Biochemical and Physiological Factors Imparting Tolerance in Safflower against Aphid, *Uroleucon compositae* (Theobald)

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**Abstract**

Development of aphid tolerant cultivars is needed in pest management in safflower, where the crop is often grown with least plant protection measures. Sixteen recombination inbred lines (RILs) of F₂ generation of the cross, CO-1 (susceptible) × EC-523368-2 (tolerant) along with parents were studied to understand the biochemical and physiological factors operating in tolerant RILs. Eight RILs were confirmed tolerant (A.I.I., 1.1 - 1.5) and 8 RILs were found highly susceptible (A.I.I., 4.5 - 5.0) to aphid. Susceptible RILs underwent more oxidative stress through more H₂O₂ (4908.0 ± 1287 nmols g⁻¹ fresh weight) production due to aphid infestation compared to tolerant RILs. Activity reactive oxygen species (ROS) enzymes, superoxide dismutase (53.63 ± 0.29 (nmols g⁻¹ fresh weight) and catalase (33.73 ± 3.3 µmols g⁻¹ fresh weight) and amount of metabolites like total phenols (169.60 ± 16.49 µg g⁻¹ fresh weight) production due to aphid infestation compared to tolerant RILs were more than susceptible ones. The tolerant RILs were physiologically more efficient with higher chlorophyll (8.45 ± 0.91 mg L⁻¹ fresh weight) and amount of metabolites like total phenols (169.60 ± 16.49 µg g⁻¹ fresh weight) were more than intolerant RILs than susceptible ones. The tolerant RILs were more antioxidative stress with higher chlorophyll (8.45 ± 0.91 mg L⁻¹ fresh weight) and amount of metabolites like total phenols (169.60 ± 16.49 µg g⁻¹ fresh weight) were more than intolerant RILs than susceptible ones. The tolerant RILs were more antioxidative stress with higher chlorophyll (8.45 ± 0.91 mg L⁻¹ fresh weight) and amount of metabolites like total phenols (169.60 ± 16.49 µg g⁻¹ fresh weight) were more than intolerant RILs than susceptible ones.

**Keywords**: Aphid, Biochemical factors, Physiological factors, Safflower, Tolerance, *Uroleucon compositae*.

**Introduction**

Safflower (*Carthamus tinctorius L.*) is an important oilseed crop, grown in India since ages for its high-quality edible oil. This is cultivated on residual soil moisture in *Rabi* (winter) season. Safflower is mainly cultivated in the states of Maharashtra, Karnataka, Telangana, Madhya Pradesh, Gujarat and parts of Andhra Pradesh under different cropping systems. India’s total safflower production is 1.22 MT from an area of 1.56 lakh ha with a productivity of 782 kg per ha (Mukta et al., 2017). Aphid *Uroleucon compositae* (Theobald) is considered as a major pest. A yield loss up to 78.5% was recorded on susceptible variety compared to a 48.5% yield loss on moderately tolerant when proper control measures were not taken (IIOR, 2015). Both nymphs and adults suck sap from a shoot and young leaves, due to which the plant growth is stunted. In case of a severe attack of the aphid, the plants start showing yellowing and drying, resulting in premature death of plants. In addition, aphid also excretes honeydew, which falls on the upper surface of below leaves on which sooty mold develops, hindering the photosynthetic activity (Balikai, 2000). Mostly, the safflower crop is grown by small and marginal farmers with low inputs and may not receive any plant protection measures many times (Hanumantharaya et al., 2007) to avoid production cost. This often results in to significant yield loss. Many authors have studied...
the reaction of safflower to aphids under natural infestation (Singh, 2008; Rajput et al., 2013; Guljar and Rajesh, 2016). Few safflower accessions were reported for their reaction under the artificial release of aphids (Sriniivas and Mukta, 2015; Mukta et al., 2017).

A stay green safflower germplasm line, EC-523368-2 has been identified, which showed higher levels of tolerance to aphid (DOR, 2012). The stay green character indicated that this tolerant genotype is physiologically more efficient than other genotypes. Tolerance is distinctive in terms of the plant’s ability to withstand or recover from herbivore injury through growth and compensatory physiological processes. The tolerant plants can compensate photosynthetically by avoiding feedback inhibition and impaired electron flow through photosystem II resulting from insect feeding. Similarly, the up-regulation of peroxidases and other oxidative enzymes during insect feeding, in conjunction with elevated levels of phytohormones, can play an important role in providing plant tolerance to insect pests (Kyle et al., 2016).

However, very little information is available on such mechanisms of tolerance in safflower against aphid. Therefore, the present investigation is undertaken to analyze the physiological and biochemical factors imparting tolerance in safflower to aphids.

Materials and Methods
The present study was carried out at ICAR- IIOR Farm, ICRISAT (17.530’N Latitude and 78.270’E Longitude) during Rabi season of 2018-2019. In previous years, a germplasm accession, EC-523368-2 was identified as stay green and highly tolerant to aphid. Based on the reaction of around 300 RILs of the cross, CO-1 X EC-523368-2 evaluated in the F$_3$ generation in the previous year, 8 tolerant and 8 susceptible recombination inbred lines (RILs) were selected. The same set of 16 RILs of F$_7$ generation and their parents were evaluated during Rabi, 2018, to confirm their reaction to aphid.

All 16 RILs along with parents (also checks) were sown on 14$^{th}$ December 2018 in two replications following a completely randomized block design. Each RIL was raised in 3 rows of 2 m in length each with a spacing of 45 x 10 cm. Infester plants of susceptible variety, CO$^-$1 was sown, one month before sowing of test entries a separate block away from the main screening block. When the test entries reached stem elongation stage (~ 40 day old), infester plants with aphids were cut and distributed @ 1 plant per 1 m row (IIOR, 2018). Aphids were moved to the test entries, multiplied and caused damage symptoms. Five plants from each RIL were randomly selected in each replication and when susceptible check, CO$^-$1 was completely got killed, the injury rating was given on a 1-5 scale based on % yellowing and drying of foliage viz., 0 to 20% = 1; 21 to 40% = 2; 41 to 60% = 3; 61 to 80% = 4 and 81 to 100% = 5 and aphid infestation index (A.I.I.) was calculated by using the following formula:

$$\text{A.I.I.} = \frac{1 \times a + 2 \times b + 3 \times c + 4 \times d + 5 \times e}{a + b + c + d + e}$$

Where, a, b, c, d and e are the actual number of plants falling in each of the 5 corresponding foliage drying grades i.e., 1 to 5. Finally, the mean of A.I.I. was calculated and the entries were classified into different grades as - highly tolerant (A.I.I. > 1.0), tolerant (A.I.I. > 0.0 to 2.0), moderately tolerant (A.I.I. > 2.0 to 3.0), susceptible (A.I.I. > 3.0 to 4.0) and highly susceptible (A.I.I. > 4.0 to 5.0).

Same set of 16 RILs were also raised in a separate block aphid as free regime that was away from main screening block to avoid migration of aphid. Recommended systemic insecticide was sprayed regularly to free the plants from aphids.

After 10 days of aphid release (DAAR), plants showed symptoms due to feeding of aphids. Top 5 cm twig portion of the plants from both aphid-free and aphid-infested plants from each replication were cut with a fine blade and the samples were brought to the laboratory and stored at -20°C in deep freezer till the plant analysis was done.

Lipid Peroxidation, Antioxidative Enzymes and Metabolites

Preparation of Various Extracts
Unless stated otherwise, all extraction procedures were carried out at 0 to 4°C. Each experiment was repeated thrice and the estimations further made in duplicate. Preliminary experiments were conducted to optimize the extraction conditions with respect to pH, molarity and type of buffer, the concentration of stabilizing agent(s) and other constituents of the extraction medium. Finally, the standardized extraction medium for superoxide dismutase (SOD) and peroxidase (POX) consisted of 0.1 M Tris-HCl buffer (pH 7.5) containing 3% (w/v) polyvinylpyrrolidone, 1 mM EDTA and 1mM CaCl$_2$. The extraction medium for Catalase (CAT) and ascorbate peroxidase (APX) consisted of 0.1 M potassium phosphate buffer (pH 7.5) in place of Tris-HCl buffer, the rest extractants being the same.

The enzymes were extracted by macerating 2 g tissue with 7.2 mL of ice-cold extraction medium in a pre-chilled pestle and mortar placing in ice bath. The homogenate was filtered through four-layered muslin cloth and the filtrate was centrifuged at 15,000 rpm for 20 minutes in a refrigerated centrifuge at 4°C. The supernatant (7.3 mL) was carefully decanted, labelled and stored in deep freezer at -20°C. Trichloro acetic acid (TCA) extract was prepared and used for the estimation of malondialdehyde (MDA) and total glutathione. One gram of tissue was ground with 5 mL of 0.1% TCA. The extract was filtered through four-layered muslin cloth and centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was collected and used for the analysis.
**Lipid Peroxidation**

MDA, the level of lipid peroxidation was measured in terms of MDA by using 2-thiobarbituric acid (TBA) reaction employing slightly modified method of Heath and Packer (1968). The MDA concentration was calculated using the molar extinction coefficient of 155 mM$^{-1}$ cm$^{-1}$ (Dipierro and Leonardis, 1997) and expressed as nmol of MDA g$^{-1}$ fresh weight. Hydrogen peroxide (H$_2$O$_2$), an important reactive oxygen species (ROS) and its higher concentration in the cell, causes lipid peroxidation. The amount of H$_2$O$_2$ was estimated by Sinha (1972) and expressed as nmol of H$_2$O$_2$ g$^{-1}$ fresh weight.

**Antioxidative Enzymes**

SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrarazolium (NBT) (Beauchamp and Fridovich, 1971). Percent inhibition was calculated by the following formula of Asada et al. (1974).

\[
\text{Percent inhibition} = \frac{(V - v)}{V} \times 100, \quad \text{Where, } V = \text{rate of assay reaction in absence of SOD, } v = \text{rate of assay reaction in presence of SOD.}
\]

The amount of SOD was expressed as nmol g$^{-1}$ fresh weight. Catalase activity was measured by slightly modified method of Sinha (1972) and the amount of CAT expressed as µmol g$^{-1}$ fresh weight. Peroxidase was assayed by determining the rate of Guaiacol oxidation in the presence of H$_2$O$_2$ at 470 nm (Rao et al., 1996). Ascorbate peroxidase was assayed by the method of Nakano and Asada (1981). The amount of POX and APX was expressed as nmols min$^{-1}$ g$^{-1}$ fresh weight.

**Metabolites**

Total phenols quantity was determined by the method of Ainsworth and Gillespie (2007) and expressed as µg g$^{-1}$ gallic acid equivalent (GAE). Total glutathione content was estimated by the method suggested by Smith (1985) and expressed as nmols g$^{-1}$ fresh weight.

**Physiological Factors**

Total chlorophyll content (Chlorophyll ‘a’ and ‘b’) was estimated from the leaf samples collected from safflower RILs at 10 DAAR, by using the method described by Arnon (1949) and expressed as mg L$^{-1}$. Net photosynthesis ($P_n$), net assimilation rate (NAR) and intrinsic water use efficiency (IWUE) were calculated by measuring amount of CO$_2$ and water vapor exchange in attached leaves through a portable gas exchange measuring system (Model LI-6400, LI-COR, USA) (Ratnakumar et al., 2013) and expressed as µmole CO$_2$ m$^{-2}$ sec$^{-1}$, µmole CO$_2$ cm$^{-2}$ leaf area and µmole CO$_2$ mol$^{-1}$ H$_2$O, respectively.

**Statistical Analysis**

The data pertaining to all tolerant RILs and susceptible RILs were pooled separately replication-wise. The data were analyzed using the analysis of variation (ANOVA) using SPSS software. Means were separated by LSD at 5% level of significance.

**Results and Discussion**

There was significant differential reaction showed by safflower RILs to aphids. Out of 16 RILs evaluated, 8 RILs were found tolerant and 8 RILs were found highly susceptible to aphid. The tolerant check, EC-523368-2 and susceptible check, CO-1 was recorded an average A.I.I of 1.3 (tolerant) and the highest A.I.I of 5.0 (highly susceptible), respectively (Table 1).

**Lipid Peroxidation (MDA, H$_2$O$_2$)**

Both MDA and H$_2$O$_2$ content indicate the extent of lipid peroxidation in the plants. The MDA content was significantly more in susceptible lines (2.60 ± 0.19 nmols g$^{-1}$ fresh weight) compared to tolerant lines after 10 days of aphid infestation. When tolerant lines were attacked by aphids MDA content was increased by 27.0%. Susceptible lines produced 9.24% more MDA compared to tolerant lines (t-test, p = 0.04) when challenged with aphid infestation. With aphid infestation, H$_2$O$_2$ content has significantly increased in both tolerant and susceptible lines by 19.0 and 12.25%, respectively compared to aphid free condition. Susceptible lines produced 10.27% more H$_2$O$_2$ than tolerant lines (Table 2). Increased lipid peroxidation was observed in both tolerant and susceptible lines whenever plants underwent stress.

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Table 1: Reaction of safflower RILs to aphid, *U. compositae* during Rabi 2018-19

<table>
<thead>
<tr>
<th>RILs</th>
<th>A.I.I.</th>
<th>Category</th>
<th>RILs</th>
<th>A.I.I.</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIL-6</td>
<td>1.1</td>
<td>Tolerant</td>
<td>RIL-81</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>RIL-14</td>
<td>1.1</td>
<td>Tolerant</td>
<td>RIL-114</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>RIL-18</td>
<td>1.1</td>
<td>Tolerant</td>
<td>RIL-152</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>RIL-34</td>
<td>1.2</td>
<td>Tolerant</td>
<td>RIL-201</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>RIL-218</td>
<td>1.3</td>
<td>Tolerant</td>
<td>RIL-210</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>RIL-222</td>
<td>1.2</td>
<td>Tolerant</td>
<td>RIL-250</td>
<td>4.5</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>RIL-235</td>
<td>1.5</td>
<td>Tolerant</td>
<td>RIL-322</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>RIL-351</td>
<td>1.2</td>
<td>Tolerant</td>
<td>RIL-358</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
<tr>
<td>EC-523368-2</td>
<td>1.3</td>
<td>Tolerant</td>
<td>CO-1</td>
<td>5.0</td>
<td>Highly susceptible</td>
</tr>
</tbody>
</table>

RIL: Recombinant Inbred Line, A.I.I- Aphid Infestation Index
due to aphid infestation but was more in susceptible lines than the tolerant lines. Lipid peroxidation has been often assessed by monitoring the changes in MDA value (Mondal et al., 2006). Increase in peroxidation of membrane lipids during oxidative stress is due to more production of reactive oxygen species (ROS) (Wise, 1995). Hydrogen peroxide is one of the ROS capable of causing oxidative damage besides other species like, superoxide radical (O$_2^-$), perhydroxy radical (HO$_2^-$), hydroxyl radical (OH$\cdot$), peroxy radical (ROO$\cdot$) and singlet oxygen (O$_2$) (Krieger-Liszkay, 2005). These ROS react with various cellular targets resulting in DNA and protein damage and in lipid peroxidation (Apel and Hirt, 2004).

**Antioxidative Enzymes (SOD, CAT, POX and APX)**

The SOD activity in tolerant lines with a mean value of 52.79 nmols g$^{-1}$ fresh weight under aphid free condition increased to mean value of 53.63 nmols g$^{-1}$ fresh weight with aphid infestation. In susceptible lines, SOD reduced by 12.7% (43.29 nmols g$^{-1}$ fresh weight) after aphid infestation. SOD was lesser in susceptible lines by 19.3% compared to tolerant lines (t-test, p = 0.002) (Table 3). SOD is the first line of defense against oxyradical-mediated injury (Van Camp et al., 1996). It catalyzes the dismutation of O$_2^•$ into H$_2$O$_2$ and plays an important role in protecting cells against superoxide derived oxidative damage (Rabinowitch and Fridovich, 1983) in plants (Giannopolitis and Ries, 1977). The increase in SOD activity was reported in wheat due to *D. noxia* feeding (Ni and Quisenberry, 2003) and in cassava due to *Tetranychus cinnabarinus* (Lu et al., 2017).

Catalase is one of the primary enzymatic defenses against oxidative stress (Zimmermann et al., 2006). In the present study, activity of CAT was increased in tolerant lines with a mean value of 33.73 µmols g$^{-1}$ fresh weight than in susceptible lines (23.66 µmols g$^{-1}$ fresh weight) after aphid infestation (t-test, p = 0.001) (Table 3). CAT activity was 29.84% higher in tolerant lines than the susceptible

**Table 2: Lipid peroxidation in tolerant vs susceptible RILs to aphid in safflower**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean + SEm</th>
<th>p &lt;= 0.05</th>
<th>% Change</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aphid free</td>
<td>Aphid infested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde (MDA) (nmols g$^{-1}$ fresh weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerant</td>
<td>1.87 ± 0.14</td>
<td>2.38 ± 0.25</td>
<td>0.05</td>
<td>27.0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>1.94 ± 0.15</td>
<td>2.60 ± 0.19</td>
<td>0.04</td>
<td>34.0</td>
</tr>
</tbody>
</table>

**Table 3: Activity of ROS enzymes in tolerant vs susceptible RILs to aphids in safflower**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean + SEm</th>
<th>p &lt;= 0.05</th>
<th>% Change</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aphid free</td>
<td>Aphid infested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide Dismutase (SOD) (nmols g$^{-1}$ fresh weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerant</td>
<td>52.79 ± 0.35</td>
<td>53.63 ± 0.29</td>
<td>0.002</td>
<td>1.6</td>
</tr>
<tr>
<td>Susceptible</td>
<td>49.58 ± 0.31</td>
<td>43.29 ± 0.64</td>
<td>0.004</td>
<td>12.7</td>
</tr>
<tr>
<td>Catalase (CAT) (µmols g$^{-1}$ fresh weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerant</td>
<td>28.93 ± 3.82</td>
<td>33.73 ± 3.3</td>
<td>0.001</td>
<td>16.59</td>
</tr>
<tr>
<td>Susceptible</td>
<td>18.72 ± 2.73</td>
<td>23.66 ± 1.96</td>
<td>0.00001</td>
<td>26.38</td>
</tr>
<tr>
<td>Peroxidase (POX) (nmols min$^{-1}$ g$^{-1}$ fresh weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerant</td>
<td>92.77 ± 9.5</td>
<td>163.36 ± 38.0</td>
<td>0.0009</td>
<td>175.0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>107.8 ± 21.6</td>
<td>328.25 ± 66.46</td>
<td>&lt;0.0001</td>
<td>204.5</td>
</tr>
<tr>
<td>Ascorbate Peroxidase (APX) (nmols min$^{-1}$ g$^{-1}$ fresh weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerant</td>
<td>112.98 ± 33.9</td>
<td>721.67 ± 179.6</td>
<td>&lt;0.0001</td>
<td>538.7</td>
</tr>
<tr>
<td>Susceptible</td>
<td>144.48 ± 36.58</td>
<td>633.67 ± 132</td>
<td>&lt;0.0001</td>
<td>339.5</td>
</tr>
</tbody>
</table>
lines after aphid infestation. This showed that catalase effectively scavenged \( \text{H}_2\text{O}_2 \) that was produced due to aphid infestation in tolerant lines compared to susceptible lines. Most of the catalase activity is associated with peroxisomes where it removes the hydrogen peroxide formed during photorespiration. Therefore, in plants, CAT is often considered to be a peroxisomal marker enzyme because of its presence in these organelles (Corpas et al., 1993). Higher activity of CAT was reported in resistant black gram genotypes than in susceptible genotypes against whiteflies, *Bemisia tabaci* (Kumar et al., 2012). CAT activity was positively related to resistance in transgenic cassava lines to *T. cinnabarinus* (Lu et al., 2017).

Peroxidases are another group of non-chloroplastic enzymes that detoxify \( \text{H}_2\text{O}_2 \) in the cytosolic part of cell. POX appeared to be a major antioxidative enzyme in safflower against aphid. When challenged with aphids, its activity was increased by 175.0 and 204.5% in tolerant and susceptible lines. With aphid infestation, POX activity was doubled (328.25 nmols min\(^{-1}\) g\(^{-1}\) fresh weight) in susceptible lines compared to tolerant lines (163.36 nmols min\(^{-1}\) g\(^{-1}\) fresh weight) (Table 3). POX would be involved in the scavenging of \( \text{H}_2\text{O}_2 \) that is not removed by CAT (Willekens et al., 1997).

Aphid infestation induced POX activity in all cultivars of wheat against *Sitobion avenae*, especially in susceptible ones Han et al. (2009).

Aphid infestation strongly induced the positive activity of APX aphid-infested plants in both tolerant and susceptible plants. With aphid feeding, APX activity was increased by 538.7% (721.67 nmols min\(^{-1}\) g\(^{-1}\) fresh weight) in tolerant lines and 339.5% (633.67 nmols min\(^{-1}\) g\(^{-1}\) fresh weight) in susceptible lines. Tolerant lines had 12.19% more APX activity than the susceptible lines when infested with aphids (Table 3). Ascorbate peroxidase is another important enzyme which plays a pivotal role in eliminating \( \text{H}_2\text{O}_2 \) from plant cells. Primary purpose of APX is to scavenge \( \text{H}_2\text{O}_2 \) before it can react with cellular biomolecules and cause damage (Shigeoka et al., 2002). Aphid-induced APX activity to a higher level in a less susceptible cultivar of triticale than in a more susceptible one against *Sitobion avenae* (Iwona et al., 2012). The oligophagous species, *Rhapalosiphum padi* caused stronger induction of APX activity in tested triticale than the monophagous species *S. avenae*.

**Metabolites (Total phenols and Glutathione)**

With aphid infestation total phenol content in safflower plants was increased in both tolerant and susceptible lines by 8.04 and 5.83%, respectively. Total phenols were more in tolerant lines (169.60 µg g\(^{-1}\) gallic acid equivalent) than susceptible lines (157.00 µg g\(^{-1}\) gallic acid equivalent) after aphid infestation. In the presence of aphid infestation, tolerant lines produced 8.02% more phenols than that of susceptible lines (t-test, p = 0.01) (Table 4). The majority of plant phenolic compounds are toxic to herbivorous insects, including aphids and impair their growth, development and fecundity (Dreyer and Campbell, 1987). An increase in total phenols with aphid infestation was earlier reported in mustard (Sharma and Rao, 2013) and cotton (Divya et al., 2017). Negative correlation between the total phenols and mustard aphid, *L. erysimi* population was reported by Ram et al. (1995) and Jat et al. (2007).

Aphid infestation induced total glutathione in susceptible and tolerant lines by 69.0% more than aphid free lines. The glutathione content has been significantly increased in susceptible lines (t-test, p = 0.0001) that were infested with aphid (11497.0 nmols g\(^{-1}\) fresh weight) than tolerant lines (10030.0 nmols g\(^{-1}\) fresh weight) (Table 4). It indicated that glutathione increased with an infestation of aphids in order to scavenge ROS produced in both tolerant and susceptible plants. Glutathione is an antioxidant also plays a role in scavenging active oxygen species (Dhindsa, 1987; Noctor and Foyer, 1998).

**Physiological Factors (Total chlorophyll, \( P_n \) NAR and IWUE)**

The impact of aphid feeding on physiological factors viz. total chlorophyll, net photosynthesis, net assimilation rate and intrinsic water use efficiency in tolerant and susceptible safflower lines was studied. Total chlorophyll content was reduced with an infestation of aphids in both the tolerant and susceptible lines. However, the loss of chlorophyll in susceptible lines was significantly more (33.0%) while tolerant lines lost only 11.0% of chlorophyll due to aphid feeding. Susceptible lines had 21.35% less chlorophyll than that of tolerant lines (t-test, p = 0.01) (Table 5). Chlorophyll levels change during plant development (Costa et al., 2001), and can alter in response to a wide variety of stresses (Lawson et al., 2001). Chlorophyll content can be reduced by insect feeding, nutritional deficiencies and pathogen infections (Ni et al., 2002). Significant chlorophyll loss in infested plants was reported in *Pisum sativum* L., *Vicia faba* L., *Trifolium pretense* L., *Medicago sativa* L. due to feeding by *Acrystosiphon pismum* (Sylvia et al., 2010) and in maize by *R. padi* and *S. avenae* (Hubert et al., 2013). Loss of chlorophyll is 40% in the susceptible soybean due to aphid, *Aphis glucines* (John et al., 2007).

In susceptible lines, net photosynthesis (\( P_n \)) was reduced by 32.0% due to aphid infestation while it is only 7.7% reduction in tolerant lines when compared to respective lines in aphid free conditions. Because of aphid infestation, net photosynthesis was reduced by 36.0% in susceptible lines compared to tolerant lines. Net assimilation rate (NAR) also followed the same trend as that of net photosynthesis. This clearly showed that the tolerant lines were more efficient photosynthetically than that of susceptible lines. This may be why the tolerant lines stay green even after aphid infestation.
Aphid-tolerant lines have more intrinsic water use efficiency (iWUE) than susceptible lines. Aphid infestation reduced iWUE of tolerant lines by 14.6% while it was 52.9% in case of susceptible lines. The study clearly showed that the tolerant lines are more efficient in water usage (0.35 µmole CO\textsubscript{2} mol\textsuperscript{-1} H\textsubscript{2}O) compared to susceptible lines (0.16 µmole CO\textsubscript{2} mol\textsuperscript{-1} H\textsubscript{2}O) when under stress of aphid infestation. Aldea et al. (2005) reported that herbivorous insects will cause water loss in the infested soybean leaves. Petitt and Smilowitz (1982) concluded that aphid feeding decreases the moisture content of infested leaves and plants under the stress of early-season infestation allocated more resources for leaf growth, but stem growth was severely retarded.

It is concluded that susceptible lines underwent more biotic stress through more lipid peroxidation through H\textsubscript{2}O\textsubscript{2} production due to aphid infestation compared to tolerant lines of safflower. Tolerant safflower lines were had more ROS enzyme activity and total phenols than susceptible ones. Also, the tolerant lines are physiologically more efficient with higher chlorophyll, net photosynthesis, net assimilation rate and intrinsic water use efficiency than the susceptible safflower lines.

**Table 4:** Plant metabolites in tolerant vs susceptible RILs to aphids in safflower

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean + SEM</th>
<th>p&lt;=0.05</th>
<th>% Change</th>
<th>ANOVA Source</th>
<th>F</th>
<th>df</th>
<th>p&lt;=0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerant</td>
<td>Aphid Free</td>
<td>156.97 ±26.3</td>
<td>169.60 ±16.49</td>
<td>0.04</td>
<td>8.04</td>
<td>Tolerance</td>
<td>61.8</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Aphid Infested</td>
<td>157.00 ±11.9</td>
<td>147.84 ±20.84</td>
<td>0.01</td>
<td>5.83</td>
<td>Aphid infestation</td>
<td>3.1</td>
</tr>
</tbody>
</table>

**Total phenols (µg g\textsuperscript{-1} Gallic Acid Equivalent)**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean ± SEM</th>
<th>p&lt;=0.05</th>
<th>% Change</th>
<th>ANOVA Source</th>
<th>F</th>
<th>df</th>
<th>p&lt;=0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerant</td>
<td>Aphid Free</td>
<td>5960±1256</td>
<td>10030±1896</td>
<td>&lt;0.0001</td>
<td>69.0</td>
<td>Tolerance</td>
<td>7075</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Aphid Infested</td>
<td>6825±1194</td>
<td>11497±1287</td>
<td>&lt;0.0001</td>
<td>68.4</td>
<td>Aphid infestation</td>
<td>44491</td>
</tr>
</tbody>
</table>

**Total Glutathione (nmols g\textsuperscript{-1} fresh weight)**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean ± SEM</th>
<th>p&lt;=0.05</th>
<th>% Change</th>
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<td>Aphid Infested</td>
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<td>&lt;0.0001</td>
<td>68.4</td>
<td>Aphid infestation</td>
<td>44491</td>
</tr>
</tbody>
</table>

**Table 5:** Comparison of different physiological factors in tolerant vs susceptible RILs to aphids in safflower

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean ± SEM</th>
<th>p&lt;=0.05</th>
<th>% Change</th>
<th>ANOVA Source</th>
<th>F</th>
<th>df</th>
<th>p&lt;=0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerant</td>
<td>Aphid Free</td>
<td>9.92 ±0.6</td>
<td>8.45 ±0.91</td>
<td>0.03</td>
<td>11.0</td>
<td>Tolerance</td>
<td>20.2</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Aphid Infested</td>
<td>9.88 ±0.78</td>
<td>6.65 ±1.34</td>
<td>0.01</td>
<td>33.0</td>
<td>Aphid infestation</td>
<td>563</td>
</tr>
</tbody>
</table>

**Total chlorophyll (mg L\textsuperscript{-1})**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean ± SEM</th>
<th>p&lt;=0.05</th>
<th>% Change</th>
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<td>0.01</td>
<td>33.0</td>
<td>Aphid infestation</td>
<td>563</td>
</tr>
</tbody>
</table>

**Net Photosynthesis (Pn) (µmole CO\textsubscript{2} m\textsuperscript{-2} sec\textsuperscript{-1})**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean ± SEM</th>
<th>p&lt;=0.05</th>
<th>% Change</th>
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<th>p&lt;=0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerant</td>
<td>Aphid Free</td>
<td>31.32 ±3.09</td>
<td>28.9 ±3.31</td>
<td>0.085</td>
<td>7.7</td>
<td>Tolerance</td>
<td>16.74</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Aphid Infested</td>
<td>27.06 ±2.62</td>
<td>18.5 ±0.07</td>
<td>0.023</td>
<td>32.0</td>
<td>Aphid infestation</td>
<td>81.33</td>
</tr>
</tbody>
</table>

**Net Assimilation Rate (NAR) (µmole CO\textsubscript{2} cm\textsuperscript{-2} leaf area)**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
<th>Mean ± SEM</th>
<th>p&lt;=0.05</th>
<th>% Change</th>
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<th>F</th>
<th>df</th>
<th>p&lt;=0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerant</td>
<td>Aphid Free</td>
<td>0.31 ±0.03</td>
<td>0.289 ±0.033</td>
<td>0.05</td>
<td>6.7</td>
<td>Tolerance</td>
<td>16.74</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Aphid Infested</td>
<td>0.27 ±0.026</td>
<td>0.185 ±0.07</td>
<td>0.01</td>
<td>31.0</td>
<td>Aphid infestation</td>
<td>81.33</td>
</tr>
</tbody>
</table>

**Intrinsic Water Use Efficiency (iWUE) (µmole CO\textsubscript{2} mol\textsuperscript{-1} H\textsubscript{2}O)**

<table>
<thead>
<tr>
<th>Nature of RILs</th>
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<th>p&lt;=0.05</th>
<th>% Change</th>
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<th>F</th>
<th>df</th>
<th>p&lt;=0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerant</td>
<td>Aphid Free</td>
<td>0.41 ±0.05</td>
<td>0.35 ±0.05</td>
<td>0.018</td>
<td>14.6</td>
<td>Tolerance</td>
<td>16.74</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Aphid Infested</td>
<td>0.34 ±0.05</td>
<td>0.16 ±0.07</td>
<td>0.05</td>
<td>52.9</td>
<td>Aphid infestation</td>
<td>81.33</td>
</tr>
</tbody>
</table>

**References**


Agricultural Sciences. 13(3), 737-740.


