Euphytica (2011) 179:461–470
DOI 10.1007/s10681-011-0351-z

Screening of wild crucifers for resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) and attempt at introgression of resistance gene(s) from *Brassica fruticulosa* to *Brassica juncea*

Sarwan Kumar · Chhaya Atri · Manjeet K. Sangha · S. S. Banga

Received: 2 August 2010 / Accepted: 17 January 2011 / Published online: 29 January 2011

Abstract A diverse array of wild and weedy crucifers was screened under laboratory conditions for their resistance to *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae). Among these, *Brassica fruticulosa* and *Brassica montana* were found to be the most promising. The availability of a synthetic amphiploid, AD-4(*B. fruticulosa* × *Brassica rapa* var. brown *sarson*) as well as a set of *Brassica juncea* lines carrying genomic introgressions from *B. fruticulosa* allowed us to investigate *B. fruticulosa* resistance in greater detail. This assessment was carried out along with susceptible check *B. rapa* ssp. brown sarson cv. BSH-1 in a series of choice and no choice experiments. The mustard aphid showed maximum preference for feeding on BSH-1 while least preference was recorded for *B. fruticulosa* followed by AD-4 as evidenced by the number of aphids that settled on circular leaf bits of these genotypes 24 and 48 h after release in a choice experiment. *Brassica fruticulosa* exhibited strong antibiosis against mustard aphid in no choice experiment and all the released aphids died within 5–8 days of their release, while the maximum survival (76.7%) was recorded on BSH-1. The survival on AD-4 (40%) was significantly lower than that on BSH-1. Almost similar trend was observed with respect to other demographic parameters of *L. erysimi* viz. development time, fecundity and longevity. In the screen house studies, there was no seedling mortality in *B. fruticulosa* and AD-4 after 30 days of aphid release while 80% mortality was observed on BSH-1. Excellent variation for aphid resistance was recorded in *B. juncea* introgression lines, emphasizing heritable nature of *fruticulosa* resistance. The biochemical analysis suggested the possibility of high concentration of lectins to be associated with low aphid infestation in *B. fruticulosa.*

Keywords Antibiosis · *Brassica montana* · Cabbage Aphid · Host Plant Resistance · Introgression lines · Wild crucifers

S. Kumar · C. Atri · M. K. Sangha · S. S. Banga (✉)
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, Punjab, India
e-mail: nppbg@pau.edu

Introduction

Aphids are the major group of insects that limit productivity of oilseed *Brassica* crops in Indian subcontinent and many other growing regions. They are economically serious pests in both field as well as green house horticulture. Among the existing taxonomic variants, *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae) is a major pest (up to 75% yield losses) on oilseeds *brassicas* in Indian subcontinent, while *Brevicoryne brassicae* (L.) has a global presence with strong yield reducing impact,

especially in vegetable brassicas. Both these are highly host specific, feeding exclusively on *Brassica* phloem sap. Retarded growth, poor seed formation and low oil content are the phenotypic manifestations of parasitic feeding and consequent source restrictions in brassica oilseeds (Singhvi et al. 1973; Bakhetia 1983, 1987; Malik and Anand 1984; Rohilla et al. 1987). As crucifer specialists, aphids have developed mechanisms to withstand or even utilize plant defensive chemicals that normally function as feeding deterrents for generalist herbivores. This is reflected in lack of correlation between herbivory of *brassica* specialist insects and defensive responses of glucosinolate–myrosinase system (Kliebenstein et al. 2002).

In the absence of resistant cultivars, the aphids are currently being managed by insecticidal sprays (Sachan and Purwar 2007). In spite of the efficacy of existing chemical controls, need for genetic solution continues to be sought. An insect resistant cultivar fits well in integrated pest management (IPM) modules as it provides the farmers with ecologically sound, effective and economical option for pest management. Even the varieties with moderate level of resistance can be integrated with other management options to reduce the pesticide application on a crop. The first step in the development of an insect resistant cultivar is the precise knowledge of sources of resistance (Stoner and Shelton 1988). A very large number of attempts have been made in the past to identify sources of resistance in primary gene pool of crop *brassica* species (Brar and Sandhu 1978; Amjad and Peters 1992; Sekhon and Ahman 1993; Bhadoria et al. 1995; Saxena et al. 1995). Despite these investigations and resultant publications claiming resistance, no germplasm that carries genetically characterized resistance factor(s) seem to be available. One of the reasons for the inability to identify suitable resistant donor(s) may have been the fact that majority of such screenings were based on pest population or avoidable yield losses under natural infestation as modes for quantification of resistance. These concepts of monitoring resistance largely quantify tolerance for a given set of aphid population pressure and prevailing weather conditions. Both of these tend to vary over the seasons. Further, ability to tolerate a particular level of aphid infestation would also be influenced by physiological status of the host plant as well as the level of heterozygosity inherent in often cross pollinated oilseed and predominantly cross pollinated vegetable forms of *Brassica* species. Heterozygosity is known to be associated with genotypic buffering. The inability to identify suitable sources of resistance has precipitated attempts at developing *Brassica* transgenics carrying genes for lectin production that offer appreciable levels of resistance against *L. erysimi* (Kanrar et al. 2002; Hossain et al. 2006). Field testing of these transgenics is still awaited.

Brassica fruticulosa—a wild relative of cultivated *Brassicas* has been reported to possess resistance against cabbage aphid, *B. brassicae* (Cole 1994a, b; Ellis and Farrell 1995; Ellis et al. 2000). High concentration of lectins was reported to be the underlying mechanism of resistance in this species (Cole 1994b). Lectins are well known for their insecticidal activity against a wide range of insects (Rahbé et al. 1995; Sadasivam and Thayumanavam 2003), especially the sap sucking insects (Foissac et al. 2000; Powell 2001). Despite the compelling evidence of resistance and preliminary elucidation of its genetics (Pink et al. 2003) there is no published evidence to suggest attempts, if any, to transfer this resistance to any of crop *brassica* species. The present studies were, therefore, undertaken to screen an array of wild crucifers to find out resistant sources against *L. erysimi*. We also report the resistance to *L. erysimi* in *B. fruticulosa*, synthesized amphiploid AD-4 (*B. fruticulosa* × *Brassica rapa* var. brown *sarson*) and a set of introgression lines of *Brassica juncea.* The occurrence of excellent resistance to mustard aphid in various accessions of *Brassica montana* is also indicated.

Materials and methods

Plant materials

The plant material comprised a germplasm collection of wild crucifers (65), amphiploid, *B. fruticulosa* × *B. rapa* and a set of *B. juncea* type introgression lines (534). 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 introgression set at the time of evaluation was in BC_1S_3 generation.

Insect culture

Culture of test insects was maintained on the susceptible host BSH-1 on potted plants in the screen house at 22 ± 1°C temperature and 10:14:: L:D photoperiod. Field collected alate adults of *L. erysimi* were released on the host plants for multiplication which were further used for experimentation. Fresh plants were infested at periodic intervals to prevent dying of colonies due to exhaustion of food material.

Germplasm screening

A total of 65 wild *Brassica* species along with the susceptible check *B. rapa* cv. BSH-1 were screened under laboratory conditions for their resistance to *L. erysimi*. The wild crucifers and associated germplasm were raised as potted plants in the screen house. Before seed sowing, earthen pots were filled with the field collected soil and peat (1:1) after applying the recommended doses of nutrients (PAU 2008). Three seeds of each test germplasm line were placed per hill and after germination one seedling each was retained. Each genotype was grown in five pots to have sufficient plants available for further evaluation. Fresh leaves of the above mentioned genotypes were placed in test tubes and five nymphs (<8 h old) were released on each leaf. A wet cotton swab was placed at petiole end of the leaf to maintain turgidity. The leaves were changed every alternate day. These test tubes were placed in biological oxygen demand (BOD) incubator at 22 ± 1°C. Daily observations on nymphal survival were made. The first aphid to reproduce in a replication was kept, the remaining ones were removed and the survival data recorded. The time interval from the date of release of a nymph till it produced a nymph was recorded as development time. The genotypes found promising in the experiment I were revaluated in experiment II to confirm their resistance reaction.

Choice test

The feeding preference of *L. erysimi* was studied in free choice tests on *B. fruticulosa*, AD-4 and the susceptible check *B. rapa* var. brown *sarson* BSH-1 in the laboratory. For this, circular leaf bits (2 cm diameter) of the test genotypes were placed in the periphery of the Petri plates. A moist filter paper was kept at the base as well as lid of the Petri plate to maintain turgidity of the leaf bits. In the centre of the Petri plate, 20 apterous aphids were placed with the help of Camel's hair brush. These Petri plates were covered completely with black paper to avoid any photo-tactic response and kept in BOD incubator at 22 ± 1°C. The experiment was laid in randomized block design with three replications and repeated thrice. The observations on number of aphids settled on leaf bits of each genotype were recorded after 24 and 48 h as the leaf bits started drying thereafter.

No choice test

The survival, development, fecundity and longevity of mustard aphid were studied in a no choice test on *B. fruticulosa*, AD-4 and BSH-1. The experimental details are the same as explained in germplasm screening. However, additional observations on fecundity and longevity were also recorded along with data on survival and development time. For this, daily counts of the number of nymphs produced in each replication were made and the neonate nymphs were removed from the test tube with a Camel's hair brush. Data on the adult longevity was recorded till death of the aphid.

Screen house studies

In the screen house studies, test genotypes of *B. fruticulosa*, AD-4 and BSH-1 were grown in the centre of the pots. At four leaf stage, 20 aphids/seedling were released on the plants of each genotype. Observations on the number of aphids on each plant were recorded at weekly intervals and consequent seedling mortality, if any.

Field assessment of introgression set

A set of 534 introgression lines along with the *B. rapa, B. juncea, Brassica napus* and *B. fruticulosa* and *B. fruticulosa* × *B. rapa* amphiploid were also screened under field conditions. Owing to very low population development under natural conditions, aphids were released artificially @ 20 aphids/plant. Data were recorded for aphid population and damage rating to calculate aphid infestation index (AII) as per Bakhetia and Sandhu (1973) at flowering and pod formation.

Statistical analysis

Means were separated using the least significant difference (LSD) test at probability of $P = 0.05$ with the statistical software OPSTAT (OPSTAT 2006). Regression analysis was carried out to find out the biochemical constituent(s) that contribute the most for resistance against *L. erysimi.*

Results

Germplasm screening

The nymphal survival varied from 0.0 to 100.0% among the test wild crucifers. Many accessions of the wild crucifers, notably belonging to *B. fruticulosa* and *B. montana* revealed 100 percent nymphal mortality (Table 1). These promising crucifers showed consistent results following reevaluation. All these species were also evaluated under field conditions for their response to *L. erysimi* infestation, but the pest population under field conditions, including that on check *B. rapa*, did not develop much and remained below the economic threshold level of 50–60 aphids/plant throughout the season. Data from field studies under the conditions of natural infestation, broadly confirmed these results.

Choice test

In the experiment I, involving only resistant accession of *B. fruticulosa*, its amphiploid with *B. rapa* (AD-4) and the susceptible check *B. rapa* cv BSH 1, the numbers of aphids settled on the leaf bits of test genotypes were statistically non-significant after 24 and 48 h of release (Table 2). In experiment II, the numbers of aphids settled on both the test genotypes were significantly lower than that on the susceptible check BSH-1 after 24 h of release. After 48 h of release, the number of aphids settled on *B. fruticulosa* (0.7) was significantly lower than *B. rapa* cv. BSH-1

Table 1 Comparative nymphal survival of *Lipaphis erysimi* on wild crucifers (Germplasm screening)

Genera	Species	Mean (±SE) Nymphal Survival (%)	Range (%)	Promising Accessions*
Brassica	*Barrelieri* (3)	43.3 ± 23.3	20–90	–
	Cretica (1)	100.0 ± 0.0	100	–
	Fruticulosa (15)	34.0 ± 9.9	0–100	6
	Incana (5)	86.0 ± 4.0	70–90	–
	Insularis (2)	0.0 ± 0.0	0–0	2
	Manurorum (1)	0.0 ± 0.0	0	1
	Montana (6)	1.7 ± 1.7	0–10	5
	Oxyrrhina (1)	30.0 ± 42.4	30	–
Diplotaxis	*Muralis* (1)	0.0 ± 0.0	0	1
	Tennuifolia (10)	25.0 ± 9.7	0–90	4
	Viminea (1)	30.0 ± 14.1	30	–
Erucastrum	*Varium* (1)	10.0 ± 14.1	10	–
Hirschfeldia	*Incana* (1)	0.0 ± 0.0	0	1
Raphanus	*Raphanistrum* (1)	90.0 ± 13.3	90	–
	Sativus (1)	100.0 ± 0.0	100	–
Rapistrum	*Rugosum* (1)	0.0 ± 0.0	0	1
Sinapis	*Alba* (9)	65.5 ± 10.3	20–100	–
	Arvensis (4)	60.0 ± 24.5	0–100	1
Sismbrium	*Orientale* (1)	0.0 ± 0.0	0	1
Brassica	*Rapa* (1) (Susc. check)	80.0 ± 0.0	80	–

Numbers in parenthesis indicate accessions evaluated, * promising accessions revealed no nymphal survival after 4 days of aphid release

Table 2 Feeding preference of mustard aphid in *B. fruticulosa* and its amphiploid with *B. rapa* as compared to susceptible check (Choice test)

Species	Genotype	Mean number of aphids on each genotype (Hrs. after release)							
		Experiment I		Experiment II		Experiment III		Pooled Mean	
		24	48	24	48	24	48	24	48
B. fruticulosa	Wild	1.0 ± 0.5	2.7 ± 2.2	1.3 ± 0.7	0.7 ± 0.7	1.3 ± 1.3	0.0 ± 0.0	1.22 ± 0.53	1.11 ± 0.67
B. fruticulosa × *B. rapa*	AD-4	4.3 ± 1.6	2.7 ± 1.7	2.3 ± 0.7	2.7 ± 1.7	4.3 ± 0.8	1.7 ± 0.3	3.63 ± 0.33	2.33 ± 0.52
B. rapa var. brown sarson (Susceptible check)	BSH 1	4.0 ± 2.0	1.0 ± 1.0	9.3 ± 2.2	5.7 ± 1.2	8.0 ± 1.5	7.3 ± 0.8	7.11 ± 1.28	4.67 ± 1.00
LSD ($P = 0.05$)		NS	NS	4.06	3.62	3.66	1.65	2.81	2.36

(5.7) while that on AD-4 (2.7) were numerically lower than those recorded on BSH-1. Almost similar trend was observed in experiment III. From the pooled data of three experiments it was evident that after 24 h of release, significantly lower number of aphids settled on *B. fruticulosa* (1.22) and AD-4 (3.63) as compared to that on BSH-1 (7.11). After 48 h of release, aphid number on *B. fruticulosa* (1.11) was significantly lower than that on BSH-1 (4.67). Aphid number on AD-4 (2.33) continued to be numerically lower than that on BSH-1. Thus, *L. erysimi* showed maximum preference for feeding on BSH-1 while the least preference was shown for *B. fruticulosa* followed by AD-4.

No choice test

Nymphal Survival

From the pooled data of two experiments, it is evident that the nymphal survival on AD-4 (40.0%) was significantly lower than that on BSH-1 (76.7%) while no survival was observed on *B. fruticulosa* (Fig. 1).

Development Time

The development time on AD-4 was significantly longer than that on BSH-1. It was 9.3 days on AD-4 compared to 7 days on BSH-1.

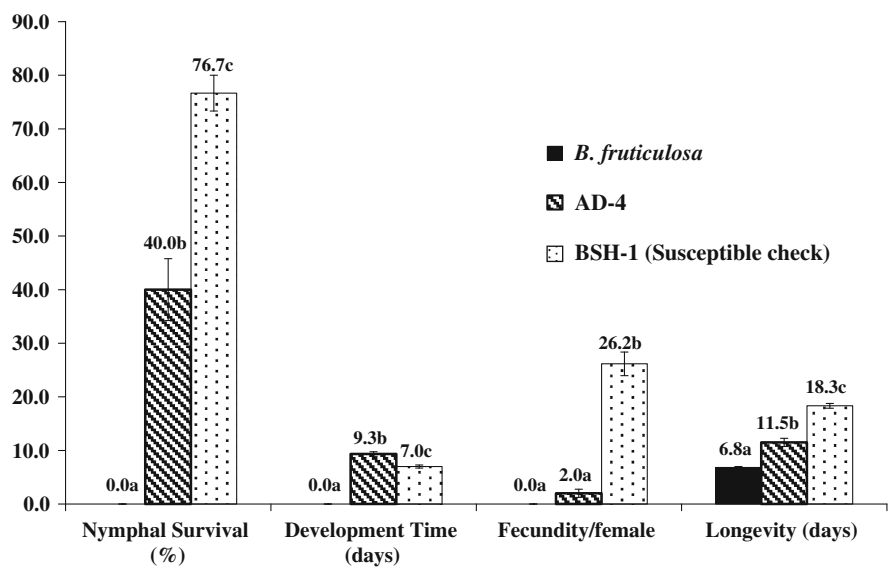

Fig. 1 Effect of *B. fruticulosa* and its amphiploid on some demographic parameters of *L. erysimi* (No choice test). Significant differences according to LSD (0.05) are indicated by *different letters*

Fecundity

The fecundity was significantly lower on AD-4 compared to BSH-1. It was 2.00 nymphs/♀ on AD-4 compared to 26.2 nymphs/♀ on BSH-1.

Longevity

On *B. fruticulosa* all *L. erysimi* nymphs started dying after 4 days of their release and on an average all the nymphs died within 7 days of release. Adult longevity on AD-4 (11.5 days) was also significantly lower than that on BSH-1 (18.3).

Seedling survival (screen house studies)

In the seedling survival study, large differences were observed in the population development on seedlings of different genotypes and the consequent seedling mortality. The population development of *L. erysimi* on BSH-1 was greater than the other two genotypes. After 7 days of release, the aphid number was maximum on BSH-1 (100 aphids/plant) which was significantly higher than that on the other two genotypes (9.11 and 17.00 aphids/plant in *B. fruticulosa* and AD-4, respectively) which were similar to each other (Table 3). Similar trend was

Table 3 Response of *Brassica* genotypes to mustard aphid infestation under screen house conditions (Screen house studies)

Genotype	Population initially released	Aphid population/plant (days after release)				Seedling mortality after 30 days (%)
		7	14	21	28	
Brassica fruticulosa	20	9.11 (2.98 ± 0.35)	8.33 (2.71 ± 0.53)	5.11 (2.44 ± 0.16)	0.00 (1.00 ± 0.00)	0.00 (0.00 ± 0.00)
AD-4	20	17.00 (4.15 ± 0.42)	15.40 (4.02 ± 0.23)	5.40 (2.41 ± 0.38)	0.00 (1.00 ± 0.00)	0.00 (0.00 ± 0.00)
BSH 1	20	100.00 (10.03 ± 0.32)	231.00 (15.15 ± 0.78)	158.00 (12.55 ± 0.57)	45.00 (6.78 ± 1.16)	80.00 (72.00 ± 18.00)
LSD ($P = 0.05$)		(1.15)	(1.76)	(1.28)	(2.08)	(32.37)

* Figures in parentheses are square root and angular transformed values for aphid population and seedling mortality, respectively

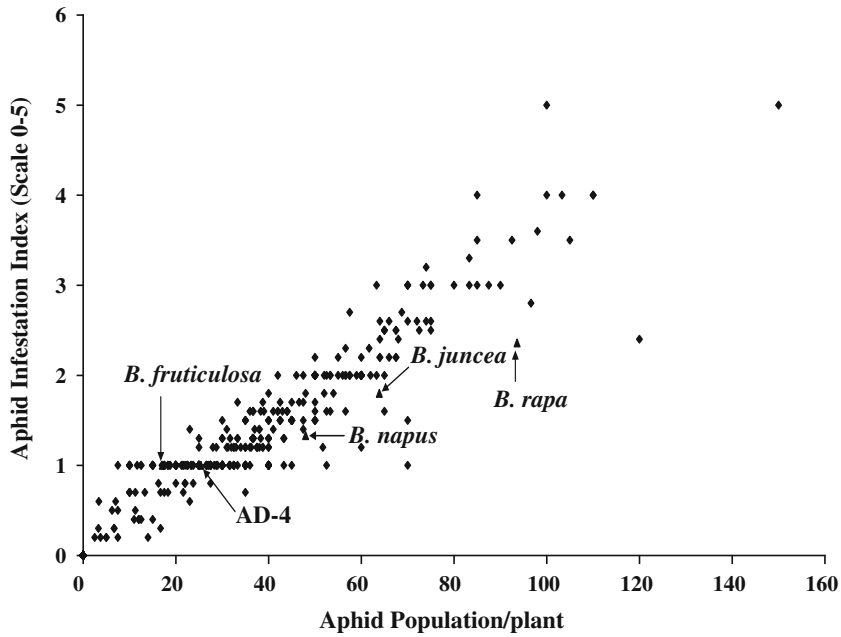

Fig. 2 Aphid Infestation Index (AII) in a set of *introgression lines* (Field assessment of introgressin set)

observed after 14, 21 and 28 days of release. The genotypes BSH-1 suffered the maximum seedling mortality (80.0%) while no seedling mortality was observed in *B. fruticulosa* and AD-4.

Field assessment of introgression set

Screening of 534 introgression lines along with the *B. rapa, B. juncea, B. napus* and *B. fruticulosa* and *B. fruticulosa* × *B. rapa* amphiploid under field conditions revealed a varied level of aphid resistance based on AII (Fig. 2). The AII ranged from <1 to as high as 5. The corresponding values for *B. fruticulosa*, amphiploid (*B. fruticulosa* × *B. rapa*) and susceptible check, *B. rapa* cv. BSH 1 were 1.0, 1.0, and 2.36. Interestingly, some of the introgression lines revealed AII lower than that recorded for *B. fruticulosa.*

Biochemical analysis

The analysis of different biochemical constituents in the past studies of our group at this University had shown that total phenols, O–OH phenols, glucosinolates and lectins were negatively correlated with the aphid population. However, multiple regression analysis of the same data suggested that of the various defensive biochemicals, the lectins' activity was the major contributory factor for resistance against mustard aphid (Table 4). The resistant genotypes of *B. fruticulosa* in present studies revealed lectin levels that ranged between 4.00 and 8.00 haemoagglutination units/ml of homogenate.

Discussion

From point of view of crop evolution, plant resistance to insects can be defined in terms of heritable characteristics that allow plants to withstand insect attack and reproduce. Several factors may constitute resistance; these may include avoidance (antixenosis), antibiosis or agronomic tolerance. For the domesticated crops, avoidance may not be sufficient since its efficacy can be compromised by multiplicity of crops and consequently the options insects may have to choose even the less preferred host for feeding or oviposition. Antibiosis does provide excellent resistance but at the same time exercises maximum pressure on insect to evolve resistant biotypes. An ideal resistance is a combination of all three mechanisms with tolerance showing least pressure on the insect to adapt (Smith 1989). The studies conducted with a large wild crucifer collection revealed occurrence of resistance in many accessions belonging to *B. fruticulosa* and *B. montana.* Resistant reaction was also recorded for *H. incana* and *Rapistrum rugosum.* While a high level of resistance against *B. brassicae* has been reported in *B. fruticulosa* (Cole 1994a, b; Ellis et al. 2000). There appeared to be no published and credible evidence for resistance to mustard aphid in *B. fruticulosa* as well as *B. montana*.

A resistant accession of *B. fruticulosa* was considered for detailed experimentation. The results of the feeding preference/choice test revealed that the mustard aphid showed maximum preference for feeding on *B. rapa* cv.BSH-1 while the least preference was shown for *B. fruticulosa*. The antixenosis to feeding in *B. fruticulosa* has earlier been reported for cabbage aphid, *B. brassicae*. The monitoring of feeding behavior of this species electronically by electrical penetration graph (EPG) showed a large reduction in the duration of passive phloem uptake on *B. fruticulosa* compared to the susceptible *Brassica oleracea* var. *capitata* cv. 'Offenham Compacta'. There was either quick withdrawal of stylets from sieve elements or disrupted phloem uptake (Cole 1994a). Similarly, Ellis and Farrell (1995) observed that very few *B. brassicae* aphids settled on the plants of *B. fruticulosa* compared to the heavy infestation on susceptible *B. oleracea* var. *italica* cultivar 'Green Glaze Glossy'. They have concluded that *B. fruticulosa* possesses very high levels of both antixenosis and antibiosis. Similarly Singh et al. (1994) discovered high levels of antixenosis and antibiosis resistance in accessions of *B. fruticulosa* against *B. brassicae*. The high level of antibiosis resistance in *B. fruticulosa* has

Table 4 Linear regression analysis for various biochemical constituents in relation to aphid population

Variable	Estimate	S. E.	*t*-Ratio
Constant	76.69	6.41	11.96
Total phenols	1.54	3.12	0.49
Ortho-hydroxy phenols	−22.65	11.99	1.88
Lectins	−16.04	7.73	2.07*
Glucosinolates	−0.039	0.22	0.18

* Significant at $P = 0.05$

also been reported against cabbage root fly, *Delia radicum* (Jenson et al. 2002).

In the no choice test conducted during present studies, there was no survival of *L. erysimi* on *B. fruticulosa* after 5–8 days of release while maximum survival was observed on BSH-1 followed by AD-4. Almost similar trend was observed w.r.t. development time, fecundity and longevity. In the screen house studies also maximum aphid population and the consequent seedling mortality was observed on BSH-1 while no seedling mortality was observed on *B. fruticulosa*. The amphiploid (AD 4) between *B. fruticulosa* and *B. rapa* produced also harboured significantly lower aphid (*L. erysimi*) population than *B. rapa* parent. It also did not show any seedling mortality. This emphasized the heritability of *fruticulosa* resistance. Phenotyping of *B. juncea* introgression lines for aphid resistance showed excellent resistance in many of the introgression lines. We propose to map the introgressed segment carrying factor(s) for aphid resistance. Pink et al. (2003) made an attempt to fix high levels of resistance in true breeding lines of *B. fruticulosa* to *B. brassicae*. The resistant inbred lines harboured on an average 3.0 aphids/plant compared to 96.0 aphids/plant on susceptible inbred lines.

Although the correlation analysis in the past studies at our university (Kaur 2008) revealed high levels of glucosinolates, total phenols, ortho-hydroxy phenols to be associated with low infestation. The reanalysis of the same biochemical data using linear regression revealed that high concentration of lectins was the major contributory factor towards aphid resistance. This conclusion was reinforced by occurrence of high levels of lectin in the resistant *B. fruticulosa* genotypes in present studies. Cole (1994b) had also reported high levels of chitin binding lectins in *B. fruticulosa* to be associated with resistance to cabbage aphid, *B. brassicae*. The biological activity of lectins against a number of sap sucking and foliage feeding insects has been well documented (Janzen et al. 1976; Shukle and Murdock 1983; Murdock et al. 1990; Powell et al. 1993; Peumans and van Damme 1995; Rahbé et al. 1995; Sadasivam and Thayumanavan 2003). The possible mechanism of lectin toxicity in insects seems to involve binding of lectin to the gut surface resulting in local lesions on the gut (Eisemann et al. 1994). Further, the incorporation of pure glucosinolates and glucosinolate extracts from *B. fruticulosa* in chemically defined artificial diets did not reduce survival of *B. brassicae* (Cole 1994a).

Thus, from the present study it can be inferred that *B. fruticulosa* possesses high levels of genetically primed antixenosis and antibiosis resistance against *L. erysimi* and is eminently qualified to be designated as source of resistance in breeding programmes aimed at development of mustard aphid resistant crop *Brassicas*. Furthermore, *B. fruticulosa* from a consumer point of view does not seem to have limiting contents of toxic or otherwise unsuitable compounds. Cooked leaves and young shoots from wild *B. fruticulosa* are consumed as vegetables in eastern part of Sicily (Branca 1995; Branca and Lapichino 1997).

Conclusion

This study demonstrated the presence of high level of resistance in *B. fruticulosa* against mustard aphid, *L. erysimi*. It was used as a resistant donor to synthesize the amphiploid AD-4. Follow up experiments demonstrated the presence of resistance in the synthesized amphiploid as well as in *B. juncea* introgression lines. Genotyping this set of introgression lines will allow us to map the introgressed segment carrying factor(s) for aphid resistance.

High concentration of lectins is suggested as the mechanism of resistance in both the wild parent and the synthesized amphiploid. Use of alien genetic resources constitute an important mean to develop insect resistant cultivars. This alternate breeding strategy is especially significant in view of the continuing concerns about the adoption of transgenic food crops and their perceived adverse effects on the environment.

Acknowledgments First and second authors contributed equally to the research. The studies were partly funded under ICAR National Professor Project "Broadening the genetic base of Indian mustard (*Brassica juncea*) through alien introgressions and germplasm enhancement" awarded to Surinder S. Banga.

References

Amjad MD, Peters C (1992) Survival, development and reproduction of turnip aphids (Homoptera: Aphididae) on oilseeds *Brassica*. J Econ Entomol 85:2003–2007

Bakhetia DRC (1983) Losses in rapeseed and mustard due to *Lipaphis erysimi* (Kalt.) in India—a literature study. Int Rapeseed Conf 6:16–22

Bakhetia DRC (1987) Insect pests of rapeseed-mustard and their management. In: Veerbhadra Rao M, Sithananthan S (eds) Plant protection in field crops. Plant Protection Association of India, Hyderabad, pp 249–259

Bakhetia DRC, Sandhu RS (1973) Differential response of *Brassica* species/varieties to the aphid (*Lipaphis erysimi* Kalt.) infestation. J Res PAU 10:272–279

Bhadoria NS, Jakhmola SS, Dhamdhere SV (1995) Relative susceptibility of mustard cultivars to *Lipaphis erysimi* in North West Madhya Pradesh (India). J entomol Res 19:143–146

Branca F (1995) Studies on some wild Brassicaceae species utilizable as vegetables in the Mediterranean areas. Plant Genet Resour Newsl 104:6–9

Branca F, Lapichino G (1997) Some wild and cultivated Brassicaceae exploited in Sicily as vegetables. Plant Genet Resour Newsl 110:22–28

Brar KS, Sandhu GS (1978) Comparative resistance of different *Brassica* species/varieties to the mustard aphid (*Lipaphis erysimi* Kalt.) under natural and artificial conditions. Indian J Agric Res 12:198–200

Cole RA (1994a) Locating a resistance mechanism to the cabbage aphid in two wild *Brassicas*. Entomol Exp Appl 71:23–31

Cole RA (1994b) Isolation of a chitin binding lectin, with insecticidal activity in chemically defined synthetic diets, from two wild brassica species with resistance to cabbage aphid, *Brevicoryne brassicae*. Entomol Exp Appl 72:181–187

Eisemann CH, Donaldson RA, Pearson RD, Cadagon LC, Vuocolo T, Pellam RL (1994) Larvicidal action of lectins on *Lucilia cuprina*; mechanism of action. Entomol Exp Appl 72:1–11

Ellis PR, Farrell JA (1995) Resistance to cabbage aphid (*Brevicoryne brassicae*) in six Brassica accessions in New Zealand. N. Z. J Crop Hortic Sci 23:25–29

Ellis PR, Kiff NB, Pink DAC, Jukes PL, Lynn J, Tatchell GM (2000) Variation in resistance to the cabbage aphid (*Brevicoryne brassicae*) between and within wild and cultivated *brassica* species. Genet Resour Crop Evol 47:395–401

Foissac X, Nguyen TL, Christou P, Gatehouse AMR, Gatehouse JA (2000) Resistance to green leaf hopper (*Nephotettix virescens*) and brown plant hopper (*Nilaparvata lugens*) in transgenic rice expressing snowdrop lectin (*Galanthus nivalis* agglutinin; GNA). J Insect Physiol 46:573–583

Hossain MA, Maiti MK, Basu A, Sen S, Ghosh AK, Sen SK (2006) Transgenic expression of onion leaf lectin in Indian mustard offers protection against aphid colonization. Crop Sci 46:2022–2032

Janzen DH, Juster HB, Liener IE (1976) Insecticidal action of the phytohemagglutinin in black beans on Bruchid beetle. Science 192:795–796

Jenson EB, Felkl G, Kristiansen K, Andersen SB (2002) Resistance to the cabbage root fly, *Delia radicum* within *Brassica fruticulosa*. Euphytica 124:379–386

Kanrar S, Venkateswari J, Kirti PB, Chopra VL (2002) Transgenic India mustard (*Brassica juncea*) with resistance to the mustard aphid (*Lipaphis erysimi* Kaltenbach). Plant Cell Rep 20:976–981

Kaur N (2008) Resistance to mustard aphid [*Lipaphis erysimi* (Kalt.)] in cultivated and wild crucifers. M.Sc. Thesis submitted to Punjab Agricultural University, Ludhiana, India, p. 82

Kliebenstein DJ, Pedersen D, Mitchell-Olds T (2002) Comparative analysis of insect resistance QTL and QTL controlling the myrosinase/glucosinolate system in *Arabidopsis thaliana*. Genetics 161:325–332

Malik RS, Anand IJ (1984) Effect of aphid infestation on the oil yielding attributes in *Brassica*. J Oilseeds Res 1:147–155

Murdock LL, Huesing JE, Nielsen SS, Pratt RC, Shade RE (1990) Biological effects of plant lectins on the cowpea weevil. Phytochemistry 29:85–89

OPSTAT (2006) Statistical Package for Agricultural Research Workers. www.hau.ernet.in/link/spas.htm

PAU (2008) Package of practices for crops of Punjab-Rabi. Punjab Agricultural University, Ludhiana, pp 45–57

Peumans WJ, van Damme EJM (1995) The role of lectins in plant defense. Histochem J 27:253–271

Pink DAC, Kift NB, Ellis PR, Mcclement SJ, Lynn J, Tatchell MG (2003) Genetic control of resistance to the aphid *Brevicoryne brassicae* in the wild species *Brassica fruticulosa*. Plant Breed 122:24–29

Powell KS (2001) Antimetabolic effects of plant lectins towards nymphal stages of the plant hoppers *Tarophagous proserpina* and *Nilaparvata lugens*. Entomol Exp Appl 99:71–77

Powell KS, Gatehouse AMR, Hilder VA, Gatehouse JA (1993) Antimetabolic effects of plant lectins and plant and fungal enzymes on the nymphal stages of two important rice pests, *Nilaparvata lugens* and *Nephotettix cinciteps*. Entomol Exp Appl 66:119–126

Rahbé Y, Sauvion N, Febvay G, Peumans WJ, Gatehouse AMR (1995) Toxicity of lectins and processing of ingested proteins in the pea aphid *Acyrthosiphon pisum*. Entomol Exp Appl 76:143–155

Rohilla HR, Singh H, Kalra VK, Kharub SS (1987) Losses caused by mustard aphid, Lipaphis erysimi (Kalt.) on various Brassica genotypes. Proc Int Rapeseed Congr 7:1077–1084

Sachan GC, Purwar JP (2007) Integrated insect pest management in rapeseed and mustard. In: Jain PC, Bhargava MC (eds) Entomology: novel approaches. New India Publishing Agency, New Delhi, pp 399–423

Sadasivam S, Thayumanavan B (2003) Molecular host plant resistance to pests. Marcel Dekker Inc, New York, pp 61–83

Saxena AK, Bhadoria SS, Gadewadikar PN, Barteria AM, Tomar SS, Dixit SC (1995) Yield losses in some improved varieties of mustard by aphid, *Lipaphis erysimi* Kalt. Agric Sci Dig 15:235–237

Sekhon BS, Ahman I (1993) Insect resistance with special reference to mustard aphid. In: Lubana KS, Banga SS, Banga SK (eds) Monographs on theoretical and applied genetics: breeding oilseed brassicas, Springer Verlag, New York Inc, pp 206–221

Shukle RH, Murdock LL (1983) Lipoxygenase, trypsin inhibitor and lectin from soybeans: effects on larval growth of *Menduca sexta* (Lepidoptera: Sphingidae). Environ Entomol 12:787–791

Singh R, Ellis PR, Pink DAC, Phelps K (1994) An investigation of the resistance to cabbage aphid in brassica species. Ann Appl Biol 125:457–465

Singhvi SM, Verma ND, Yadav TP (1973) Estimation of losses in rapeseed (*Brassica campastris* L. var toria) and mustard, *Brassica juncea* (L.). Ann Biol 16:145–148

Smith CM (1989) Plant resistance to insects: a fundamental approach. Wiley, New York, p 286

Stoner KA, Shelton AM (1988) Role of non-preference in the resistance of cabbage varieties to the onion thrips (Thysanoptera: Thripidae). J Econ Entomol 81:1062–1067