresh plants were infested at periodic intervals to maintain regular supply of test insects for the experimentation.

Antixenosis experiments (choice test)

I_8, I_{79}, I_{82} were investigated for antixenosis along with *B. fruticulosa*, *B. juncea* cv. PBR-210 and *B. rapa* cv. BSH-1 under both laboratory and field conditions.

Choice test: a laboratory study

The feeding preference of *L. erysimi* was studied under laboratory conditions. Circular leaf discs (2-cm diameter) of the test genotypes were placed at the periphery of the Petri plates (2.5-cm height, 10 cm diameter). Young leaves of similar size were detached from the plants at the onset of flowering (45 days after sowing), the most susceptible stage of plant to aphid infestation (Kumar and Singh 2015). Discs were cut from young leaves of similar size that were detached from test plants. A moist filter paper was kept at the base of the Petri plate to maintain the turgidity of the leaf discs. 20 apterous aphids were placed in the centre of the Petri plate using a camel's hair brush. Petri plates were covered with black paper to avoid photo-tactic variations and kept in an incubator at 22 ± 1 °C with relative humidity of 62–67% and 8 h dark cycles (Kumar et al. 2011). The experiment was laid in a completely randomized design with four replications and repeated three times. For layout of the experiment refer to supplementary file Online Resource 1a. The observations on the number of aphids settled (i.e. when they visited, stayed and fed upon) on leaf discs of each genotype were recorded after 24 and 48 h as the leaf discs started drying after that. Aphids found settled on the top/sides of Petri plate were excluded from data recording.

Choice test: the field study

Test ILs were sown in large plots (12 m^2), under field conditions, following a randomized complete block design with four replications as presented in supplementary file Online Resource 1b. In all, there were 24 plots in four blocks with six plots in each block. The plots and blocks were isolated by 1.5 and 3.0 m of open space, respectively. Sowing was deliberately delayed until 17 November 2016 to ensure heavy build-up of aphids under natural conditions (Kumar et al. 2011; Atri et al. 2012). The row to row and plant to plant spacing was maintained at 30 × 15 cm, respectively, according to the agronomic practices recommended by the Punjab Agricultural University (https://www.pau.edu/content/ccil/pf/pp_rabi.pdf). Uniform doses of nitrogen and phosphorous were applied at the time of sowing. Weeds were removed manually at about 20 days after sowing. No insecticide spray was applied. At the time of pest appearance, 10 plants from 6 middle rows of each plot were selected at random and weekly data on aphid populations were collected from the top 10 cm portion of central twig. Damage ratings were also assigned in the terms of Aphid Infestation Index (AII) as per the equation reported by Bakhetia and Sandhu (1973).

Antibiosis experiment (no-choice test)

The effect of antibiosis resistance was studied under laboratory conditions in a no-choice test. For this, 10 nymphs (less than 8 h old) were placed on the fresh leaves of each genotype in the test tubes using a camel's hair brush. A wet cotton swab was placed at the petiole end of the leaf to maintain turgidity and the test tubes were plugged with cotton plugs as shown in supplementary file Online Resource 2. These leaves were replaced every alternate day and the test tubes were placed in an incubator at 22 ± 1 °C, relative humidity of 62–67% and 8 h dark cycles. Data on the nymphal survival were recorded daily. Black coloured nymphs which did not show any response to probing with fine hair brush were considered dead. We also recorded data on nymphal development, fecundity and longevity of *L. erysimi* to work out treatment means from live individuals in each replication. The number of nymphs surviving until the initiation of reproduction in the first aphid was recorded as measure of nymphal survival, while the time taken from the date of release of a nymph till it produced a nymph was recorded as a development period. For fecundity data, the number of neonate nymphs produced in each replication was recorded daily and these neonate nymphs were removed from the test tube using a camel's hair brush. Data on the adult longevity were recorded daily until mortality. The experiment was laid in a completely randomized design with four replications as shown in supplementary file Online Resource 1c and repeated three times.

Tolerance study

For this study, the aforementioned genotypes were sown in two sets in plots (12 m^2) in the factorial randomized complete block design under insecticide protected and unprotected conditions with four replications as shown in supplementary file Online Resource 1d. The genotypes served as the first factor while infestation level (uninfested and infested) served as the second factor. All genotypes were sown in four blocks. Each block comprised 12 plots in sets of two (sub plots) per genotype. One subplot of each genotype in each block served as infested plot, while the other served as uninfested plot. To avoid aphid infestation, the uninfested plots were sprayed with thiamethoxam 25WG at

the rate of 100 g ha^{-1} while natural infestation was allowed to occur in unprotected plots. Thiamethoxam is a systemic neonicotinoid insecticide recommended for the control of *L. erysimi* in Punjab (https://www.pau.edu/content/ccil/pf/pp_rabi.pdf). Plots and blocks were separated by 1.5 and 3.0 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 unprotected/infested plots.

At the time of pest appearance, 10 plants from 6 middle rows of each plot were selected at random and weekly data on aphid population from the top 10 cm portion of central twig was recorded according to Bakhetia and Sandhu (1973). Data on the grain yield were recorded from the middle 6 rows of each plot at the time of harvesting.

Statistical analysis

The data were first subjected to normality test using Kolmogorov–Smirnov test and then subjected to analysis of variance (ANOVA) through the PROC GLM using the statistical software SAS 9.1 (SAS Institute 2005). Least square differences (LSD) were used to compare treatment means when *F* test was significant ($p < 0.05$). Cv. BSH-1 was used for maintaining aphid population. Hence, it was excluded from the data analysis for laboratory experiments to avoid pre-imaginal conditioning (Mphosi and Foster 2010). The Spearman correlation coefficient was used to check the agreement between the laboratory and field data obtained from free choice experiments.

Results

Antixenosis experiments (choice test)

Feeding preference of *L. erysimi* on different genotypes under laboratory conditions

In experiment I, number of aphids settled on circular leaf discs of *B. fruticulosa* and I_{82} were significantly lower than those on PBR-210 after 24 h of release (Table 1). A similar trend was observed after 48 h, where a significantly lower number of aphids was also recorded on I_8 along with these 2 genotypes compared to PBR-210. In experiment II, also a significantly lower number of aphids settled on *B. fruticulosa*, I_8 and I_{82} compared to PBR-210 after 24 h of release. After 48 h of release, number of aphids settled on leaf discs was significantly lower on *B. fruticulosa*, I_8, I_{79} and I_{82} compared to PBR-210. In experiment III, number of aphids settled on leaf discs of all genotypes was significantly lower than those on PBR-210 after 24 and 48 h of release.

Preference of *L. erysimi* for feeding and colonization on different genotypes under field conditions

Significant differences in *L. erysimi* population were observed among the genotypes at all the observation intervals. The aphid population on *B. fruticulosa*, I_{82}, I_{79} and I_8, was significantly lower than that recorded on the BSH-1 and PBR-210 during 2nd Standard Meteorological Week (SMW). Almost similar trend was observed during 3rd to 6th SMW where *B. fruticulosa,* I_8, I_{79} and I_{82} harboured significantly lower aphid populations than that on BSH-1 and PBR-210 (Table 2). However, during 7th SMW, a significant increase in the aphid population was recorded on I_8 that was statistically at par with that recorded on BSH-1 and PBR-210. Experiments were terminated at 8th SMW

Table 1 Feeding preference of *Lipaphis erysimi* on different genotypes under laboratory conditions (Choice test) at Ludhiana, Punjab, India during 2017

Genotypes	Mean number of aphids on each genotype (Hrs. after release) (Means ± SE)					
	Experiment I		Experiment II		Experiment III	
	24	48	24	48	24	48
I_8	3.5 ± 0.5b	1.5 ± 0.7a	1.3 ± 0.3a	1.8 ± 0.6a	2.0 ± 0.4ab	1.5 ± 0.3a
I_{79}	4.5 ± 0.5b	5.5 ± 0.8b	7.3 ± 1.6c	3.8 ± 1.1a	3.5 ± 0.9b	3.0 ± 0.4b
I_{82}	1.5 ± 0.5a	1.5 ± 0.3a	1.8 ± 0.3a	1.8 ± 0.3a	2.3 ± 0.3ab	1.8 ± 0.5a
B. fruticulosa	0.8 ± 0.8a	1.5 ± 0.3a	0.8 ± 0.5a	1.3 ± 0.5a	1.0 ± 0.4a	1.0 ± 0.0a
PBR-210	4.2 ± 0.3b	4.5 ± 0.8b	4.3 ± 0.3b	6.8 ± 1.6b	5.5 ± 0.9c	5.5 ± 0.5c
F	10.29	9.12	12.29	5.81	7.24	9.12
P	0.0003	0.0006	0.0001	0.0049	0.0018	0.0006

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$

Total number of aphids in each replicate trial was 20

Table 2 Relative population of *Lipaphis erysimi* during different standard meteorological weeks (SMWs) on different genotypes in the field under free choice conditions at Ludhiana, Punjab, India during 2017

Genotypes	Number of aphids per top 10 cm twig (Means ± SE)					
	IInd SMW	IIIrd SMW	IVth SMW	Vth SMW	VIth SMW	VIIth SMW
I_8	1.1 ± 0.6a	7.2 ± 4.1a	40.6 ± 20.3a	70.5 ± 42.0a	197.0 ± 24.8c	870.3 ± 435.3b
I_{79}	1.3 ± 0.5a	6.2 ± 2.7a	24.3 ± 10.1a	48.8 ± 22.6a	116.0 ± 35.5bc	282.2 ± 131.7a
I_{82}	0.9 ± 0.3a	3.8 ± 0.9a	6.9 ± 1.7a	12.3 ± 0.9a	58.2 ± 6.1ab	133.3 ± 51.3a
B. fruticulosa	0.0 ± 0.0a	0.9 ± 0.9a	0.8 ± 0.8a	0.5 ± 0.3a	15.3 ± 4.0a	37.0 ± 10.1a
BSH-1	65.7 ± 2.8b	93.7 ± 6.5b	358.4 ± 239.7b	568.0 ± 104.1b	837 ± 7.1d	1104.7 ± 24.1b
PBR -210	77.2 ± 3.1c	193.2 ± 26.4c	592.3 ± 44.3b	723.9 ± 107.3b	1154.8 ± 49.5e	1237.7 ± 12.9b
F	39.10	45.10	6.35	30.87	369.20	7.14
P	< 0.0001	< 0.0001	0.0066	< 0.0001	< 0.0001	0.0043

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$

A sudden decline in population was observed during VIIIth and IXth SMW due to thundershowers which did not develop further

due to thundershowers and a consequent decline in the aphid population.

Aphid Infestation Index (AII) was measured on the basis of the degree of damage to host plants (Bakhetia and Sandhu 1973). I_8 and I_{79} exhibited resistance response till 5th SMW, while *B. fruticulosa* and I_{82} exhibited it until 6th SMW (Fig. 1) compared with highly susceptible responses of BSH-1 and PBR-210. The exceptionally high aphid pressure led to the breakdown of resistance even in the ILs at the later stages of crop growth. However, *B. fruticulosa* maintained its resistance until maturity. The Spearman rank correlation coefficient indicated strong correlation between the resistance responses between laboratory and field evaluations at all observation intervals, barring experiment II at 24 h interval (Table 3).

Antibiosis experiments (no-choice test)

The effect of different genotypes on nymphal survival of *L. erysimi*

In experiment I, nymphal survival on *B. fruticulosa* and I_{79} was significantly lower than that on the other genotypes after 3 days of release (DAR) (Table 4). A general decline in nymphal survival was observed with the passage of time and the genotypic differences became more evident. After 6 days of release, the nymphal survival on *B. fruticulosa*

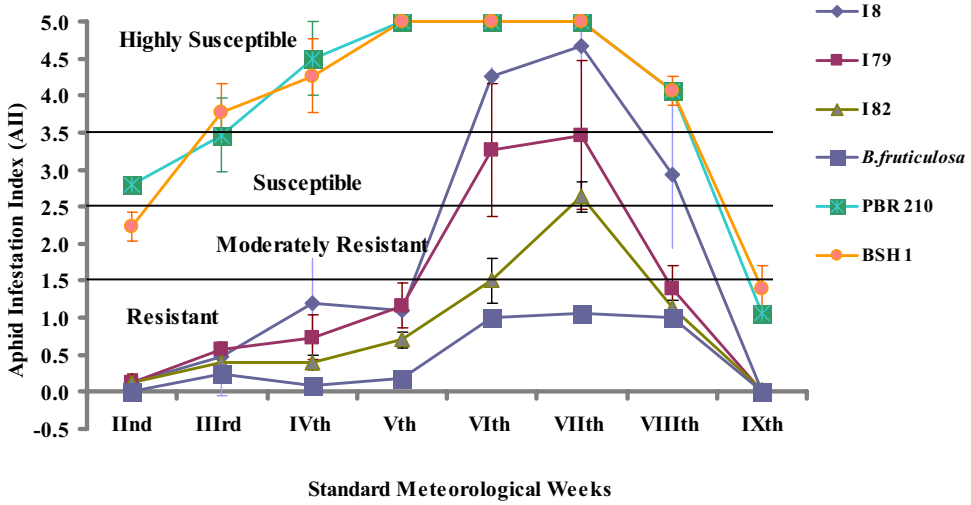

Fig. 1 Temporal variation in resistance response of introgression lines in the field under free choice conditions based on Aphid Infestation Index (AII). Introgression lines I_8 and I_{79} maintained their resistance response till 5th Standard Meteorological Week (SMW) while I_{82} till 6th SMW as against susceptible reaction observed in BSH 1 and PBR 210. After this, exceptionally high aphid pressure development lead to the breakdown of resistance.AII 0.0–1.5: Resistant; 1.51–2.5: Moderately resistant; 2.51–3.5: Susceptible and > 3.5: Highly susceptible (Bakhetia and Sandhu 1973)

Table 3 Spearman rank correlation between the aphid population under laboratory and field conditions in free choice test

Field population	Exp I		Exp II		Exp III	
	24 h	48 h	24 h	48 h	24 h	48 h
IInd SMW	0.90*	0.90*	0.80*	0.97*	0.90*	0.90*
IIIrd SMW	0.70*	0.70*	0.50	0.83*	0.70*	0.70*
IVth SMW	0.70*	0.70*	0.50	0.83*	0.70*	0.70*
Vth SMW	0.70*	0.70*	0.50	0.83*	0.70*	0.70*
VIth SMW	0.70*	0.70*	0.50	0.83*	0.70*	0.70*
VIIth SMW	0.70*	0.70*	0.50	0.83*	0.70*	0.70*

*Significant at $p \leq 0.05$

and I_8, I_{79} and I_{82} was significantly lower than that recorded on PBR-210. A further decline in nymphal survival was observed after 9 days of release with no survival observed on *B. fruticulosa* and only 15.0, 22.5, and 25.0% survival on I_8, I_{82}, and I_{79}, respectively, which was significantly lower than that on PBR-210. Almost similar trend was observed after 12 days of release. In the experiment II, the nymphal survival on *B. fruticulosa,* I_8, I_{79} and I_{82} was significantly lower than on BSH-1 and PBR-210 at the observation intervals. A decline in nymphal survival was observed after 6 days of release with significantly lower survival on *B. fruticulosa,* I_8, I_{79} and I_{82}, compared to PBR-210. A progressive decline in nymphal survival was observed after 9 and 12 days of release with a trend similar to that observed after 6 days of release. Thus, after 12 days of release only 12.5, 17.5, 20.0, and 22.5 per cent nymphs survived on *B. fruticulosa*, I_{82}, I_{79} and I_8, respectively, as against significantly high survival (65.0%) on PBR-210. Almost similar trend was observed in experiment III. Nymphal survival was significantly lower on *B. fruticulosa,* I_8, I_{79} and I_{82} than that on PBR-210.

The effect of different genotypes on nymphal development period of *L. erysimi*

Significant differences in the nymphal development period were observed among the genotypes in all 3 experiments. In experiment I, nymphal development period was not determined on *B. fruticulosa* due to complete nymphal mortality. Nymphs took significantly longer to complete their development on I_{79}, I_8, and I_{82} as compared to that on PBR-210 (Fig. 2a). Almost similar trend was observed in experiment II and III.

Effect of different genotypes on *L. erysimi* fecundity

In experiment I, there was no nymphal survival on *B. fruticulosa*, fecundity could not be determined. However, female fecundity on I_{82}, I_8, and I_{79} was significantly lower than that observed on PBR-210 (Fig. 2b). A similar trend was observed in experiment II and III.

Effect of different genotypes on adult longevity of *L. erysimi*

In experiment I, *L. erysimi* adults survived for a maximum of only 3.5, 3.8, and 3.9 days when reared on I_8, I_{82} and I_{79}, respectively, as against significantly higher adult longevity on PBR-210 (7.5 days) (Fig. 2c). In experiment II, *L. erysimi* adults survived for a significantly lower number of days when reared on I_{82}, *B. fruticulosa* and I_8 compared to that on PBR-210. Almost similar trend was observed in experiment III with significantly lower adult longevity on I_{82}, I_8 and *B. fruticulosa* than that on PBR-210.

The tolerance study under protected and unprotected conditions

The aphid population on different genotypes ranged from 46.6 to 202.0 plant^{-1}. In unprotected set, the mean aphid population on I_{79}, I_{82} and I_8 was significantly lower than that on PBR-210 and BSH-1 (Table 5).

In the protected set, the seed yield among the different genotypes ranged from 1031.2 to 1855.5 kg per hectare. The maximum seed yield of 1855.5 kg per hectare was obtained in PBR-210 followed by I_{82} (1759.5 kg/ha), I_{79} (1664.5), I_8 (1658.0) and BSH-1 (1031.2). The corresponding yield in unprotected set was 1398.7, 1679.2, 1556.7, 1621.7, and 672.0 kg hectare^{-1}. These 3 genotypes suffered significantly lower loss in seed yield compared to BSH-1 and PBR-210. I_8 suffered merely 2.2 per cent loss in seed yield followed by 4.6 and 6.5 per cent in I_{82} and I_{79} as compared to significantly high yield loss in PBR-210 and BSH-1 (24.6 and 34.8%, respectively). Further comparison of the three ILs with PBR-210 (check) in unprotected set revealed that the yield of ILs was significantly higher than that recorded for PBR-210, these lines harboured significantly lower aphid population than PBR-210. Correlation analysis revealed significantly negative correlation between the aphid population in unprotected set with yield ($r = -0.91$). First principal component showed maximum eigenvalue (1.9052), explaining 95 per cent of the observed phenotypic variation (95.26%). Genotypic differentiation between the resistant ILs and susceptible cv. PBR 210 and their stability of

Table 4 Effect of different genotypes on nymphal survival of *Lipaphis erysimi* at Ludhiana, Punjab, India during 2017

Genotypes	Nymphal survival % (Mean ± SE)											
	Experiment I				Experiment II				Experiment III			
	3DAR#	6DAR	9DAR	12DAR	3DAR	6DAR	9DAR	12DAR	3DAR	6DAR	9DAR	12DAR
I_8	90.0 ± 4.1bc	40.0 ± 7.1b	15.0 ± 2.9ab	15.0 ± 2.9a	90.0 ± 5.8bc	50.0 ± 4.1b	22.5 ± 4.8ab	22.5 ± 4.8a	67.5 ± 4.8b	30.0 ± 4.1a	22.5 ± 2.5a	10.0 ± 5.8a
I_{79}	75.0 ± 6.5ab	50.0 ± 7.1b	25.0 ± 5.0b	25.0 ± 5.0ab	85.0 ± 6.5bc	50.0 ± 5.8b	30.0 ± 4.1b	20.0 ± 4.1a	52.5 ± 4.8a	40.0 ± 4.1a	27.5 ± 2.5a	15.0 ± 6.5a
I_{82}	85.0 ± 2.9bc	55.0 ± 2.9b	22.5 ± 6.3b	22.5 ± 6.3ab	62.0 ± 18.0b	52.5 ± 4.8b	25.0 ± 2.9ab	17.5 ± 4.8a	57.5 ± 4.8ab	35.0 ± 2.9a	22.5 ± 2.5a	7.5 ± 4.8a
B. fruticulosa	67.5 ± 7.5a	20.0 ± 4.1a	0.0 ± 0.0a	0.0 ± 0.0a	55.0 ± 2.9a	32.5 ± 4.8a	12.5 ± 4.8a	12.5 ± 4.8a	70.0 ± 4.1b	30.0 ± 4.1a	20.0 ± 2.5a	20.0 ± 4.1a
PBR-210	97.5 ± 2.5c	82.5 ± 4.8c	62.5 ± 7.5c	62.5 ± 7.5c	97.5 ± 2.5c	77.5 ± 2.5c	65.0 ± 2.9c	65.0 ± 2.9b	95.0 ± 2.9c	77.5 ± 2.5b	65.0 ± 2.9b	62.5 ± 2.5b
F	5.49	17.57	20.86	20.87	14.58	12.70	25.38	24.16	14.43	30.97	40.93	21.13
P	0.0062	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$

[#]DAR: Days after release

performance was also apparent from principal component analysis (Please see supplementary file Online Resource 3 for details).

Discussion

Aphid infestations can be a major cause of concern for mustard growers due the adverse consequences in terms of production loss and impaired seed quality. Many abiotic and biotic factors regulate aphid-plant attraction, colonization, and damage. These include phenological stage of host-plant at the time of infestation, host preference, insect population density, weather and prevalence of aphid natural enemies. Damage is higher when the infestation occurs at flowering and pod formation stages. Aphid settling and ease of reproduction on resistant versus susceptible host plants are the key determinants of pest population density. This process involves host recognition, defence signalling and intercellular trafficking of macromolecules. Studies have shown that dominant cues controlling plant preference and initiation of reproduction are recognized early during the stylet penetration, well before initial contact with the phloem (Klingauf 1987; Powell et al. 2006). Aphids use volatile organic compounds (VOCs) such as nitriles, isothiocyanates and monoterpenes as olfactory cues (Visser and Piron 1997). However, there are conflicting views regarding the role of volatile organic compounds and epicuticular waxes in defining resistance responses to herbivores (Heil 2014; Wójcicka 2015; Hondelmann et al. 2020; Canassa et al. 2020). Further, *L. erysimi* can also distinguish between virus infected and healthy brassica plants (Adhab et al. 2019), but fortunately, it does not transmit any plant virus in oilseed brassica in this part of the country. Productivity losses can be minimized if plants are endowed with the capacity to reduce survival and reproduction of aphids (antibiosis). Damage can also be avoided if there is non-preference for the host (antixenosis).

Many reports show variability for both the factors in Indian mustard germplasm (Teotia and Lal 1970; Yadava et al. 1985; Angadi et al. 1987; Chetterjee and Sengupta 1987; Kalra et al. 1987; Rohilla et al. 1999). However, none of these reported sources of variation has helped in the evolution of resistant varieties because the levels of reported resistance were non-reproducible and non-heritable. Thus, it was considered important to use the resistance to *L. erysimi* available in the wild *Brassicaceae* species *B. fruticulosa*. As described earlier, the ILs used in this study were selected after 8 years of rigorous field screening under artificial infestation conditions for resistance to *L. erysimi*. Present studies were conducted to analyse mechanism(s) of resistance. We strived to minimize the influence of various biotic or abiotic factors on resistance responses of the test genotypes by substantiating field outcomes with laboratory

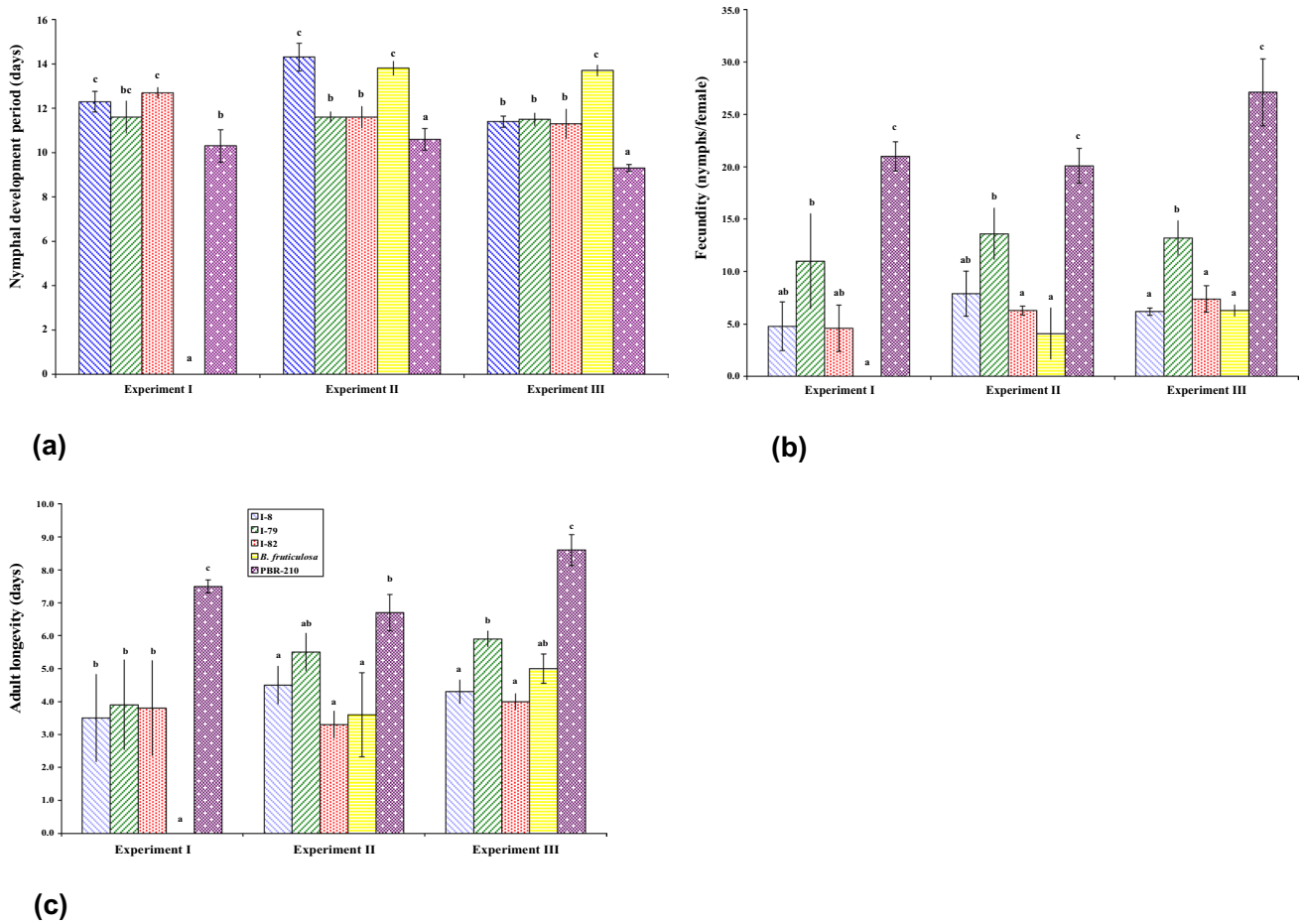

Fig. 2 Effect of different genotypes on some of the demographic parameters of *Lipaphis erysimi* (no choice test under laboratory conditions): **a** Nymphal development period **b** Fecundity and **c** Adult longevity.—Nymphs took significantly higher number of days to complete their development when reared on three introgression lines and *B. fruticulosa* compared to BSH 1 and PBR 210 (see part **a**) while opposite was true for fecundity and adult longevity (see part **b** and **c**)—Bars with same alphabet at the top did not differ significantly at $p < 0.05$ according to LSD

Table 5 Comparative aphid population on and yield of different genotypes under protected and unprotected conditions (Tolerance study) at Ludhiana during 2017

Genotypes	Mean no of aphids plant^{-1*}		Yield (kg/ha) (Means ± SE)		Yield loss
	Protected†	Unprotected	Protected†	Unprotected	
I_8	0.6 ± 0.2a	54.5 ± 2.6a	1658.0 ± 48.5b	1621.7 ± 42.7c	2.2a
I_{79}	0.4 ± 0.0a	46.6 ± 3.1a	1664.5 ± 51.2b	1556.7 ± 32.2c	6.5a
I_{82}	0.3 ± 0.0a	47.9 ± 2.6a	1759.5 ± 33.2b	1679.2 ± 67.0c	4.6a
BSH-1	0.4 ± 0.2a	202.0 ± 2.5c	1031.2 ± 25.0a	672.0 ± 20.7a	34.8b
PBR-210	0.3 ± 0.7a	180.2 ± 10.1b	1855.5 ± 45.2c	1398.7 ± 41.7b	24.6b
F	1.08	392.22	49.17	72.15	13.31
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*Mean of five observations

†Protected set was sprayed with thiamethoxam 25 WG at the rate of 100 g ha^{-1}

Means within a column followed by the same alphabet did not differ significantly at $p \leq 0.05$

evaluations. Present studies involved a series of free choice and no choice tests under field and laboratory conditions. Free choice tests, conducted under both laboratory and field conditions, demonstrated that the introgressed resistance resulted from high levels of antixenosis. Same were true for the resistance donor species, *B. fruticulosa*. *L. erysimi*

showed least preference for feeding and colonization on the 3 ILs and *B. fruticulosa*. Although there were some variations in the number of aphids settling on leaf discs of ILs under laboratory conditions, such variations were not observed on intact plants in the free choice field study. I_8 and I_{82} showed consistent resistance response in all 3 experiments except for I_8 at 24 h observation interval when it was at par with PBR-210. Detached leaf assay is a rapid, reliable method to screen insect resistance in many crops (Girousse and Bournoville 1994; Sharma et al. 2005; Ulusoy and Olmez-Bayhan 2006; Kumar et al. 2011; Chen et al. 2012; Shankar et al. 2013; Chongtham et al. 2017; Novak et al. 2019). The use of leaf discs instead of intact plants may reduce the intensity of the resistance effect, but sufficiently replicated experiments normally result in similar conclusions (Kloth et al. 2015). Spearman rank correlation analysis in our studies also revealed a significant positive correlation of aphid data under laboratory conditions with that under field conditions on intact plants except for 24 h interval in experiment II. Antixenosis to feeding on *B. fruticulosa* has been reported earlier for both *L. erysimi* (Kumar et al. 2011) and *B. brassicae* (Cole 1994). This mode of resistance resulted in a reduced duration of passive phloem uptake and quicker withdrawal of aphid stylets from the phloem on *B. fruticulosa* compared to *B. oleracea* var. *capitata* cv. 'Offenhafm Compacta' (Cole 1994). Resistance to pea aphid (*Acyrthosiphon pisum*) in two genotypes of *Medicago sativa* was due to lower exudation rates compared to susceptible genotypes (Girousse and Bournoville 1994). In oilseed *Brassica*, flowering is the most susceptible stage for aphid infestation (Kumar and Singh 2015). The crop season in 2016–17 witnessed exceptionally high aphid pressure. Despite that, ILs I_8 and I_{79} maintained their resistance response until the 5th SMW while I_{82} maintained it until the 6th SMW. After this, exceptionally high population pressure and massive migration of aphids from the plants dried up due to excessive aphid feeding overwhelmed the resistance responses of three ILs which were still green (See supplementary file Online Resource 4). Exceptionally high pest densities are known to overwhelm resistance responses ("association susceptibility") (White and Whitham 2000; Santolamazza-Carbone et al. 2014). Biochemical studies in the test ILs also showed the downregulation of glucosinolates and upregulation of total phenols. Reverse was true for susceptible genotypes PBR-210 and BSH-1 (Palial et al. 2018). Significant differences in the nymphal survival, nymphal development period, female fecundity and adult longevity were noted following release of neonate nymphs on the detached leaves. *B. fruticulosa*, I_8, I_{79} and I_{82} exhibited strong antibiosis resistance, with < 20 per cent nymphal survival. Similarly, nymphal development period was significantly longer, while female fecundity and adult longevity were significantly lower than BSH-1 and PBR-210. The minimum feeding preference for *B. fruticulosa* by *B. brassicae* was previously observed by Ellis and Farrel (1995) who reported a significantly lower number of aphids on *B. fruticulosa* as compared to the susceptible *B. oleracea* var. *italica* cultivar 'Green Glaze Glossy'. Other studies have also shown very high levels of antixenosis and antibiosis resistance in *B. fruticulosa* (Singh et al. 1994; Pink et al. 2008). Tolerance seemed to be of little importance as a mechanism of resistance since high level of antixenosis and antibiosis resistance in the 3 ILs and *B. fruticulosa* did not allow the aphid population to develop even in unprotected plots and aphid population remained below the economic threshold level of 60 aphids plant^{-1} (https://www.pau.edu/content/ccil/pf/pp_rabi.pdf) both in the protected and unprotected plots.

Plant resistance is a valuable tool to manage aphid pests (Bhatia et al. 2011; Kumar et al. 2011; Kumar and Banga 2017). Unfortunately, it has not been possible to discover any source of resistance to *L. erysimi* in the primary gene pool of crop brassicas. The loss of ancestral defensive traits is a ubiquitous outcome of domestication and a long history of directed selection for enhanced productivity. High level of resistance to many biotic and abiotic stresses has been almost always noted in wild *Brassicaceae* species, where natural selection favours survival under adverse growing conditions (Gupta and Banga 2020). Alien introgressions can help recover lost genes by moving desirable genes from wild to cultivated plants (Dosdall and Kott 2006). However, introgression of resistance from wild species is a long and tedious process with uncertain outcomes. Then, there is the problem of concurrent introgression of unwanted variation, also known as linkage drag. It is now possible to enhance the value of wild genetic resources following integration with modern tools of plant biotechnology (Zhang and Batley 2020; Agrawal et al. 2021). Our studies have provided significant insights into proven aphid resistance responses of *B. juncea-B. fruticulosa* ILs by establishing antixenosis and antibiosis as mechanisms of host-plant resistance. This study is an important milestone in breeding for resistance as understanding the mechanisms of resistance and its components is the key to resistant cultivar development. Moreover, the development of genotypes with varied constellation of introgressed genes for resistance will be useful for understanding the role of genetic background on efficiency and durability of resistance. Shotgun sequences of these ILs are now available. Initial studies have allowed the identification of 17 candidate genes. These included the genes associated with jasmonate regulated plant defence pathways and glucosinolate accumulation in response to phloem-feeding, wounding and oxidative stresses (Banga unpublished). Such information is necessary to develop an effective framework for marker-assisted transfer of introgressed resistance to the cultivated backgrounds and to reduce linkage drag that often masks introgressed variation. The longevity of such resistant

germplasm can be extended if it is used as a component of integrated pest management strategy rather than an approach used in isolation.

Author contributions

SP conducted experiments and collected the data, SK designed the experiments and wrote the manuscript, SS helped in statistical analysis of data, SSB and CA developed the *B. juncea-B. fruticulosa* introgression lines used in the studies. SSB edited the manuscript.

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Declarations

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

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