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# INTEGRATED PHENOTYPIC, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF MUSTARD APHID *(LIPAPHIS ERYSIMI)* RESISTANCE IN RAPESEED-MUSTARD GENOTYPES

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**ABSTRACT**
Rapeseed-mustard is one of the significant oil seed crops of India but the productivity is limited due to mustard aphid (*Lipaphis erysimi*). Resistant plants are one of the measures towards sustainable management of aphid. The present study was carryout at Dholi, Bihar on 40 advanced rapeseed–mustard genotypes with two susceptible and resistant checks under natural field infestation during *rabi* 2022-2023. Aphid population and responses to aphid damage were measured at the flowering and pod formation stages of development and aphid resistance categories were assigned by aphid populations, damage and resistance indices. In addition, selected genotypes were screened for the total phenol and soluble sugar content. In addition, mitochondrial cytochrome c oxidase subunit I (mtCOI) sequencing was used to support the identification of collected aphid specimens. The lowest population of the aphid (41.82) was observed on KMR(E) 21-1 which was considered as Resistant which had almost equivalent value with the resistant check Kranti (43.98). There were 11 moderately resistant genotypes, 25 susceptible and four highly susceptible genotypes. Biochemical estimation showed high negative correlation between total phenol content and aphid infestation index ($r = -0.972$, $p < 0.01$), while soluble sugars did not show significant positive correlation. The molecular analysis revealed 98.00-98.04% sequence similarity with *Lipaphis erysimi pseudobrassicae*. These resultants are useful in testing materials for resistance and integrated mustard aphid management programs.

**KEYWORDS:** Rapeseed-mustard, *Lipaphis erysimi*, genotype screening, total phenols, soluble sugars, mtCOI sequencing.

## 1. INTRODUCTION

Rapeseed-mustard (Brassica juncea, Brassica rapa and Brassica napus) is a significant group of oilseeds in the Indian sub-continent and Indian mustard is the one main source of edible oil in India (Sandeep Raj et al., 2017). Insect pests can lead to reductions in crop performance and yield of Brassica crops (Kular & Kumar 2011). Pests are an important constraint for rapeseed-mustard production among these species mustard aphid Lipaphis erysimi (Kaltenbach) significantly affects the crop depending on growing conditions and genotype (Bhatia et al. 2011; Palial et al. 2022). Lipaphis erysimi feeds by inserting its stylets into plant tissues and withdrawing phloem sap (Goggin, 2007). Aphid infestations can be seen on shoots, inflorescences and developing pods, often leading to pod setting, decreased production, inflorescence damage and yellowing of the leaves (Sandeep Raj et al., 2017; Dhillon et al., 2018). Pesticides residues have entered the food chain and bioaccumulated in tropical agroecosystems (Baliyan et al., 2025). Damage can vary depending on the host genotype, season and location; therefore, finding genetically resistant or less susceptible material is also a valuable part of mustard aphid management (Kular & Kumar, 2011; Palial et al., 2022). Integrated pest management (IPM) can be augmented with host-plant resistance, as this decreases the vulnerability of the crop to attack by the pest. The genotype screening under natural infestation of pests puts plants at risk from the pressure of the pest attack and environment conditions in the target production system. The differences between aphid counts and plant damage scores with susceptible and resistant checks may help distinguish genotypic responses when grown under this set of conditions (Dhillon et al., 2018). There is also a report of variability for aphid resistance among cultivated varieties of Brassica and lines from the wild or introgression which implies continued search for germplasm with increased resistance (Bhatia et al., 2011; Palial et al., 2022). Some of the variation in host suitability explained by biochemical properties. Phenolic compounds have been identified as involved in plant defense chemistry and could be linked to decreased insect settling, feeding or performance, while soluble sugars

have been linked to the nutritional properties of host tissues and could affect the performance of insects (Awmack & Leather, 2002; Goggin, 2007). Relationships between biochemical composition and feeding damage by the aphids, however, do not necessarily define a resistant mechanism. Determination of the phenol and soluble-sugar content can thus be used as a preliminary means of evaluating the relationship of known responses of different aphid species with particular phenotypes in different cultivars. Accurate identification of the aphid is also important when field specimens are used for biological studies. The use of molecular markers as a tool for morphological identification includes the region of the mitochondrial cytochrome c oxidase subunit I (COI) gene, which is commonly employed for animal identification and for the taxonomy of aphids (Hebert et al., 2003; Foottit et al., 2008; Coeur d'Acier et al., 2014). The present study does not aim to identify plant-resistance genes or to dissect molecular mechanisms of resistance, but rather was aimed to assist with species identification through COI sequencing. Based on this, the present investigation resulted in screening of 40 advanced rapeseed-mustard genotypes along with susceptible and resistant checks under the influence of natural infection of L. erysimi. The objectives were to evaluate aphid population and damage reactions at the flowering and pod filling stage, to classify the genotypes based on the resistance indices reported in the literature, to compare the total phenol and total soluble-sugar content among selected genotypes, and to employ the mitochondrial COI sequences to aid the identification of morphologically examined aphid samples.

## 2. MATERIAL AND METHODS

### 2.1 Plant material and field experiment

Forty rapeseed-mustard genotypes (Table 1), along with Yellow sarson (66-197-3) (Susceptible Check), Kranti (National Resistant Check), were acquired from All India Coordinated Research Project (AICRP) on Rapeseed and Mustard, Sewar, Bharatpur (Rajasthan), India. The experiment was conducted under natural field conditions at research farm of Tirhut college of Agriculture, Dholi, during rabi 2022-23 crop season in Randomized Block Design (RBD) and replicated three times in a plot size (3 × 1.5m) of paired rows with 30 x 15 cm row to row and plant to plant spacing. Two rows of susceptible check 'Yellow sarson' (66-197-3) were sown after every ten entries and also in beginning and end of each replication.

**Table 1 List of rapeseed-mustard genotypes screened against mustard aphid.**

| Genotype No. | Genotype Name | Genotype No. | Genotype Name |
|---|---|---|---|
| G1 | KMR(E) 21-1 | G22 | IJ16R1168 |
| G2 | DRMRHT 13-13-5-4 | G23 | 46 S 46 (Check) |
| G3 | DRMRHT 13-13-5-5 | G24 | PBR 939 |
| G4 | RH 1999-42 | G25 | RH 1975 |
| G5 | ORM 2019-25 | G26 | ACN 237 |
| G6 | PRE 2018-10 | G27 | NPJ 252 |
| G7 | RH- 725 | G28 | KMR 21-3 |
| G8 | DRMRHJ 1117 | G29 | DRMRIJ 20-157 |
| G9 | DRMR 2018-19$ | G30 | IJ19R5004 |
| G10 | DRMRHJ 430 | G31 | PDZ-14 |
| G11 | NPJ 250 | G32 | KANTI |

| | | | |
|---|---|---|---|
| G12 | NPJ 251 | G33 | RH 1974 |
| G13 | BSH-1 | G34 | KGMH 9198 |
| G14 | NPJ 253 | G35 | SKM-1801 |
| G15 | RHH 2101 | G36 | DRMR 2018-37 |
| G16 | DRMRHJ 2518 | G37 | DRMRCI(Q) 47 |
| G17 | NAMJH 21-01 | G38 | PDZ-15 |
| G18 | 18J408C | G39 | RH 1934 |
| G19 | SVJH-70 | G40 | RGN 524 |
| G20 | RMX 9922 | G41* | Yellow sarson (66-197-3) |
| G21 | SVJH-71 | G42* | Kranti |

**2.2 Aphid population assessment and resistance indices**

The observations on population of mustard aphid, Aphid Population Index (API), Aphid Damage Index (ADI) and Aphid Resistance Index (ARI) were recorded at flowering and pod stage based on the scale given by Dhillon et al. (2018). For this, the aphid population count was taken from ten randomly selected plants of top 10 cm inflorescence in each replication. The ARI were determined based on API and ADI values and ultimately derived the resistance category of each genotype.

**2.3 Biochemical analysis**

**2.3.1 Total phenol estimation**

The total phenols in the inflorescences of the selected rapeseed-mustard genotypes were estimated using the method as reported by Swain and Hillis (1959). In the present study, 0.25 g inflorescence was homogenized in 10 mL of 80% Methanol and placed in the water bath for 2 hrs. This was filtered through Whatman No. 1 filter paper and the volume brought down to 10 mL. A 0.5 ml aliquot was transferred to test tubes and evaporated in a boiling water-bath. Residue was dissolved in 6.5 mL distilled water and 0.5 mL Folin-Ciocalteu reagent was added to it. After 5 min, 1 mL saturated solution of Na2CO3 was added to the tubes and they were incubated in the dark for 1 hour. Absorbance was measured at 760 nm. Gallic acid (10-100 µg) was used as the standard, and phenol content was expressed as mg/g gallic acid equivalent.

2.3.2 Total soluble sugar estimation

Estimating total soluble sugars followed the procedure of Yemm and Willis (1954). Anthrone reagent solution was prepared by dissolution of 0.4 g of anthrone in 100 mL concentrated H2SO4. 300 mg of inflorescences were collected from each genotype and then homogenized in 80% methanol. The filter was collected and refluxed thrice for 15 minutes in 80% methanol hot water bath. The supernatant from each extraction was cooled and decanted into another test tube and diluted to 5 ml with 80 per cent methanol. The resulting extract was used to calculate total soluble sugars. An aliquot of 0.2 ml was taken in test tubes and evaporated to dryness in a boiling water bath. The residue in the tube was dissolved in 1 ml of distilled water and mixed with 4.0 ml of the anthrone reagent after cooling and heated in water bath for 5 min, cooled and absorbance at 620 nm was measured with a Spectrophotometer. The concentration of the total sugars was calculated from the standard curve of glucose (10-100 µg).

**2.4 Molecular identification of Lipaphis erysimi**

**2.4.1 Sample collection and morphological confirmation**

After morphological confirmation of L. erysimi samples collected from different locations across India with varying altitudes, they were further characterized at molecular level. Five adult aphids from each location were used for genomic DNA isolation.

**2.4.2 Genomic DNA extraction by CTAB**

Protocol for genomic DNA isolation of L. erysimi adults was standardized in the Molecular laboratory, Department of Entomology and Molecular laboratory II, Department of Plant Breeding and Genetics, PG College of Agriculture, RPCAU, Pusa. CTAB method was modified to isolate the genomic DNA (Cubero et al., 1999). Five adults from each

location were taken and placed on a heating block to allow the ethanol to evaporate. The samples were crushed with micro-pestles in 500µl of 2% CTAB buffer containing 100 mM Tris HCl (1.576 g), 1.4 M NaCl (8.1816 g), 20 mM EDTA (0.744 g), 2% CTAB (2 g; pH 8.0) that had been preheated at 65oC for 10 minutes and was added with 10µl of 20% Sodium Dodecyl Sulphate (SDS), 10µl of 0.1% 2-mercaptoethanol and 10µl of Proteinase-K. The suspension was incubated for 45 minutes at 65oC with the tubes being inverted for every 15 minutes. Equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added after being cooled at room temperature. The suspension was then centrifuged at 14000 rpm for 10 minutes at 4oC. The upper aqueous layer was separated in a new micro centrifuge tubes by adding an equal volume of ice-cold isopropanol and stored at -20°C for overnight and the precipitated pellet was cleaned with 70 per cent chilled ethanol with centrifugation at 10000 rpm for 10 minutes at 4oC. The ethanol was then removed and the genetic material was allowed for air dry for few minutes before being dissolved in 50 µl 1X TE buffer.

**2.4.3 DNA quality assessment**

For qualitative DNA assessment, a 0.8% agarose gel was prepared by dissolving 0.8 g agarose in 100 mL TAE buffer (Tris-acetate-EDTA). After cooling, 5.0µL EtBr (Ethidium Bromide) was added. Gel was casted in gel casting tray. DNA samples together with tracking dye (6x Methanol blue) were loaded in the well. Gel was run in the electrophoretic unit for 45 min at 100 V. After 45 min and placed into Syngene Gel Documentation system. Quantitative assessment was performed with a Biophotometer by measuring at both wavelengths (A260/A280 and A260/A230) and A260/A280 values between 1.8 and 2.0 indicate that the DNA is of high quality. If a higher concentration of DNA is discovered, it is diluted to obtain a working solution of 100 ng/µl (Sambrook et al., 1989).

**2.4.4 PCR amplification of mtCOI and sequencing**

In a Gradient Thermal Cycler (Agilent technologies TM), the PCR reaction was performed using 1µl of template DNA, 0.75µl of forward (LCO 1490 5'- GGTCAACAAATCATAAAGATATTGG-3') and reverse (HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primers, 5 µl of Hi-SYbr PCR master mixture and 3.5µl of nuclease-free water were subjected to a thermocycling programme. The amplified products were run on a 2.5 per cent Agarose gel (2.5 gm Agarose dissolved in 100 ml 1X TAE buffer) with a 100 bp DNA ladder stained with 6 µl Ethidium Bromide. The product was sent to Biokart India pvt. Ltd. Bangaluru for sequencing. Thermal conditions for amplification of mtCOI primers are follows: initial denaturation at 94°C for 2.30 sec; denaturation at 94°C for 45 sec; annealing for 30 sec; extension at 72°C for 5 min; final extension at 72°C for 10 min; and incubation at 4°C indefinitely, with 35 cycles indicated for the denaturation/annealing/extension stages.

## 3. RESULTS AND DISCUSSION

### 3.1 Field screening of Brassica genotypes

The mean aphid population at 50% flowering varied from 32.55 on KMR(E) 21-1 to 88.19 on KGMH 9198 in the table 2. The six genotypes that had the lowest aphid population at this stage were KMR(E) 21-1, DRMRHT 13-13-5-4, DRMRHT 13-13-5-5, ORM 2019-25, DRMRHJ 1117 and KANTI with values ranging from 32.55 to 43.91. Yellow sarson (66-197-3), the susceptible check, had 89.23. At pod formation, the mean aphid population ranged from 51.1 in KMR(E) 21-1 to 187.39 on KGMH 9198; KMR(E) 21-1, DRMRHT 13-13-5-4, DRMRHT 13-13-5-5, ORM 2019-25, KANTI and RH 1999-42 were among the genotypes with the lowest values, while Yellow sarson had 183.73. In the two observation stages, the cumulative mean aphid population ranged from 41.82 in KMR(E) 21-1 to 137.79 in KGMH 9198. KMR(E) 21-1, DRMRHT 13-13-5-4, DRMRHT 13-13-5-5, DRMRHJ 1117, KANTI and ORM 2019-25 had a mean population of 41.82, 44.99, 49.60, 51.95, 52.94 and 53.33, respectively. Yellow sarson, the susceptible check, had 136.48, while the national resistant check Kranti had 43.98. Mean API ranged from 1.83 to 3.26 and mean ADI from 1.03 to 4.80, while the reported mean ARI ranged from 1.43 in KMR(E) 21-1 to 4.03 in KGMH 9198. The table 3 of resistance categories classified KMR(E) 21-1 and Kranti as resistant, 11 genotypes as moderately resistant, 25 genotypes as susceptible and four as highly susceptible; no genotype was classified as highly resistant according to the reported ARI categories.

Similarly, Singh et al., (2014) tested wild and cultivated Brassica for resistance towards L. erysimi and found Brassica fruticolosa (0.7) and B. spinescens (1.0) having the lowest AII in wild crucifers and grouped them to be resistant, and the highest AII (4.0 and 5.0) in BSH-1 and Rohini and grouped them as susceptible ones. Mukherjee et al., (2017) assessed sixty-six improved rapeseed-mustard breeding genotypes for ability to resist L erysimi and found the genotypes Varuna, YST 151, Bhawani, DRMR 1165-40, PDZ 4, CS 700-3-3-6, DRMRIJ 13-38, RGN 337 to have the lowest AII (1.2). Depending on the degree of infestation. According to Maurya et al., (2018) observed that GSC-6, NRCDR 601, Vardhan, and Pusa Jagnath had the lowest infestation. Sreedhar et al., (2019), found the NPJ-194 genotype having the lowest average aphid index (1.0) and was recognised as highly resistant.

**Table 2: Screening of Brassica genotypes against Mustard aphi**

*API= Aphid Population Index, ADI= Aphid Damage Index, ARI= Aphid Resistance Index.*

| | Genotypes | Mean aphid population at 50% Flowering stage* | Mean aphid population at Pod stage* | Cumulative mean aphid population* | Mean API* | Mean ADI** | Mean ARI |
|---|---|---|---|---|---|---|---|
| 1 | KMR(E) 21-1 | 32.55 | 51.1 | 41.82 | 1.83 | 1.03 | 1.43 |
| 2 | DRMRHT 13-13-5-4 | 32.77 | 57.22 | 44.99 | 2.31 | 2.63 | 2.47 |
| 3 | DRMRHT 13-13-5-5 | 37.27 | 61.94 | 49.60 | 2.37 | 2.52 | 2.44 |
| 4 | RH 1999-42 | 46.28 | 63.56 | 54.92 | 2.42 | 2.49 | 2.45 |
| 5 | ORM 2019-25 | 43.91 | 62.76 | 53.33 | 2.41 | 2.67 | 2.54 |
| 6 | PRE 2018-10 | 59.89 | 87.56 | 73.72 | 2.68 | 2.91 | 2.79 |
| 7 | RH- 725 | 51.2 | 74.99 | 63.09 | 2.54 | 2.68 | 2.61 |
| 8 | DRMRHJ 1117 | 41.73 | 62.18 | 51.95 | 2.34 | 2.61 | 2.47 |
| 9 | DRMR 2018-19 | 46.81 | 71.55 | 59.18 | 2.49 | 2.72 | 2.60 |
| 10 | DRMRHJ 430 | 57.53 | 83.78 | 70.65 | 2.62 | 2.79 | 2.70 |
| 11 | NPJ 250 | 71.09 | 178.87 | 124.98 | 3.16 | 3.42 | 3.29 |
| 12 | NPJ 251 | 61.29 | 98.41 | 79.85 | 2.74 | 3.04 | 2.89 |
| 13 | BSH-1 | 64.38 | 149.49 | 106.93 | 3.04 | 3.48 | 3.26 |
| 14 | NPJ 253 | 48.43 | 79.23 | 63.83 | 2.54 | 2.74 | 2.64 |
| 15 | RHH 2101 | 83.93 | 143.43 | 113.68 | 3.07 | 3.47 | 3.27 |
| 16 | DRMRHJ 2518 | 59.63 | 112.89 | 86.26 | 2.86 | 3.09 | 2.97 |
| 17 | NAMJH 21-01 | 46.72 | 71.27 | 58.99 | 2.47 | 2.63 | 2.55 |
| 18 | 18J408C | 50.78 | 71.09 | 60.93 | 2.41 | 2.59 | 2.5 |
| 19 | SVJH-70 | 73.45 | 105.6 | 89.52 | 2.87 | 3.14 | 3.00 |
| 20 | RMX 9922 | 62.06 | 117.89 | 89.97 | 2.86 | 3.18 | 3.02 |
| 21 | SVJH-71 | 78.91 | 183.67 | 131.29 | 3.21 | 3.54 | 3.37 |
| 22 | IJ16R1168 | 64.21 | 176.98 | 120.59 | 3.14 | 3.51 | 3.32 |
| 23 | 46 S 46 (Check) | 50.12 | 110.27 | 80.19 | 2.64 | 2.87 | 2.75 |
| 24 | PBR 939 | 67.81 | 146.91 | 107.36 | 3.04 | 3.34 | 3.19 |
| 25 | RH 1975 | 51.76 | 104.53 | 78.14 | 2.72 | 2.98 | 2.85 |
| 26 | ACN 237 | 63.91 | 127.19 | 95.55 | 2.93 | 3.21 | 3.07 |
| 27 | NPJ 252 | 63.52 | 123.29 | 93.40 | 2.91 | 3.32 | 3.11 |
| 28 | KMR 21-3 | 49.91 | 89.12 | 69.515 | 2.61 | 2.93 | 2.77 |
| 29 | DRMRIJ 20-157 | 73.47 | 152.92 | 113.19 | 3.09 | 3.43 | 3.26 |
| 30 | IJ19R5004 | 42.79 | 69.05 | 55.92 | 2.43 | 2.71 | 2.57 |
| 31 | PDZ-14 | 73.19 | 161.88 | 117.53 | 3.12 | 3.47 | 3.29 |
| 32 | KANTI | 42.59 | 63.29 | 52.94 | 2.31 | 2.52 | 2.41 |
| 33 | RH 1974 | 44.89 | 69.26 | 57.07 | 2.44 | 2.51 | 2.47 |
| 34 | KGMH 9198 | 88.19 | 187.39 | 137.79 | 3.26 | 4.8 | 4.03 |
| 35 | SKM-1801 | 67.54 | 139.22 | 103.38 | 3.02 | 3.4 | 3.21 |
| 36 | DRMR 2018-37 | 41.22 | 76.98 | 59.10 | 2.31 | 2.78 | 2.54 |
| 37 | DRMRCI(Q) 47 | 84.78 | 179.12 | 131.95 | 3.21 | 3.82 | 3.51 |
| 38 | PDZ-15 | 64.31 | 149.04 | 106.67 | 3.04 | 3.53 | 3.28 |
| 39 | RH 1934 | 82.67 | 182.91 | 132.79 | 3.22 | 3.79 | 3.50 |
| 40 | RGN 524 | 62.93 | 141.03 | 101.98 | 3.01 | 3.41 | 3.21 |
| 41 | Yellow sarson (SC) | 89.23 | 183.73 | 136.48 | 3.25 | 3.84 | 3.54 |
| 42 | Kranti (NRC) | 33.21 | 54.75 | 43.98 | 2.0 | 1.93 | 1.96 |
| | **CD (P=0.05)** | 8.09 | 16.41 | 12.22 | 0.36 | 0.40 | 0.38 |
| | **SEm(±)** | 2.870 | 5.82 | 4.33 | 0.12 | 0.14 | 0.13 |
| | **CV (%)** | 8.42 | 8.9 | 8.7 | 8.08 | 8.15 | 8.12 |

** Top 10 cm inflorescence, ** whole plant; Susceptible Check (SC), National Resistant Check (NRC).*

**Table 3: Categorization of Brassica genotypes on the basis of Aphid Infestation Index (AII).**

| Sl. No | Number of Genotypes | Genotypes (with ARI) | Scale | Resistance category |
|---|---|---|---|---|
| **1.** | 0 | NIL | 0.0-1.0 | Highly Resistant (HR) |
| **2.** | 2 | KMR(E) 21-1 (1.43), Kranti (NRC) (1.96) | 1.1-2.0 | Resistant (R) |
| **3.** | 11 | DRMRHT 13-13-5-4 (2.47), DRMRHT 13-13-5-5 (2.44), RH 1999-42 (2.45), DRMRHJ 1117 (2.47), 18J408C (2.50), KANTI (2.41), RH 1974 (2.47), DRMR 2018-37 (2.54), ORM 2019-25 (2.54), NAMJH 21-01 (2.55), IJ19R5004 (2.57) | 2.1-2.5 | Moderately resistant (MR) |
| **4.** | 25 | PRE 2018 -10 (2.79), RH- 725 (2.61), DRMR 2018-19$ (2.60), DRMRHJ 430 (2.70), NPJ 250 (3.29), NPJ 251 (2.89), BSH-1 (3.26), NPJ 253 (2.64), RHH 2101 (3.27), DRMRHJ 2518 (2.97), SVJH-70 (3.00), RMX 9922 (3.02), SVJH-71 (3.37), IJ16R1168 (3.32), 46 S 46 (2.75), PBR 939 (3.19), RH 1975 (2.85), ACN 237 (3.07), NPJ 252 (3.11), KMR 21-3 (2.77), DRMRIJ 20-157 (3.26), PDZ-14 (3.29), SKM-1801 (3.21), PDZ-15 (3.28), RGN 524 (3.21). | 2.6 - 3.5 | Susceptible (S) |
| **5.** | 4 | KGMH 9198 (4.03), DRMRCI(Q) 47 (3.51), RH 1934 (3.51), Yellow sarson (SC) (3.54). | 3.6-5.0 | Highly susceptible (HS) |

*Susceptible Check (SC), National Resistant Check (NRC).*

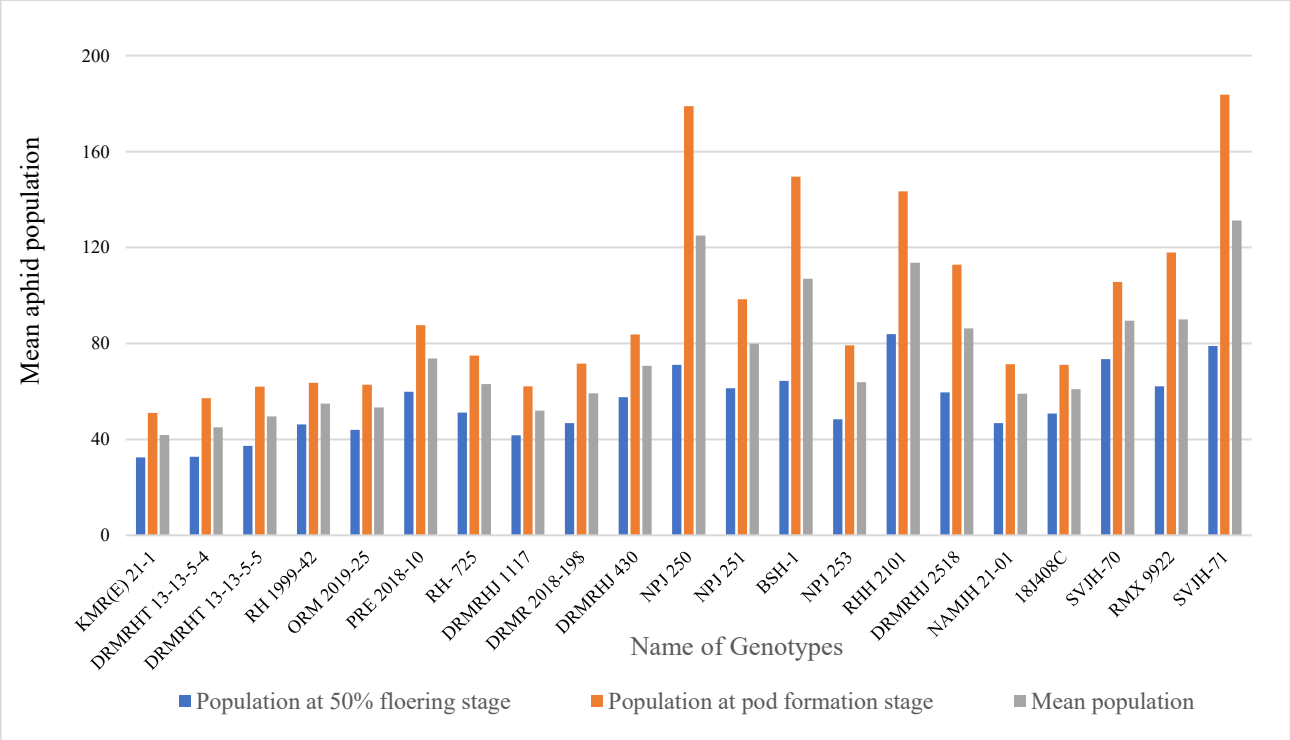
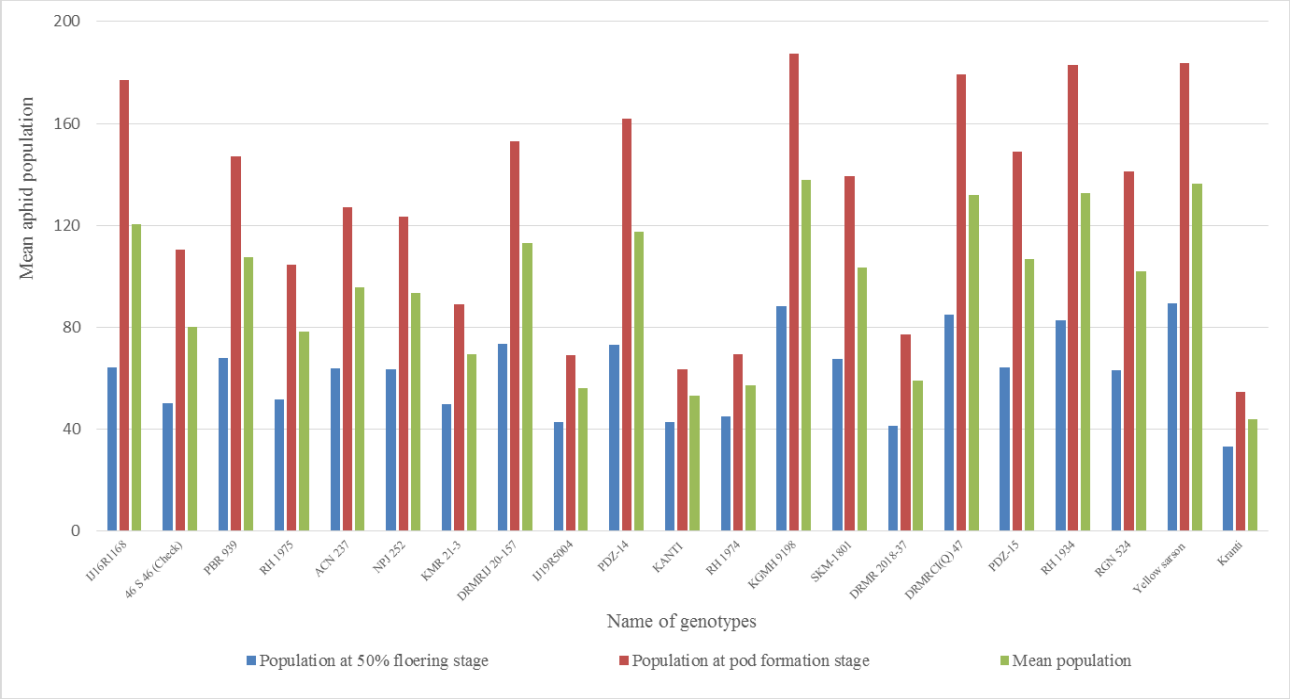



**Fig 1: Overall mean population of mustard aphid (Lipaphis erysimi) in different Brassica genotypes during rabi 2022-23**




**Continued figure 1**

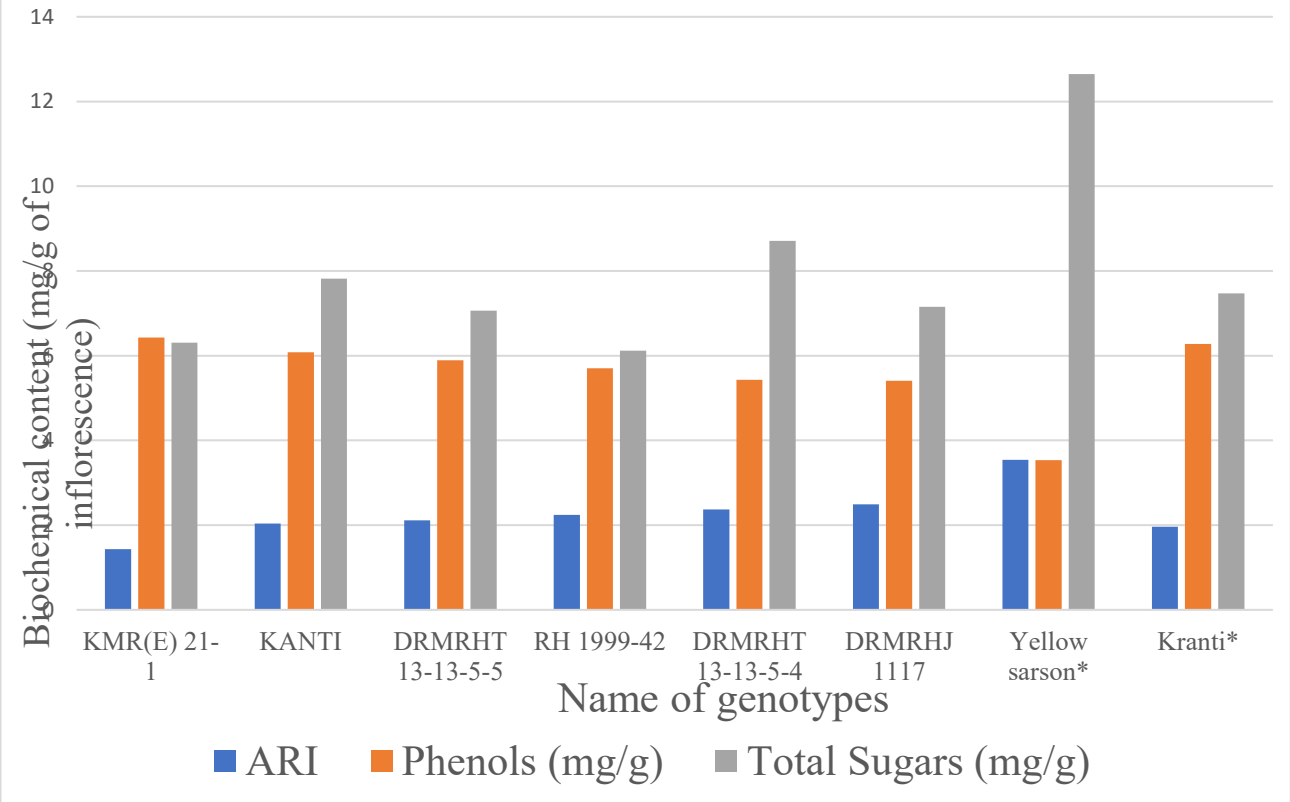



**Figure 2: Correlation of different biochemical parameters with the aphid resistance index of six promising Brassica genotype**

### 3.2 Biochemical analysis of selected genotypes

Six genotypes for biochemical comparison were identified namely KMR(E) 21-1, KANTI, DRMRHT 13-13-5-5, RH 1999-42, DRMRHT 13-13-5-4, DRMRHJ 1117 and Yellow sarson and Kranti as checks. The phenol contents of six selected genotypes were recorded as 6.43, 6.08, 5.89, 5.70, 5.43 and 5.41 mg/g respectively while Yellow sarson and Kranti recorded 3.53 and 6.28 respectively. The corresponding total sugar values were 6.31, 7.82, 7.06, 6.12, 8.71 and 7.15 mg/g, compared with 12.65 mg/g for Yellow sarson and 7.47 mg/g for Kranti (table 4).

From the correlation analysis reported in Table 5 it may be seen that phenols have a strong negative correlation with the Aphid Infestation Index (AII) with (-0.972**) significant at (p=0.01), while Total Sugars had positive but insignificant correlation with Aphid Infestation Index (AII) with (0.685 NS). These associations suggest that the measured variables are related to each other; they do not indicate a special biochemical mechanism.

The following studies from several researchers support these results; In their studies of the biochemical components of L. erysimi resistance in several rapeseed-mustard genotypes, according to Kumar and Sangha's (2017) assessment into the biochemical mechanisms behind mustard genotype that resist infestation of Lipahis erysimi. Kumar et al., (2017) discovered that BSH-1 and YSH-401 had the lowest phenol concentration. According to Palial et al., (2018) increased levels of total phenols served as a biochemical basis of resistance in the three Brassica introgression lines (I8, I79 and I82). Total phenols revealed an inverse relationship with aphid invasion, Similarly, Joseph and Peter (2007) observed the low amount of total sugar in resistant lines of cowpea against aphid infestation. Zhang and Liu (2011) found that susceptible wheat cultivars to Macrosiphum avenae had low sugar content. According to Xu et al., (2016), susceptible wheat cultivars to grain aphid infestation had significantly lower sugar levels than resistant cultivars. Kumar et al., (2017) observed the higher sugar content in susceptible variety of mustard (BSH-1).

**Table 4: Biochemical constituents of promising Rapeseed-mustard genotypes in relation to aphid infestation**

| Genotypes | ARI | Phenols (mg/g) | Total Sugars (mg/g) |
|---|---|---|---|
| KMR(E) 21-1 | 1.43 | 6.43 | 6.31 |
| KANTI | 2.04 | 6.08 | 7.82 |
| DRMRHT 13-13-5-5 | 2.11 | 5.89 | 7.06 |
| RH 1999-42 | 2.24 | 5.70 | 6.12 |
| DRMRHT 13-13-5-4 | 2.37 | 5.43 | 8.71 |
| DRMRHJ 1117 | 2.49 | 5.41 | 7.15 |
| Yellow sarson* | 3.54 | 3.53 | 12.65 |
| Kranti* | 1.53 | 6.28 | 7.47 |

**Genotypes taken as susceptible and resistant checks.*

**Table 5: Correlation between biochemical constituents of promising Rapeseed-mustard genotypes and Aphid Infestation Index (AII)**

| Variable | Phenols | Total sugars |
|---|---|---|
| Aphid Infestation Index (AII) | -0.972** | 0.685 $^{NS}$ |

**3.3 Molecular identification of aphids in Brassica genotypes.**

The present study aimed at isolation of DNA from aphid species from mustard crop and mitochondrial cytochrome oxidase gene was amplified by using two primers HCO 2198 and LCO 1490 followed by sequencing. The sequences of aphid species Lipaphis erysimi pseudobrassicae was taken through sanger sequencing. These obtained sequences of aphids were subjected to analyse the species through NCBI BLAST to confirm the species. During NCBI BLAST it was found that sample nucleotide sequence of sample 1 has 98.04% similarity with Lipaphis erysimi pseudobrassicae while sample 2 has 98% similarity with the same species.

After species confirmation with the NCBI BLAST, the nucleotides were subjected to submission in NCBI GenBank to get the accession numbers. The BLASTN results of sequences were confirmed with morphological characters of aphids.

The morphological identification of the aphids was confirmed by mtCOI analysis of sequence data. COI is a marker established in animal DNA barcoding, and for aphids, it helps identify species for a wide range of taxa (Hebert et al. 2003; Foottit et al. 2008). The present sequences exhibited 98.04 and 98% similarity to that deposited in NCBI as Lipaphis erysimi pseudobrassicae and are shown as ORO145045 and ORO145046, respectively. The results agree with the morphological identification in the study and provide molecular confirmation.

**Table 4.5: mtCOI sequence records, GenBank accession numbers and similarity values reported for two aphid samples.**

| Sample No. | Nucleotide sequence | Accession number | Similarity % |
|---|---|---|---|
| 1 | AAAATTTAAATTTGAAACTTAAAAAATTAATTAC GCTGTTATCCCTAAAGTAACTTTTTCCTTTTAAT TAAAAATTTTAATTCAAATATTCATTAAATAATG TAAAATTTTAAAAAAAGTTATTTAAATTTTTTTA TCACCCCAATAAAATAAATATTAAAATTAAAATA TTTATTTTCCAAAAAAATTAAAATTAATATTTAT AAAGTTTTATAGGGTCTTATCGTCCCTTTAAATA ATTTAAGCTTTTTAACTTAAAAATAAAATTCTAA TTATATAAATAATAAAGTCTATTTCTCATCAAAT CTTTCATACAAGTCCTCAATTAAAAGACTAATTA TTATGCTACCTTTGCACAGTCAAAATACTGCAGC TATTTAATAAATCATTGAGCAGATCCTATATTAA ATTAAACTTAATACAATGTTTTGATAAAACAGGG TGA | ORO145045 | 98.04 |
| 2 | GGTTTTTTTTATATAGGATCTGCTCATGATTTATT AAATAGCTGCAGTATTTTGACTGTGCAAAGGTAGC ATAATAATTAGTCTTTTAATTGAGGACTTGTATGA AAGATTTGATGAGAAATAGACTTTATTATTTATAT AATTAGAATTTTATTTTTAAGTTAAAAAGCTTAAA TTATTTAAAGGGACGATAAGACCCTATAAAACTTT ATAAATATTAATTTTAATTTTTTTGGAAAATAAAT ATTTTAATTTTAATATTTATTTTATTGGGGTGATA AAAAAATTTAAATAACTTTTTTTAAAATTTTACAT TATTTAATGAATATTTGAATTAAAATTTTTAATTA AAGGAAAAAGTTACTTAGGGATAACAGCGTAATTA ATTTTTTAAGTCAAATTTAAAAATTAGTTTGCGAC CTCGATGTTGAATAAG | ORO145046 | 98 |

**CONCLUSION**

Among the various genotypes tested, KMR(E) 21-1 proved to be a promising resistant one and its performance against mustard aphid (Lipaphis erysimi) was comparable with the resistant check (Kranti). There was strong negative correlation between total phenol content and aphid incidence, indicating that this could play a role in aphid resistance. mtCOI sequencing was also used to aid in the identification of aphids. The findings can inform resistance breeding programmes and integrated pest management, but more multi-location and multi-season testing is required.

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