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

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Introduction

- **Turnip aphid is a serious pest of rapeseed-mustard in Indian subcontinent and many other countries of the world.**
- **Losses range from 19-96 % (Bakhetia and Sekhon, 1989; Singh and Sharma, 2002).**

- **At present, there is no genetic solution to this pest and it is largely managed by insecticidal chemicals which have their own adverse effects.**

➢ **Thus, the need for alternate pest management strategy is always sought after.**

- **Host plant resistance (HPR) is considered important alternate pest management strategy – offers effective, economical and environment friendly method of pest management.**

- **One wild species *Brassica fruticulosa* has been reported to be resistant to this pest (Kumar *et al.* 2011).**

- **A set of introgression lines were developed from *B. fruticulosa* x *B. juncea* with the objective to transfer resistant trait to *B. juncea* background.**

- **The resistant introgression lines can serve as an important breeding tool in development of aphid resistant cultivars.**

Objectives of the study

1. To screen a set of *Brassica juncea - fruticulosa* introgression lines for resistance against turnip aphid.
2. To study the mechanism of resistance in the introgressed material.

EXPERIMENTS

- **Field screening of introgression lines for resistance against *Lipaphis erysimi* (Phenotyping for aphid resistance)**

- **Study of mechanism of resistance in the selected introgression lines**

  - **Study of antixenosis**
  - **Study of antibiosis**
  - **Study of tolerance**

Field screening (Plant Phenotyping)

- **A set of 133 introgression lines were screened under field conditions along with**
  - ***B. fruticulosa* (male parent, resistant donor),**
  - ***B. juncea* var. PBR 210 (female parent, susceptible, cultivated variety),**
  - ***B. rapa* var. BSH 1 (susceptible check) as per the standard procedure (Bakhetia and Sandhu, 1973).**
- **Aphids were artificially released on 10 randomly selected plants in each genotype @ 20 apterae/ plant and covered with nylon mesh bags (40 mesh) (10 x 20 inch).**

Field screening of introgression lines after artificial release of aphids

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Study of mechanism of resistance

- For the study of mechanism of resistance, the three introgression lines were evaluated under field and laboratory conditions in a series of choice (both field and lab study) and no choice tests (lab study).

Set I: Study of Antixenosis (Free Choice experiment)

a) Field Experiment:

- **Plot size: 4 x 3 m** **Design: RBD**
- **Replications: 4** **Entries: I_8, I_{79}, I_{82}, *B. fruticulosa*, PBR 210 and BSH 1**

- **All the six genotypes were sown under field conditions and natural infestation was allowed to take place (free choice condition).**
- **Weekly data on number of aphids from top 10 cm central twig of 10 randomly selected plants in each plot were recorded.**

b) Laboratory Experiment

Design: CRD **Replications: Four**

Entries: I_8, I_{79}, I_{82}, *B. fruticulosa*, PBR 210 and BSH 1

- **Under laboratory conditions, circular leaf bits (2 cm diameter) of these lines were placed in periphery of the Petri plate (10 cm diameter) on moist filter paper.**

- **In the centre of the Petri plate, 20 apterous aphids maintained in the culture were placed with the help of Camel's hair brush (Kumar *et al* 2011)**
- **The Petri plates were covered with black paper to avoid any phototactic response and kept in BOD incubator at 22±1°C**

- **The experiment was repeated thrice**

Observations

- **The number of aphids settled on circular leaf bits of introgression lines were recorded after 24 and 48 hours.**

Set II: Study of Antibiosis (No Choice Experiment)

Design: CRD **Replications:** Four

- For the study of antibiosis, fresh leaves of introgression lines to be tested, were placed in test tubes and five nymphs (<8 h old) were released on each leaf.
- 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
- The leaves were changed every alternate day and the test tubes were placed in B.O.D. incubator at 22±1°C.

Observations:

- Daily observations on the nymphal survival, nymphal development, fecundity and adult longevity were made.
- The experiment was repeated thrice.

Set III: Study of Tolerance

Plot size: 4 x 3 m
Treatments: Two (Protected and Unprotected)
Design: RBD **Replications:** four

- For the study of tolerance, two sets (protected and unprotected) of introgression lines were sown under field conditions

- At the appearance of turnip aphid, the protected set was sprayed with thiamethoxam 25WG @ 100 g per ha while the other set was left unsprayed

Observations:

- ✓ Weekly data on aphid population from top 10 cm central twig of 10 randomly selected plants was recorded
- ✓ Yield data recorded at harvest

RESULTS

- **Out of 133 introgression lines evaluated, three introgression lines I_8, I_{79} and I_{82} exhibited consistent resistant reaction after two years of field screening (2015-16, 2016-17)**

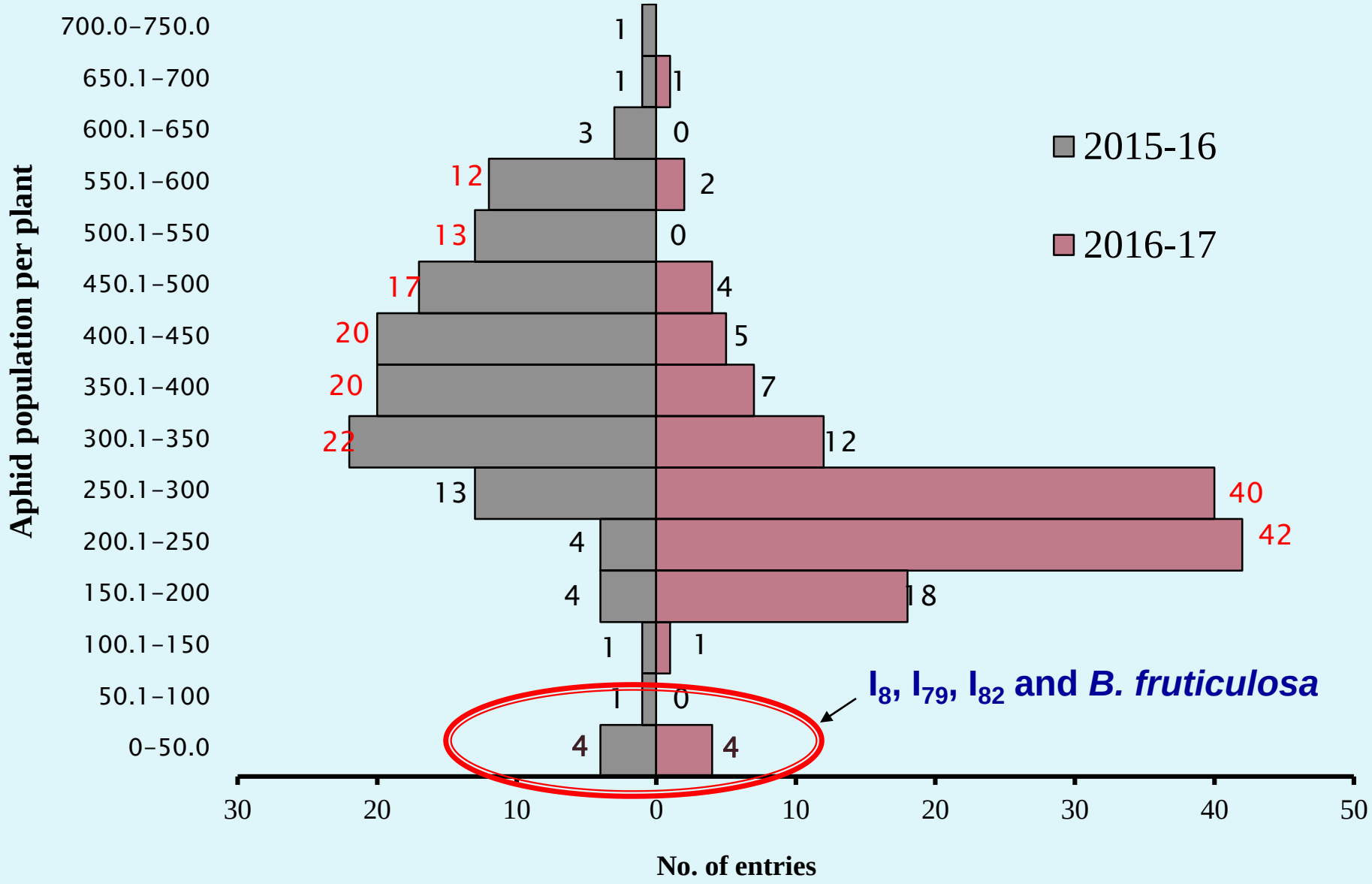

Fig. 1. Reaction of introgression lines to turnip aphid infestation based on aphid population

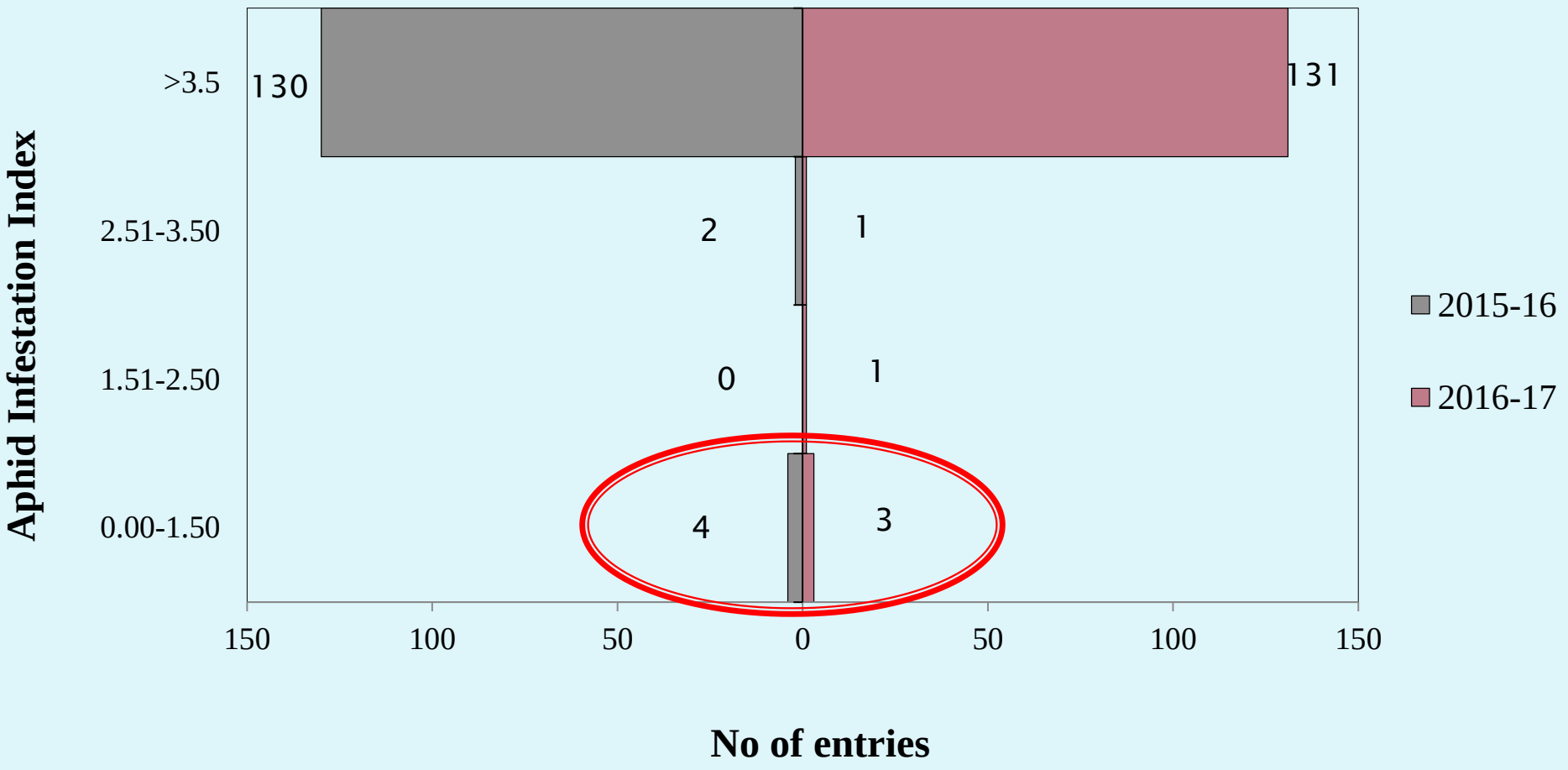

Fig. 2. Reaction of introgression lines to turnip aphid infestation based on Aphid Infestation Index (AII)

Aphid Infestation Index (AII): 0.00-1.50: Resistant
1.51-2.50: Moderately Resistant
2.51-3.50: Susceptible
>3.5 : Highly susceptible (Bakhetia and Sandhu, 1973)

A general view of the field showing all the lines dried up after severe aphid damage while the resistant introgression lines are still green

Resistant

Susceptible

Close up of resistant and susceptible lines

FREE CHOICE FIELD EXPERIMENT

Table 1. Relative population of *Lipaphis erysimi* on different genotypes in the field under free choice conditions

Geno-types	Number of aphids per plant on top 10 cm central twig								
	Standard Meteorological Week (SMW)								
	IInd	IIIrd	IVth	Vth	VIth	VIIth	VIIIth	IXth	Pooled mean
I_8	1.1±0.6a	7.2±4.1a	40.6±20.3a	70.5±42.0a	197.0±24.8c	870.3±435b	173.7±113b	0.4±0.4a	226.6±72.0b
I_{79}	1.3±0.5a	6.2±2.7a	24.3±10.1a	48.8±22.6a	116.0±35.5 bc	282.2±132.a	38.3±10.6a	0.2±0.2a	85.9±34.6a
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	29.7±2.6a	0.4±0.4a	40.7±8.3a
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	13.5±1.8a	0.0±0.0a	11.4±2.6a
BSH 1	65.7±2.8b	93.7± 6.5b	358.4±239.7b	568.0±104b	837±7.1d	1104.7±24b	143.7±27.9b	43.7±6.4b	592.5±54.8c
PBR 210	77.2±3.1c	193.2±26.4c	592.3±44.b	723.9±107b	1154.8±49.e	1237.7±13b	102.0±2.4b	37.7±1.0b	667.3±18.5d

In a column, means followed by same alphabet are not significantly different at $p<0.05$

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

FREE CHOICE LAB EXPERIMENT

Table 2. Feeding preference of *Lipaphis erysimi* on different genotypes under laboratory conditions (Choice test)

Geno-types	Mean number of aphids settled on circular leaf bits of each genotype (Hrs. after release)							
	Experiment I		Experiment II		Experiment III		Pooled Mean	
	24	48	24	48	24	48	24	48
I_8	3.5±0.5b	1.5± 0.7b	1.3±0.3c	1.8± 0.6c	2.0±0.4bc	1.5± 0.3d	2.3±0.3b	1.6±0.2c
I_{79}	4.5±0.5ab	5.5± 0.8a	7.3±1.6a	3.8± 1.1c	3.5±0.9b	3.0± 0.4c	5.1±0.9a	4.1±0.5b
I_{82}	1.5±0.5c	1.5± 0.3b	1.8±0.3c	1.8± 0.3c	2.3±0.3bc	1.8± 0.5cd	1.8±0.2b	1.7±0.2c
B.fruticulosa	0.8±0.8c	1.5± 0.3b	0.8±0.5c	1.3± 0.5c	1.0±0.4c	1.0± 0.0d	0.8±0.2b	1.3±0.2c
BSH-1	5.5± 0.5a	5.5± 1.0a	4.8± 1.0b	4.8± 0.5ab	5.8±0.8a	7.3± 0.8a	5.3± 0.6a	5.8± 0.3a
PBR-210	4.2±0.3ab	4.5± 0.8a	4.3±0.3b	6.8± 1.6a	5.5±0.9a	5.5± 0.5b	4.7±0.3a	5.9±0.9a

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

NO CHOICE EXPERIMENT

Table 3. Effect of different genotypes on nymphal survival of *Lipaphis erysimi*

Genotypes	Nymphal survival (%) Days after release (DAR)							
	Experiment I				Experiment II			
	3DAR	6DAR	9DAR	12DAR	3DAR	6DAR	9DAR	12DAR
I_8	90.0±4.1c	40.0 ±7.1b	15.0 ±2.9b	15.0 ±2.9b	90.0±5.8bc	50.0±4.1b	22.5±4.8ab	22.5 ±4.8a
I_{79}	75.0±6.5ab	50.0 ±7.1b	25.0 ±5.0b	25.0 ±5.0b	85.0± 6.5b	50.0±5.8b	30.0 ±4.1b	20.0 ±4.1a
I_{82}	85.0±2.9bc	55.0 ±2.9b	22.5 ±6.3b	22.5 ±6.3b	62.0±18.0b	52.5±4.8b	25.0±2.9ab	17.5 ±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
BSH 1	90.0±0.0c	87.5 ±2.5c	77.5 ±2.5d	75.0 ±2.9c	90.0±0.0bc	82.5 ±2.5c	75.0 ± 5.0c	70.0±4.1b
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

Means within a column followed by the same letter are not significantly different at p≤0.05 according to DMRT

contd…

Table 3 continued

Genotypes	Nymphal survival (%)							
	Experiment III				Pooled Mean			
	3DAR	6DAR	9DAR	12DAR	3DAR	6DAR	9DAR	12DAR
I_8	67.5 ±4.8b	30.0± 4.1a	22.5± 2.5a	10.0 ±5.8a	82.5±2.1b	40.0±2.4b	20.0±3.0b	15.8 ±4.4a
I_{79}	52.5 ± 4.8a	40.0 ±4.1a	27.5± 2.5a	15.0 ±6.5a	70.8 ±3.9a	46.7±4.3c	27.5±3.2c	20.0 ±1.9a
I_{82}	57.5±4.8ab	35.0± 2.9a	22.5± 2.5a	7.5 ±4.8a	68.2 ±5.2a	47.5±0.8c	23.3±1.4bc	15.8 ±2.5a
B. fruticulosa	70.0±4.1b	30.0 ±4.1a	20.0±2.5a	20.0 ±4.1a	64.2±3.7a	27.5±0.8a	10.8±2.1a	10.8 ±2.1a
BSH 1	97.5 ±2.5c	85.0±2.9b	80.0±4.1c	80.0 ±4.1c	92.5 ±0.8c	85.0±0.9d	77.5±1.6e	75.0 ±2.2c
PBR 210	95.0 ± 2.9c	77.5 ±2.5b	65.0±2.9b	62.5±2.5b	96.7±1.4c	79.2±1.6d	64.1±2.5d	63.3 ±3b

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

Table 4. Effect of different genotypes on nymphal development period of *L. erysimi*

Genotypes	Developmental period (Days) (Means ± SE)			
	Experiment I	Experiment II	Experiment III	Pooled mean
I_8	12.3±0.5d	14.3±0.6c	11.4±0.3b	12.7±0.2c
I_{79}	11.6±0.7cd	11.6±0.2b	11.5±0.3b	11.6±0.2b
I_{82}	12.7±0.2d	11.6±0.5b	11.3±0.7b	11.8±0.3b
B. fruticulosa	0.0±0.0a	13.8±0.3c	13.7±0.3c	13.8±0.3d
BSH-1	9.8±0.2b	9.7±0.5a	9.1±0.2a	9.6±0.3a
PBR-210	10.3±0.7bc	10.6±0.5ab	9.3±0.2a	10.1±0.4a

- Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD.

Table 5. Effect of different genotypes on fecundity of *L. erysimi*

Genotypes	Fecundity per female			
	Experiment I	Experiment II	Experiment III	Pooled Mean
I_8	4.8±2.3ab	7.9±2.1a	6.2±0.4a	6.3±0.7b
I_{79}	11.0±4.5b	13.6±2.4b	13.2±1.7b	12.6±0.3c
I_{82}	4.6±2.2ab	6.3±0.4a	7.4±1.3a	6.1±0.5b
B. fruticulosa	0.0±0.0a	4.1±2.4a	6.3±0.5a	3.5±0.9a
BSH-1	21.0±0.5c	21.9±0.2c	24.7±0.8c	22.6±0.4d
PBR-210	21.0±1.4c	20.1±1.7c	27.1±3.2c	22.7±0.9d

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

Table 6. Effect of different genotypes on adult longevity of *L. erysimi*

Genotypes	Adult longevity (days)			
	Exp. I	Exp. II	Exp. III	Pooled Mean
I_8	3.5±1.3b	4.5±0.6abc	4.3±0.4a	4.1±0.5b
I_{79}	3.9±1.4b	5.5±0.6bcd	5.9±0.2bc	5.1±0.3c
I_{82}	3.8±1.4b	3.3±0.4a	4.0±0.2a	3.7±0.3ab
B. fruticulosa	0.0±0.0a	3.6±1.3ab	5.0±0.4ab	2.9±0.4a
BSH-1	6.9±0.2c	6.0±0.1cd	6.2±0.1c	6.9±0.1e
PBR-210	7.5±0.2c	6.7±0.5d	8.6±0.5d	7.6±0.2d

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

- ***L. erysimi*** **showed least preference for feeding and colonization on these three introgression lines both under field and laboratory evaluation**

- **These introgression lines along with *B. fruticulosa* showed adverse effect on nymphal survival, nymphal development, female fecundity and adult longevity**

Table 7. Comparative aphid population on and yield of different genotypes under protected and unprotected conditions (Tolerance study)

Genotypes	Mean number of aphids/ plant*			Yield (kg/acre)			Yield loss (%)
	Protected†	Unprotected	Mean	Protected†	Unprotected	Mean	
BSH 1	11.2±0.2	202.0±2.5	106.6±95.7	412.5±10	268.8±8.3	340.7±72	34.8
PBR210	9.7±0.7	180.2±10.1	95.0±85.5	742.2±18.1	559.5±16.7	650.8±91.6	24.6
B. fruticulosa	0.1±0.0	11.2±1.1	5.6±5.6	179.0±4.4	173.7±4.2	177.1±2.0	2.2
I8	0.6±0.2	54.5±2.6	27.5±27.0	663.2±19.4	648.7±17.1	641.2±22.1	6.6
I82	0.3±0.0	47.9±2.6	24.2±23.9	703.8±13.3	671.7±26.8	685.7±18.2	5.2
I79	0.4±0.0	46.6±3.1	23.5±23.2	665.8±20.5	622.7±12.9	653.8±12	3.6
Mean	3.7±2.2	90.4±33.2		561.1±90.6	488.7±87.1		
CD (p =0.05)	Genotypes			6.2		33.4	
	Protection level			3.6		19.3	
	Genotypes x Protection level			8.8		47.3	

* Mean of five observations

†Protected set was sprayed with thiamethoxam 25 WG @ 100 g ha^{-1}

Aphid population on the three introgression lines and *B. fruticulosa* did not cross economic threshold level (60 aphids/ plant) even under unsprayed conditions. Hence, tolerance as a mechanism of resistance was of little importance.

Conclusion

- **The three introgression lines (I_8, I_{79} and I_{82}) showed resistance to *L. erysimi* both under field and laboratory conditions under choice and no choice experiments.**
- **Mechanism of resistance was a combination of antixenosis and antibiosis.**
- **Tolerance was not manifested as mechanism of resistance as these lines did not allow the turnip aphid to develop even on unsprayed set.**

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Thank you!