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Reliable screening technique for evaluation of wild crucifers against mustard aphid *Lipaphis erysimi* (Kalt.)

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Wild crucifers namely *Arabidopsis thaliana*, *Brassica fruticulosa*, *B. rugosa*, *B. spinescens*, *B. tournefortii*, *Camelina sativa*, *Capsella bursa-pastoris*, *Crambe abysinnica*, *Cronopus didymus*, *Diplotaxis assurgens*, *D. gomez-campoi*, *D. muralis*, *D. siettiana*, *D. tenuisiliqua*, *Enatharocarpus lyratus*, *Lepidium sativum* and *Sinapis alba* along with five cultivated *Brassica* species including *B. rapa* (BSH-1), *B. juncea* (Rohini), *B. napus* (GSC-6), *B. carinata* (DLSC-2) and *Eruca sativa* (T-27) were screened against mustard aphid *Lipaphis erysimi* (Kalt.) with a standardized technique under definite level of aphid pressure developed using specially designed cages. Observations have revealed that *B. fruticulosa*, *B. spinescens*, *Camelina sativa*, *Crambe abysinnica* and *Lepidium sativum* were resistant to mustard aphid *L. erysimi* with aphid infestation index (AII) ≤1. *Capsella bursa-pastoris* was highly susceptible to bean aphid, *Aphis fabae* during its vegetative stage (with 100% mortality). Other genotypes were found in the range of 'susceptible' to 'highly susceptible' with AII ranging 3-5.

Keywords: Aphid infestation index, *Aphis fabae*, *Brassica* spp., *Camelina sativa*, *Capsella bursa-pastoris*, *Crambe abysinnica*, Insect pest, *Lepidium sativum*, Rapeseed, Resistance, Susceptibility

Family *Cruciferae* (*Brassicaceae*) worldwide comprises more than 380 genera and 3200 species having oilseed and vegetable importance. They include mainly rapeseed-mustard, cabbage, cauliflower, kale, turnip, brussel sprouts, broccoli, radish, etc.[1] Rapeseed (*sarson* and *toria*) and mustard (*rai*) are prominent oil producing crops being grown in India as well as in 52 other countries throughout the world. Rapeseed comprises five different crops namely, *Brassica rapa* var. *brown sarson*, *B. rapa* var. *yellow sarson*, *B. rapa* var. *toria*, *B. napus* and *Eruca sativa* while under mustard there are two crops *i.e. B. juncea* and *B. carinata,* rich in fats, vitamins and minerals[2]. The estimated area, production and yield of rapeseed-mustard worldwide during 2009-10 was 30.74 million hectares, 59.93 million tonnes and 1,950 kg/ha, respectively[3]. India accounts for 21.7 and 10.7% of the total acreage and production, respectively, with productivity 1145 kg/ha, far behind the world's average productivity[3]. Though Indian mustard has yield potential of 1500-3000 kg/ha, the actual yield is low hampered mainly by biotic stress[4]. During 2006-07, total oil consumption in India was 12.5 million tonnes and per capita availability of edible oil was 30.6 g/day which is far below the world's average consumption. However, India had to import more than 50% of required edible oil to meet the growing demand[5]. During 2012-13, India imported a record edible oil about 10.6 million tonnes[6].

The rapeseed-mustard crops are vulnerable to insect pest attack. More than three dozen insect pests are reported to infest these crops at various phonological stages in India[7]. Among them, mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae) having worldwide presence, is the most devastating pest on *Brassica*[8] causing yield loss ranging from 65-96% and 15% oil reduction[9,10]. Adults and nymphs of *L. erysimi* suck the sap of tender leaves, twigs, stems, inflorescence and pods. They secrete excessive amount of honey dew which interferes with the photosynthetic process of the plant[7]. Apart from sucking on the phloem, they also transmit single-stranded RNA luteoviruses while feeding[11]. With the lack of identified reasonable resistant source among the cultivated *Brassica,* hazardous chemical pesticides are the only practical tool to contain this dreaded pest[12,13], despite the fact that they are associated with harmful side effects

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including emergence of pesticide resistance[14]. Wild germplasm is a potential source of resistance against many dreaded insect-pests[15]. As no resistant source is available for mustard aphid till date and the existing field screening techniques of *Brassica* germplasm often generate inaccurate actual resistant status of the test material, we evaluated certain wild sources of crucifer against mustard aphid adopting an innovative reliable screening technique with a view to tap effective resistance traits which can be further exploited in breeding resistant variety programme of cultivated *Brassica.*

Materials and methods

Seventeen wild crucifers, namely *Arabidopsis thaliana*, *Brassica fruticulosa, B. rugosa, B. spinescens, B. tournefortii, Camelina sativa, Capsella bursa-pastoris*, *Crambe abysinnica*, *Cronopus didymus, Diplotaxis assurgens, D. Gomez-campoi, D. muralis, D. siettiana, D. tenuisiliqua, Enatharocarpus lyratus, Lepidium sativum*, and *Sinapis alba* along with five cultivated ones including *Brassica rapa* (BSH-1), *B. juncea* (Rohini), *B. napus* (GSC-6), *B. carinata* (DLSC-2) and *Eruca sativa* (T-27) were evaluated against mustard aphid, *Lipaphis erysimi* (Kalt.) in pots under definite aphid pressure (no choice/force feeding technique). The study was conducted for two successive years, 2010-11 and 2011-12. Three pots per species were covered with iron cages (200×90×45 cm height; lower and upper diameter, respectively) having muslin cloth covering. Twenty mustard aphids, *L. erysimi* were released per plant one week before pre-flowering and allowed them to settle well with the advancement of plant growth. Mustard aphid population were recorded at three stages *i.e.* pre-flowering, full-flowering and full pod formation and Aphid Infestation Index (AII) was calculated as suggested by Bakhetia and Sandhu[16] based on symptoms of injury to plants and aphid colony developed (Table 1).

Table 1—Aphid Infestation Index (AII) as advocated by Bakhetia and Sandhu (1973)

AII	Symptoms of injury
0	Free from aphid infestation.
1	Few aphids along with little or no symptoms of injury, normal growth, no curling or yellowing of leaves.
2	Aphid colony established curling and yellowing of few leaves, average plant growth, flowering and fruiting.
3	Bigger aphid colony, plant growth below average, curling and yellowing of the leaves on some branches, plant showing less pod setting.
4	Very poor plant growth due to heavy aphid infestation, heavy curling and yellowing of the leaves, stunting of plant, a little or no flowering.
5	Plant full of aphids, severe stunting of plant, curling, crinkling and yellowing of almost all the leaves, no flowering and pod formation.

Results

The screening technique was perfectly standardized and used to screen 17 wild crucifers and 5 cultivated *Brassica* spp. with a meagre initial population of mustard aphid and without any chance for escape under natural condition consecutively for two years. The population of 20 aphids multiplied into thousands on the susceptible host grown in pots within the period of 15 days (Table 2).

Evaluation of 17 wild crucifers and five cultivated ones in pots under definite level of mustard aphid, *L.erysimi* pressure during both the years revealed that *Brassica fruticulosa, B. spinescens, Camelina sativa, Crambe abysinnica* and *Lepidium sativum* were resistant to mustard aphid, *Lipaphis erysimi* with aphid infestation index ≤1 (Fig. 1, Table 2). *Capsella bursa-pastoris* was highly susceptible to bean aphid, *Aphis fabae* during its vegetative stage (with 100% mortality of plants) during the second year. In the first year, few plants survived and shown resistance to mustard aphid (Table 2). Other genotypes namely, *B. tournefortii, B. rugosa, C. sativa, Cronopus didymus, Diplotaxis muralis, D. assurgens, D. sieattiana, D. gomez-campoi, D. tenuisiliqua, Enatharocarpus lyratus and Sinapis alba* along with five cultivated crucifers were either 'susceptible' or 'highly susceptible' against mustard aphid with aphid infestation index ranging 3-5 (Fig. 2, Table 2).

Discussion

Breeding resistant variety programme in *Brassica* against the mustard aphid, *L. erysimi* targets to tap the best source of resistance in this genetically diverse species[17]. It requires a perfect screening technique as the usual field trial approach often proves inaccurate. The field trial which is commonly practiced in our country to evaluate the germplasm of *Brassica* and related species against mustard aphid under natural conditions harbours poor aphid pressure and sometimes test entries escape the uniform aphid pressure even when the susceptible checks are planted

Table 2—Evaluation of wild crucifers against mustard aphid, *L. erysimi*. Year I (2010-11), Year II (2011-12)

Wild/cultivated crucifers	Initial aphid release (no./plant)	Number of aphids per plant						Aphid infestation index (AII)		Remarks
		Pre-flowering		Full flowering		Full pod formation				
		Year I	Year II	Year I	Year II	Year I	Year II	Year I	Year II	
Wild crucifers										
Arabidopsis thaliana	20	150	*	300	*	800	*	4	*	Susceptible
Brassica fruticulosa	20	18.3	17.7	14.3	14.3	10	0	1	0.7	Resistant
B. rugosa	20	4666.7	4000	-	-	-	-	5	5	Highly Susceptible
B. spinescens	20	12	16	18	20	13.3	11	1	1	Resistant
B. tournifortii	20	150	160	1000	1000	2833.3	3000	5	5	Highly Susceptible
Camelina sativa	20	13.3	16.7	10	12.7	7	8	1	1	Resistant
Capsella bursa-pastoris	20	10	$	12.7	$	6.7	$	1	$	Bean aphid, *Aphis fabae* attacked at vegetative stage but shown resistant reaction to *L. erysimi*
Crambe abysinica	20	14	18	17.3	13.3	8.3	0	1	0.7	Resistant
Cronopus didymus	20	5000	4666.7	-	-	-	-	5	5	Highly Susceptible
Diplotaxis assurgens	20	61.7	56.7	151.7	153.3	258.3	263.3	3	3	Moderately Susceptible
D. gomez-campoi	20	63.3	65.3	150	160	516.7	566.7	4	4	Susceptible
D. muralis	20	53.3	50	160	150	266.7	300	3	3	Moderately Susceptible
D. siettiana	20	50	55	150	153.3	633.3	667.7	4	4	Susceptible
D. tenuisiliqua	20	160	163.3	300	350	800	800	4	4	Susceptible
Enatharocarpus lyratus	20	100	110	200	226.7	300	317	3	3	Moderately Susceptible
Lepidium sativum	20	14	9.7	3.3	4	0	0	0.7	0.7	Resistant
Sinapis alba	20	600	633.3	2000	2000	5000	5000	5	5	Highly Susceptible
Cultivated crucifers										
B. carinata (DLSC-2)	20	293.3	317	600	633.3	800	933.3	4	4	Susceptible
B. juncea (Rohini)	20	1200	1000	4000	3000	5000	5000	5	5	Highly Susceptible
B. napus (BSC-6)	20	100	123.3	213.3	208.3	333.3	310	3	3	Moderately Susceptible
B. rapa (BSH-1)	20	400	412.5	816.7	800	1000	1066.7	4	4	Susceptible
Eruca sativa (T-27)	20	60	83.3	123.3	160	1000	966.7	4	4	Susceptible

*not included in the second year study as the seeds were destroyed by rodents

- means 100% mortality

$ 100% mortality by bean aphid, *Aphis fabae*

among the test material. This happens because aphid infestation starts from the peripheral plants in the field and does not spread uniformly in the entire plot which ultimately leads to inaccurate results. On the other hand, to inoculate the entire test population with mustard aphid is impractical as it requires a large aphid population. Mass multiplication of mustard aphid in glass houses is expensive in terms of space, labour, time and financial requirement[17].

Kumar *et al.*[18] adopting screening by feed preference *i.e.* choice test/no-choice test and field evaluation reported that *B. fruticulosa* and *B. montana* are least preferred by mustard aphid. The aphid pest *L. erysimi* on *B. fruticulosa* did not survive after 5-8 days of release. In the in-house screening study also, *B. fruticulosa* harboured significantly lower population of *L. erysimi* and did not show any seedling mortality[18]. They further reported that high

Fig. 1—Wild crucifers resistant to mustard aphid.

Fig. 2—Wild crucifers susceptible to mustard aphid.

concentrations of lectins are probably associated with low aphid infestation in *B. fruticulosa*. Similarly, studies on resistance against cabbage aphid *Brevicoryne brassicae* in *B. fruticulosa*[19-21] reveal that *B. fruticulosa* possess high level of antixenosis and antibiosis resistance through high level of chitin binding lectins against *B. brassicae*. Others have also reported a high level of antixenosis resistance in accession of *B. fruticulosa* against *B. brassicae* and cabbage root fly, *Delia radicum*[22,23]. Ellis *et al.*[24] reported *B. spinescens* resistance to *D. radicum*. A defence responsive gene in wild crucifer, *Rorippa indica* against mustard aphid, *L. erysimi* was also identified by Bandopadhyay *et al.*[4]. Atri *et al.*[25] developed an artificially synthesized amphiploid, AD-4 (*B. fruticulosa* × *B. rapa* var. brown *sarson*) for use as a bridge species to transfer *B. fruticulosa* resistance to *B. juncea*. They conclude that *B. juncea-B. fruticulosa* introgression set may be a powerful breeding tool for aphid resistance related QTL/gene discovery and fine mapping of the desired genes/ QTLs to facilitate marker assisted transfer of identified gene(s) for mustard aphid resistance in the background of commercial mustard genotypes.

In the present study, screening was done with meagre aphid population collected from field and further augmented on the plants of wild and cultivated crucifers grown in pots and covered by the cages just before the aphid release. This technique hardly allows any escape under natural condition consecutively for two years. As discussed here, our results corroborate well with earlier studies regarding resistant status of wild crucifer, particularly *B. fruticulosa* against mustard aphid apart from other wild crucifers *i.e. B. spinescens, C. sativa, C. abysinnica* and *L. sativum*. Successfully tested varieties, viz. *B. fruticulosa, B. spinescens, C. sativa, C. abysinnica* and *L. sativum* resistant to *L. erysimi* can be further exploited to identify the responsible QTLs through population development and transfer to cultivated *Brassica* spp. using MAS (Marker assisted selection). Similarly, stage specific metabolic profiling and its correlation to candidate gene expression will provide important information regarding regulation chemistry and expression patterns.

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