Standardization of screening techniques for resistance to *Lipaphis erysimi* (Kalt.) in rapeseed-mustard under field conditions

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The population and damage by aphid, *Lipaphis erysimi* (Kalt.) in *Brassica* spp. is highly variable across seasons and regions, wherein screening of rapeseed-mustard genotypes under natural infestation conditions has not been rewarding for aphid resistance. Since no reliable screening technique is in place, we developed and evaluated various screening techniques to differentiate diverse mustard genotypes for resistance to *L. erysimi* under field conditions. Artificial infestation at bud formation stage with 20 mixed stage aphids pinned with bell pins on the top third branch near inflorescence was found most appropriate and effective for establishment of aphids at inoculation site. Evaluation of mustard genotypes under multi-choice natural infestation revealed maximum variability in *L. erysimi* resistance indices, but plot cage artificial screening technique was found appropriate over natural infestation for multi-choice assays. Genotypes Heera and PDZM 31 showed susceptible to highly susceptible reaction against *L. erysimi* under all the artificial infestation screening techniques. However, PM 30, PM 21, Pusa Bold and Pusa Vijay displayed variable resistance reactions under different screening techniques. Although no-choice twig cage and plant cage techniques showed significant differences in test mustard genotypes for various aphid resistance indices, the twig cage technique revealed maximum variability and could differentiate them at slightest variation in levels of tolerance/susceptibility to *L. erysimi*. The rate of *L. erysimi* multiplication on test mustard genotypes was highly variable under plant cage as compared to twig cage. The twig cage technique also successfully differentiated the double low erucic acid and total glucosinolate, single low erucic acid, and conventional varieties with high erucic acid and total glucosinolate groups of mustard genotypes for *L. erysimi* resistance. The multiplication rate and ease in scouting of aphids, easy handling and cost of the cage, and natural plant growth conditions are some of the most favourable factors, suggesting twig cage technique more precise, realistic, economical, and efficient for artificial screening of rapeseed-mustard for resistance against the aphid *L. erysimi* infestation.

**Keywords:** Aphids resistance screening, *Brassica*, Mustard aphid, Twig cage technique

Rapeseed-mustard is one of the most important oilseed crops in India, which occupies third place after soybean and palm in the world1. In India, it occupies second position in edible oilseed production after groundnut contributing to about 27.8% of the Indian oilseed economy2. It is grown on an area of 5.8 million ha with production of 6.28 million tons in India3. The average productivity of rapeseed-mustard in India (1176 kg/ha) is about two-third of world’s (1695 kg/ha) average yield2. Mainly four oleferous rapeseed-mustard species viz., *Brassica juncea*, *B. rapa*, *B. napus* and *B. carinata* are grown under different agro-climatic conditions in India, of which *B. juncea* is the frontrunner occupying >80% of rapeseed-mustard area4. Production and productivity of rapeseed-mustard is highly variable across crop growing states of India due to variable agroclimatic conditions, cropping systems, production technologies, biotic and abiotic stresses. Among the biotic stresses, mustard aphid, *Lipaphis erysimi* (Kalt.) is considered as most important pest of cruciferous crops worldwide5-8, and is a major constraint for the production of rapeseed-mustard across agro-ecological region of India. The damage by *L. erysimi* vary from 10-90% depending upon the climatic conditions, intensity of population build up and crop growth stage9. Mustard aphid, *L. erysimi* is a specialist *Brassica* feeder, and the oviparity and viviparity modes of reproduction makes it important and difficult to control them. Oviparity in *L. erysimi* is for migration and host finding, while viviparity mode of reproduction for establishment and multiplication. Shorter generation time, parthenogenetic viviparity and higher multiplication, and population growth rates make it economically most significant and important pest of rapeseed-mustard10.

Mustard aphid is not only a major threat to Indian rapeseed-mustard, it has also been reported one of the
most destructive pest in other rapeseed-mustard growing countries Bangladesh and Pakistan\textsuperscript{11-14}. Both the adults and nymphs cause damage to mustard at vegetative, flowering and pod formation stages by sucking sap from the plant. Continuous aphid feeding inhibits plant growth resulting in poor pod formation, less seed set, low oil content, and reduced seed yield\textsuperscript{15,16}. \textit{L. erysimi} has also been reported to transmit about 13 different viruses, including important viruses of the Brassicaceae, such as \textit{Beet mosaic virus}, \textit{Cabbage black ring spot virus}, \textit{Cauliflower mosaic virus} and \textit{Radish mosaic virus}\textsuperscript{17,18}. In case of severe infestation, leaves become curled, plant fails to develop pods, and young pods if developed do not mature and produce unhealthy seeds. Although several aphid management tools like adjustment of sowing dates, yellow sticky traps, biological control, etc. are being talked about, but it is currently being managed by insecticide applications. The insecticide sprays not only increase cost of cultivation, but also disrupt the aphid-natural enemy balance, pollination services, and leave harmful residues in the food and the environment\textsuperscript{19}. To minimize insecticide use an alternate, effective, economic and environmentally safe method of aphid management is highly desirable. In this context, development and cultivation of \textit{L. erysimi} resistant rapeseed-mustard varieties gains attention as it imparts inherent insect control.

Rarely a researcher is able to evaluate and interpret insect damage accurately under natural infestation as either there are insufficient insect numbers to cause adequate damage or insects occur at an inappropriate phenological stage of the crop growth\textsuperscript{20}. Therefore, standardization of an efficient and reliable screening technique is highly desirable for identification of sources of resistance and evaluation of breeding materials for resistance to insect pests. Earlier efforts of rapeseed-mustard evaluation against aphids indicate that the sources of resistance in the primary gene pool are rare bearing low to moderate levels of resistance to \textit{L. erysimi}\textsuperscript{21,25}. These efforts have relied on natural infestation conditions, wherein several factors interplay to counterfeit in identification of resistant/tolerant rapeseed-mustard genotypes. Moreover, the variation in population and damage by \textit{L. erysimi} in rapeseed-mustard across seasons and regions, and influence of environmental factors on it’s biology and behavior, has made it difficult to identify reliable sources of resistance under natural infestation conditions. No reliable screening technique has been developed yet which can differentiate aphid resistant genotypes from susceptible ones. Thus, it was highly desirable to develop/standardize a dependable screening technique to evaluate rapeseed-mustard for resistance to \textit{L. erysimi} under field conditions. Therefore, we developed and evaluated different artificial screening techniques for their efficiency in differentiating diverse mustard genotypes for resistance to \textit{L. erysimi} under field conditions.

**Materials and Methods**

The experiments were conducted at ICAR-Indian Agricultural Research Institute, New Delhi (Latitude 28°38’23” N and Longitude 77° 09’27”E, height above mean sea level 228.61 meters) during 2013-15 \textit{Rabi} (winter) seasons under field conditions. We standardized artificial aphid infestation method and evaluated diverse mustard genotypes using different cage screening techniques for their appropriateness to use in identification of sources of resistance to \textit{Lipaphis erysimi}, and further deployment in aphid resistance breeding program.

**\textit{L. erysimi} culture and standardization of artificial infestation technique**

Aphids can be reared in large numbers on natural food in the laboratory or can be directly obtained from the field to inoculate the test plant material to ascertain uniform insect infestation. Standardization of a technique to infest the material at the susceptible stage with uniform insect density is essential for successful evaluation of the test plant material under artificial infestation conditions. While devising a technique to inoculate the plant material, it is important to take into consideration of (i) the stage of the insect and inoculation procedure; (ii) the number of insects and time of inoculation; and (iii). susceptible stage of the crop and the site of inoculation. It is well known that rapeseed-mustard is more prone to \textit{L. erysimi} damage from bud initiation to pod formation stages, resulting in extensive yield loss. Our efforts to standardize susceptible stage and appropriate plant site revealed that the bud formation stage is most appropriate which enable aphids to hide and establish on the mustard plant. Since, both nymphs and adults are damaging stages and exit together on the plant, we found that mixed stages of \textit{L. erysimi} are more appropriate to inoculate on the mustard plants. \textit{Lipaphis erysimi} is easily available in enough numbers on rapeseed-mustard crop in the field during this season, thus we used field collected aphids for artificial infestation on test mustard genotypes. We
compared two infestation methods, (i) inoculation with 10 individual *L. erysimi* adults transferred with camel hair brush; and (ii) the *L. erysimi* infested mustard twigs cut into small pieces accommodating around 20 mixed stage aphids (nymphs and adults) were pinned to the inoculation site with the help of bell pins (Nickel plated, solid head, needle point pins) on the third branch from top of the rapeseed-mustard plant. The ease of inoculation and establishment of aphids on the site of inoculation were taken as criteria for selection of appropriate inoculation technique. Transfer of individual aphids with camel hair brush was difficult to handle, time consuming, and experienced with poor aphid establishment. Conversely, the inoculation through aphid infested twigs was found easy to handle and the establishment/transfer of aphids on the site of inoculation was found effective and satisfactory. Thus, we used aphid infested mustard twig method of artificial inoculation for further studies.

**Plant materials**

We used six diverse mustard genotypes *viz.*, Heera and PDZM 31 (double zero low in erucic acid and total glucosinolate), PM 21 and PM 30 (low in erucic acid only), and Pusa Bold and Pusa Vijay (conventional varieties high in erucic acid and total glucosinolate) procured from Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi. We planted four sets of these test genotypes one each for the test screening techniques in randomized complete block design in three replications. The seeds of test mustard genotypes were sown in four row plots of 5 meter row length having 15 cm plant-plant and 30 cm row-row spacing during 2013-15 *Rabi* seasons under field conditions. The crop was raised following all the recommended cultivation practices, except insecticide application.

**Cage screening techniques**

The population and damage by aphid, *L. erysimi* in rapeseed-mustard varies across seasons and regions, and is difficult to identify genotypes with resistance to this pest under natural infestation conditions. Caging the test plants with insects is another dependable method of screening for insect resistance under field conditions. (Fig. 1A). Under caging, considerable control is exercised on maintaining the uniform insect

![Fig. 1 — Glimpses of different screening techniques: (A) Overview of no-choice caging techniques (Inset: Plant and Twig cages); (B) Twig cage technique; (C) Plant cage technique; (D) Plot cage technique; and (E) Open field natural infestation technique.](image-url)
pressure on the test genotypes, and plants are inoculated at the same phenological stage. This method of screening also protects the test insect from natural enemies and prevents its migration away from the test plants. Therefore, we designed and evaluated two cage techniques for no-choice (twig cage and whole plant cage), and two techniques for multi-choice (artificial inoculation in plot cage and open natural infestation) conditions for their effectiveness to screen rapeseed-mustard genotypes for resistance to *L. erysimi* under field conditions (Fig. 1A). Further, the evaluation criteria and resistance indexing proposed by earlier workers was found unsuitable in terms of capturing plant stage-specific *L. erysimi* population build up and translation in to plant damage and yield loss. Thus, we modified and used this newly developed evaluation system encompassing various aphid population and damage indices to categorize rapeseed-mustard genotypes for resistance to *L. erysimi* (Table 1).

**No-choice cage screening techniques**

Two types of no-choice cages viz., cylindrical twig cage (15 cm diameter × 60 cm height mounted on 3 rings one each at top, middle and bottom of the structure; Fig. 1B) and whole plant cage (45 cm diameter × 90 cm height mounted on 3 rings one each at top, middle and bottom of the structure; Fig. 1C) were designed using three ring light muslin cloth bag stitched at one end and open at another end. Quality of muslin cloth were such that air and light can easily penetrate the cage, and normal photosynthetic activity takes place in the plant and conducive for the growth and establishment of the aphids. Ten randomly selected plants of each test mustard genotype from middle two rows in each replication were tagged and inoculated using aforesaid inoculation method, i.e., *L. erysimi* infested mustard twig method of artificial infestation at bud initiation stage for both no-choice cage screening techniques. Before inoculation it was ensured that there were no aphids and other non-target insects including natural enemies on the test plants. The third branch from the top of each test plant was inoculated with aphids for both twig and whole plant cage techniques. After inoculation with *L. erysimi*, in case of twig cage technique only the inoculated twig was covered with twig cage; while in whole plant cage technique, full test plant was covered with plant cage. The lower ends of the cages were tied together to close entry or exit of aphids and other non-target insects. The plant and twig cages were hooked on the bamboo sticks (to provide support) as per the desired height and fixed in the soil near test plants.

**Multichoice screening techniques**

For multi-choice evaluation of mustard genotypes for resistance against *L. erysimi*, we used two screening techniques viz., plot cage and open natural infestation conditions. For multi-choice plot cage technique, we designed a plot cage comprising of iron pipes (6 m length × 5 m width × 2.7 m height; Fig. 1D) clamped together to make the structure and covered with fine mosquito net restricting the in and out movement of aphids. The size of the plot net cage can be extended up to the coverage of complete test genotypes which can also be partitioned with a sheet of same material. For multi-choice natural infestation, the test genotypes were kept open to natural

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**Table 1 — Aphid population, damage and resistance indices to categorize rapeseed-mustard for resistance to Lipaphis erysimi**

<table>
<thead>
<tr>
<th>Aphid Population Index (API)</th>
<th>Aphid Damage Index (ADI)</th>
<th>Aphid Resistance Index (ARI)</th>
<th>Resistance Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = No or less than 20 aphids on the inflorescences of test plants</td>
<td>1 = Normal plant growth, no symptoms of injury, no curling or yellowing of leaves</td>
<td>0.1-1.0 (API+ADI/2)</td>
<td>0.0-1.0 = Resistant</td>
</tr>
<tr>
<td>2 = upto 25% inflorescences have 21-100 aphids on the test plants</td>
<td>2 = Average plant growth, curling and yellowing of few leaves, flowering and fruiting</td>
<td>1.1-2.0 (API+ADI/2)</td>
<td>1.1-2.0 = Moderately resistant</td>
</tr>
<tr>
<td>3 = upto 50% of inflorescences have 101-250 aphids across test plants</td>
<td>3 = Poor plant growth, curling and yellowing of leaves on some branches, drying of few flowers and poor pod setting</td>
<td>2.1-3.0 (API+ADI/2)</td>
<td>2.1-2.5 = Tolerant</td>
</tr>
<tr>
<td>4 = upto 75% inflorescences have 251-500 aphids across test plants</td>
<td>4 = Stunted plant growth, heavy curling and yellowing of leaves all through the plant, drying and curling of almost half the inflorescence with poor flowering and rare pod setting</td>
<td>3.1-4.0 (API+ADI/2)</td>
<td>2.6-3.5 = Susceptible</td>
</tr>
<tr>
<td>5 = 100% of inflorescences have more than 500 aphids across test plants</td>
<td>5 = Severe stunting and ragged plant appearance, yellowing and curling of almost all the leaves, complete drying of inflorescence without any flower and immature drying of pods if any</td>
<td>4.1-5.0 (API+ADI/2)</td>
<td>3.6-5.0 = Highly susceptible</td>
</tr>
</tbody>
</table>
infestation by *L. erysimi* (Fig. 1E). Ten randomly selected plants of each test genotype from middle two rows in each replication were inoculated using aforesaid twig method of artificial infestation at bud initiation stage for multi-choice cage screening technique, while for natural multi-choice test the genotypes received natural *L. erysimi* infestation. Before inoculation it was ensured that there were no aphid and other non-target insects including natural enemies on the test plants.

**Observations recorded**

The observations were recorded on number of aphids, aphid population index (API), aphid damage index (ADI), and aphid resistance index (ARI) for all the test screening techniques. The observations on API were recorded after 21 days of inoculation, while ADI at completion of pod formation as per the index methods described in Table 1. The ARI were determined based on API and ADI values, and ultimately derived at resistance category (Table 1). In case of no-choice screening techniques, total numbers of aphids were also counted on the inoculated twig in twig cage technique and on the inoculated plants in case of whole plant cage technique after 21 days of inoculation. These total numbers of aphids per plant or twig for respective cage techniques were used to calculate aphid multiplication rate (AMR) = (Total number of aphids/Number of aphids released) × 100.

**Data analysis**

The data were subjected to analysis of variance (ANOVA) using the statistical software SAS® version 9.2. The data on different aphid screening techniques were analyzed in a factorial design with genotypes as the main treatment, and the screening techniques and seasons as sub-treatments. The significance of differences between genotypes, screening techniques, seasons, and their interactions were measured by F test, and the treatment means were compared using the least significant difference (LSD) at *P* = 0.05.

**Results**

**Standardization of artificial infestation and screening techniques**

Our initial efforts to standardize susceptible stage and appropriate plant site to infest aphids revealed that the artificial infestation of *L. erysimi* at bud formation in mustard crop is most appropriate stage and site to enable aphids to hide and establish. Considering the ease of inoculation and establishment of aphids, inoculation with the *L. erysimi* infested mustard twig pieces having ~20 mixed stage aphids pinned with bell pins on the top third branch was found effective and satisfactory, thus used for artificial infestation. Caging of mustard plants with *L. erysimi* protected them from natural enemies and restricted their migration away from the plants. Among the screening techniques, cage techniques for no-choice (twig cage and whole plant cage) and multi-choice (plot cage) conditions were found effective to screen rapeseed-mustard genotypes for resistance to *L. erysimi* as compared to natural infestation conditions. Many a times, the high *L. erysimi* population at flowering and pod setting doesn’t translate into considerable plant damage and yield loss, on the other hand low *L. erysimi* population some time translates into severe plant damage and yield loss. Looking at these situations, certain damage evaluation procedures were devised and translated into various indices to differentiate test rapeseed-mustard genotypes into different *L. erysimi* resistance categories (Table 1).

**Lipaphis erysimi** resistance indices under no-choice screening techniques

There were significant differences in aphid population index (*F*<sub>5,70</sub> = 61.39; *P* <0.001), aphid damage index (*F*<sub>5,70</sub> = 12.6; *P* <0.001), and aphid resistance index (*F*<sub>5,70</sub> = 44.61; *P* <0.001) between mustard genotypes, under different no-choice screening techniques (API: *F*<sub>5,70</sub> = 14.43, *P* = 0.017; ADI: *F*<sub>5,70</sub> = 2.99, *P* = 0.017; ARI: *F*<sub>5,70</sub> = 10.08, *P* <0.001), and across seasons (API: *F*<sub>10,70</sub> = 20.82, *P* <0.001; ADI: *F*<sub>10,70</sub> = 5.81, *P* <0.001; ARI: *F*<sub>10,70</sub> = 15.70, *P* <0.001) evaluated for *L. erysimi* resistance under artificial infestation conditions in the field (Table 2). Under twig cage technique, the aphid population index, aphid damage index and aphid resistance index were significantly higher in Heera as compared to other test genotypes (Table 2). Further, PDZM 31 and PM 21, and PM 30, Pusa Bold and Pusa Vijay were on par in aphid population index and aphid resistance index; while PM 21, PM 30, Pusa Bold and Pusa Vijay were on par in aphid damage index (Table 2). However, aphid damage index in Heera, PDZM 31, PM 30 and Pusa Bold; and aphid population index and aphid resistance index among Heera and PDZM 31, PM 30 and Pusa Bold, and PM 21 and Pusa Vijay were significantly on par under whole plant cage technique (Table 2). The *L. erysimi* damage parameters viz., aphid population index (*F*<sub>2,70</sub> = 85.89; *P* <0.001), aphid damage index
<table>
<thead>
<tr>
<th>Genotype</th>
<th>No-choice</th>
<th>Multi-choice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2013</td>
<td>2014</td>
</tr>
<tr>
<td>Aphid Population Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heera</td>
<td>4.40</td>
<td>3.40</td>
</tr>
<tr>
<td>PDZM 31</td>
<td>3.97</td>
<td>4.10</td>
</tr>
<tr>
<td>PM 21</td>
<td>2.97</td>
<td>2.67</td>
</tr>
<tr>
<td>PM 30</td>
<td>3.67</td>
<td>4.23</td>
</tr>
<tr>
<td>Pusa Vijay</td>
<td>3.47</td>
<td>3.20</td>
</tr>
<tr>
<td>Pusa Bold</td>
<td>3.67</td>
<td>4.30</td>
</tr>
<tr>
<td>Mean</td>
<td>3.69</td>
<td>3.65</td>
</tr>
<tr>
<td>Aphid Damage Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDZM 31</td>
<td>4.03</td>
<td>4.27</td>
</tr>
<tr>
<td>PM 21</td>
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</tr>
<tr>
<td>Pusa Bold</td>
<td>3.37</td>
<td>3.57</td>
</tr>
<tr>
<td>Pusa Vijay</td>
<td>3.87</td>
<td>4.50</td>
</tr>
<tr>
<td>Mean</td>
<td>3.78</td>
<td>3.87</td>
</tr>
<tr>
<td>Aphid Resistance Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heera</td>
<td>4.53</td>
<td>3.53</td>
</tr>
<tr>
<td>PDZM 31</td>
<td>4.00</td>
<td>4.18</td>
</tr>
<tr>
<td>PM 21</td>
<td>2.98</td>
<td>2.83</td>
</tr>
<tr>
<td>PM 30</td>
<td>3.70</td>
<td>4.23</td>
</tr>
<tr>
<td>Pusa Bold</td>
<td>3.42</td>
<td>3.38</td>
</tr>
<tr>
<td>Pusa Vijay</td>
<td>3.77</td>
<td>4.40</td>
</tr>
<tr>
<td>Mean</td>
<td>3.73</td>
<td>3.76</td>
</tr>
</tbody>
</table>

Table 2 — Evaluation of mustard genotypes for resistance to aphid, Lipaphis erysimi under no-choice artificial infestation in (A) twig and (B) plant cages; and multi-choice in (C) plot cage artificial infestation and (D) natural infestation conditions in the field.

(F_{2,70} = 170.13; P < 0.001), and aphid resistance index (F_{2,70} = 160.48; P < 0.001) also varied significantly between test genotypes during different crop seasons (Table 2). The aphid population index (F_{1,70} = 1.95; P = 0.166), aphid damage index (F_{1,70} = 0.43; P = 0.512), and aphid resistance index (F_{1,70} = 1.47; P = 0.229) under test no-choice screening techniques, and the techniques × season interactions (API: F_{2,70} = 1.45, P = 0.242; ADI: F_{2,70} = 0.60, P = 0.552; ARI: F_{2,70} = 1.00, P = 0.373) were found statistically non-significant, indicating season-neutral efficacy of these no-choice screening techniques to evaluate mustard genotypes for resistance to L. erysimi under field conditions. The interactions between no-choice screening techniques × genotypes × seasons also showed significant variability in aphid population index (F_{10,70} = 13.46; P < 0.001), aphid damage index (F_{10,70} = 2.40; P = 0.016), and aphid resistance index (F_{10,70} = 8.58; P < 0.001) between mustard genotypes for L. erysimi resistance under artificial infestation conditions in the field (Table 2). Furthermore, the twig cage technique revealed maximum variability...
among mustard genotypes for aphid population index (2.33 to 4.63 vs. 2.38 to 3.88; Table 2), aphid damage index (2.79 to 4.24 vs. 2.89 to 3.73; Table 2), and aphid resistance index (2.67 to 4.44 vs. 2.64 to 3.81; Table 2) as compared to plant cage technique. These findings indicate that the twig cage artificial screening technique is more efficient in differentiating mustard genotypes for slightest variation in levels of tolerance/susceptibility to *L. erysimi*.

Reproduction of *L. erysimi* under no-choice screening techniques

There were significant differences between mustard genotypes for aphid population and rate of aphid multiplication (*F*<sub>5,70</sub> = 1138.77; *P* < 0.001), under different no-choice screening techniques (*F*<sub>5,70</sub> = 68.02, *P* < 0.001), and across seasons (*F*<sub>10,70</sub> = 80.54, *P* < 0.001) evaluated for *L. erysimi* resistance under artificial infestation conditions in the field (Table 3). The numbers of *L. erysimi* and their multiplication rate under both twig cage and plant cage techniques were significantly higher on Heera, being lower on Pusa Vijay as compared to other genotypes (Table 3). Aphid population and aphid multiplication rates on PM 30 and Pusa Bold were significantly at par under twig cage technique, while under plant cage technique these were on par with each other on PM 21 and PM 30 (Table 3). The numbers of *L. erysimi* and their multiplication rate during different crop seasons (*F*<sub>2,70</sub> = 379.29; *P* < 0.001) under test no-choice screening techniques (*F*<sub>1,70</sub> = 4.05; *P* = 0.048), and for techniques × season interactions (*F*<sub>2,70</sub> = 45.29, *P* < 0.001) varied significantly between test mustard genotypes (Table 3). These results indicate significant effect of screening technique and cropping season on the rate of *L. erysimi* multiplication on different mustard genotypes even under artificial infestation conditions in the field. The interactions between screening techniques × genotypes × seasons also showed significant variability in number of *L. erysimi* and multiplication rate (Table 3). Highly variable rate

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Twig cage</th>
<th>Plant cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2013</td>
<td>2014</td>
</tr>
<tr>
<td>Aphid Population (No. of aphids)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heera</td>
<td>1742</td>
<td>937</td>
</tr>
<tr>
<td>PDZM 31</td>
<td>644</td>
<td>563</td>
</tr>
<tr>
<td>PM 21</td>
<td>1178</td>
<td>397</td>
</tr>
<tr>
<td>PM 30</td>
<td>379</td>
<td>410</td>
</tr>
<tr>
<td>Pusa Bold</td>
<td>680</td>
<td>319</td>
</tr>
<tr>
<td>Pusa Vijay</td>
<td>399</td>
<td>473</td>
</tr>
<tr>
<td>Mean</td>
<td>837</td>
<td>517</td>
</tr>
<tr>
<td>Aphid Multiplication Rate (x times)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heera</td>
<td>87.1</td>
<td>46.8</td>
</tr>
<tr>
<td>PDZM 31</td>
<td>32.2</td>
<td>28.2</td>
</tr>
<tr>
<td>PM 21</td>
<td>58.9</td>
<td>19.8</td>
</tr>
<tr>
<td>PM 30</td>
<td>19.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Pusa Bold</td>
<td>34.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Pusa Vijay</td>
<td>19.9</td>
<td>23.7</td>
</tr>
<tr>
<td>Mean</td>
<td>41.8</td>
<td>25.8</td>
</tr>
</tbody>
</table>

LSD (*P* = 0.05) for comparing Aphid Population (No. of aphids) Aphid Multiplication Rate (x times)

| Technique (T) | 18.04 |
| Genotype (G)  | 31.25 |
| Season (S)    | 22.1  |
| T × G         | 44.19 |
| T × S         | 31.25 |
| G × S         | 54.12 |
| T × G × S     | 76.54 |
of *L. erysimi* multiplication under plant cage (17.3 to 77.3) as compared to twig cage (20.6 to 64.1) across mustard genotypes (Table 3), further indicate that the twig cage technique is proficient and précised for artificial screening of mustard genotypes for resistance to *L. erysimi*.

**L. erysimi** resistance indices under multi-choice screening techniques

Screening of mustard genotypes under multi-choice conditions revealed that the aphid population index (F\(_{5,70} = 108.19; P < 0.001\)), aphid damage index (F\(_{5,70} = 30.32; P < 0.001\)), and aphid resistance index (F\(_{5,70} = 75.04; P < 0.001\)) varied significantly among genotypes, screening techniques (API: F\(_{5,70} = 27.30, P < 0.001\); ADI: F\(_{5,70} = 3.41, P = 0.017\); ARI: F\(_{5,70} = 13.57, P < 0.001\)), and seasons (API: F\(_{10,70} = 5.94, P < 0.001\); ADI: F\(_{10,70} = 21.44, P < 0.001\); ARI: F\(_{10,70} = 37.26, P < 0.001\)) for *L. erysimi* resistance under field conditions (Table 2). The aphid population index, aphid damage index and aphid resistance index were significantly higher in Heera followed by PDZM 31 and PM 21 under plot cage artificial infestation conditions. These studies thus indicate that the plot cage artificial screening technique is more appropriate to differentiate mustard genotypes for resistance to *L. erysimi* under controlled release than under natural infestation conditions.

Resistance categorization of test mustard genotypes against *L. erysimi*

The evaluation of double low erucic acid and total glucosinolate, single low erucic acid, and conventional varieties with high erucic acid and total glucosinolate mustard genotypes for *L. erysimi*

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**Table 4** — Categorization of test mustard genotypes for *Lipaphis erysimi* using aphid population and damage indices

<table>
<thead>
<tr>
<th>Screening techniques</th>
<th>Mustard genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twig cage</td>
<td>Heera</td>
</tr>
<tr>
<td></td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>Plant cage</td>
<td>PDZM 31</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Plot cage</td>
<td>PM 21</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Across cage techniques</td>
<td>PM 30</td>
</tr>
<tr>
<td></td>
<td>Pusa Bold</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>Pusa Vijay</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Natural infestation</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td></td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

---
resistance showed that the aphid population index (Fig. 2A), aphid damage index (Fig. 2B), aphid resistance index (Fig. 2C), and aphid multiplication rate (except under multi-choice cage and natural infestation conditions for this parameter; Fig. 2D) were significantly higher in double low erucic acid and total glucosinolate genotypes across the test screening techniques as compared to other group of genotypes (Fig. 2). There was no significant difference for aphid damage index (Fig. 2B) and aphid multiplication rate (Fig. 2D) on single low erucic acid, and conventional varieties with high erucic acid and total glucosinolate group of mustard genotypes under either of the screening techniques, except under twig cage technique. Genotype Heera was found susceptible to *L. erysimi* under plot cage technique, while highly susceptible under no-choice screening techniques and natural infestation (Table 4). Genotype PDZM 31 was found consistently susceptible to highly susceptible to *L. erysimi* under no-choice and multi-choice artificial infestation conditions, while it showed tolerant reaction under natural infestation conditions, could be due to migration to more preferred susceptible staged plants. Genotypes PM 21, Pusa Bold and Pusa Vijay were found susceptible under both no-choice cage screening techniques as well as under natural infestation conditions, while these genotypes showed tolerant to moderately resistant reaction under plot cage technique, might be due to lateral spread of inoculated aphids to adjacent non-inoculated plants (Table 4). Genotype PM 30 was found tolerant under multi-choice plot cage and natural infestation conditions, however susceptible under no-choice cage screening techniques, indicating that the no-choice cage techniques are more reliable to identify resistant genotypes as compared to multi-choice natural or artificial infestation techniques. Furthermore, both the no-choice cage screening techniques were although equally effective and consistent, the ease of handling, cost of the cage (as twig cage costs half the plant cage), and plant growth under natural microclimatic conditions (except the infested twigs) are some of the important factors which sounds the twig cage technique more précised and appropriate for artificial screening of rapeseed-mustard for resistance to *L. erysimi*.

**Discussion**

The population and damage by aphid, *L. erysimi* in rapeseed-mustard varies across seasons and regions, and is difficult to identify genotypes with resistance to this pest under natural infestation conditions. Several techniques have been in use for infestation and evaluation of the test material in the field for many crops. However, due to lack of such dependable technique to screen rapeseed-mustard for resistance against *L. erysimi*, aphid resistance breeding program in this crop has not been rewarding. The amount of food available, multiplication rate and the insect density influence expression of resistance, thus optimum and uniform level of infestation is emphasized to get maximum differences between the resistant and susceptible genotypes. Standardization of techniques to inoculate the rapeseed-mustard plants at the susceptible stage with uniform insect density and damage evaluation procedures is essential for success of...
the insect resistance screening program. Present studies
found that the artificial infestation of mustard genotypes
with *L. erysimi* at bud formation stage is most
appropriate for the establishment of aphids at the
inoculation site. Further, the inoculation with *L. erysimi*
infested mustard twig pieces having around 20 mixed
stage aphids pinned with bell pins on the top third
branch near inflorescence was found effective and
satisfactory for artificial infestation. Certain resistance
evaluation procedures encompassing *L. erysimi* population
and damage levels were also devised to differentiate
test mustard genotypes into different resistance categories.

The screening for insect resistance can be carried out
under multi-choice, dual-choice, or no-choice conditions using appropriate artificial infestation and
cage techniques. The choice or no-choice insect
resistance screening techniques have earlier been
reported to be successful in identifying sources of
resistance to sucking pests in several crops. Immense efforts have also been made in the past to
identify sources of resistance to aphids in *Brassica*
species, which also indicate that the resistance
in the primary gene pool of rapeseed-mustard to
*L. erysimi* are rare bearing low to moderate levels of
resistance. But most of these studies have been under
natural infestation conditions, wherein several factors
interplay to counterfeit to identify the resistant/
tolerant genotypes. Sucking insects particularly the
aphids have specialized feeding habit and ingest
enormous phloem fluid to fulfill the desired nutritional
requirement. The aphid bioassay techniques like
detached plant part bioassay and pot cage screening
technique have also been used for evaluation of
rapeseed-mustard for aphid resistance. The detached
plant parts like leaves, inflorescence, pods, or fruits
are inefficient to cater the nutritional requirement of
aphids as do the naturally growing plants. The
bioassays with attached vs. excised leaves of alfalfa
for resistance to spotted alfalfa aphid, *Theroioaphis*
maculata (Buckton) although revealed significant
differences in nymphal survival but it was greater on
excised leaves than on intact leaves, suggesting that
the excised leaves underestimate the levels of
resistance in the test plants. The bioassays with
excised leaves have also been reported associated
with induced resistance, and is not representative of
the plant organ. Pot cage bioassays are although
useful for evaluation of mustard for aphid resistance,
the abiotic and edaphic plant growing conditions,
physiological and nutritional deviation from naturally
grown host plants, and their limitation to use for large
number of genotypes restrict the scope of this
technique in final decision making on the test plant’s
resistance/susceptibility reaction.

Caging the mustard plants with *L. erysimi* is
dependable method of screening for insect resistance
under field conditions, wherein considerable control is
exercised to maintain uniform insect pressure by
artificial inoculation at the same plant phenological
stage. It also protects the aphids from natural enemies
and restricts their movement away from the test plants.
The cages can be designed to cover the whole plants or
only the plant parts that are more prone to insect
damage. Present studies revealed significant differences
in aphid population index, aphid damage index, and
aphid resistance index between mustard genotypes,
under no-choice cage screening techniques and across
seasons for *L. erysimi* resistance under artificial
infestation conditions in the field. The non-significant
differences for various aphid resistance indices under
no-choice screening techniques and the techniques ×
season interactions indicate season-neutral efficacy of
these techniques to evaluate mustard genotypes for
resistance to *L. erysimi*. However, significant variation
in *L. erysimi* damage parameters for techniques × season
interactions indicated seasonal effect on the efficacy
of multi-choice screening techniques to evaluate
mustard genotypes for resistance to *L. erysimi* under
field conditions. Furthermore, evaluation under multi-
choice natural infestation conditions although
revealed maximum variability in aphid resistance
parameters among the test mustard genotypes, but it
was unregulated, inconsistent and higher damage
levels as compared to those under plot cage artificial
infestation conditions. Efforts have also been made
earlier to assess the resistance in several oleferous
rapeseed-mustard species by observing aphid settling
and alate production in *L. erysimi* after inoculation via
infested inflorescence under field conditions. These
studies observed significant differences in aphid
settling and alate formation on the test rapeseed
mustard species, however no further reference has
been made on standardization, validation or use of
infested inflorescence method of aphid inoculation.
Thus, plot cage artificial screening technique for
multi-choice assays is more appropriate as compared
to natural infestation conditions.

The numbers and multiplication rate of *L. erysimi*
were significantly higher on Heera, being lower on
Pusa Vijay as compared to other genotypes under
both twig cage and plant cage techniques. However,
the numbers and multiplication rate of *L. erysimi* on
PM 30 and Pusa Bold under twig cage technique and on PM 21 and PM 30 under plant cage technique were statistically on par with each other. These variations indicate significant effect of screening technique and cropping season on the rate of L. erysimi multiplication on different mustard genotypes even under artificial infestation conditions in the field. Genotype PDZM 31 was found consistently susceptible to highly susceptible to L. erysimi under no-choice and multi-choice artificial infestation conditions, while it showed tolerant reaction under natural infestation conditions. However, tolerant reaction of PM 30 under multi-choice plot cage and natural infestation conditions and susceptible under no-choice cage conditions, indicate that the no-choice cage screening techniques are more reliable than the multi-choice ones to identify L. erysimi resistant mustard genotypes. Furthermore, maximum variability among test mustard genotypes for all the aphid damage parameters under twig cage technique as compared to plant cage technique, suggest that the twig cage artificial screening technique is more appropriate to differentiate mustard genotypes at slightest variation in levels of tolerance/susceptibility to L. erysimi. The of twig cage technique also efficiently differentiated the double low erucic acid and total glucosinolate, single low erucic acid, and conventional varieties with high erucic acid and total glucosinolate groups of mustard genotypes for L. erysimi resistance. The high variability in multiplication rate of aphids across test mustard genotypes and difficulty in scouting of aphids throughout the test plant under plant cage technique as compared to twig cage technique further endorse the appropriateness and precision of twig cage technique for artificial screening of mustard for resistance to L. erysimi. The no-choice cage techniques were although equally effective, but ease of handling, cost of the cage and plant growth under natural microclimatic conditions (except only the aphid inoculated twigs) are some additional indicators which also support that the twig cage technique is more precise, realistic, economical, and efficient for artificial screening of mustard for resistance to L. erysimi.

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References


