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RESEARCH ARTICLE

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 F_7 generation of the cross, CO-1 (susceptible) X 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 H_2O_2 (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} gallic acid equivalent) were more intolerant RILs than susceptible ones. The tolerant RILs were physiologically more efficient with higher chlorophyll (8.45 ± 0.91 mgL^{-1}), net photosynthesis (28.9 ± 3.31 µmole CO_2 m^{-2} sec^{-1}), net assimilation rate (0.289 ± 0.033 µmole CO_2 cm^{-2} leaf area) and intrinsic water use efficiency (0.35 ± 0.05 µmole CO_2 $mole^{-1}$ H_2O) than the susceptible RILs.

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

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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 (Srinivas 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_6 generation in the previous year, 8 tolerant and 8 susceptible recombination inbred lines (RILs) were selected. The same set of 16 RILs of F7 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 14th 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., >1.0 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 (p^H 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_2O_2), an important reactive oxygen species (ROS) and its higher concentration in the cell, causes lipid peroxidation. The amount of H_2O_2 was estimated by Sinha (1972) method and expressed as nmol of H_2O_2 g^{-1} fresh weight.

Antioxidative Enzymes

SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich, 1971). Percent inhibition was calculated by the following formula of Asada *et al.* (1974). Percent inhibition = $[(V - v)/V] \times 100$, Where, V = rate of assay reaction in absence of SOD, v = 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_2O_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_2O, 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_2O_2)

Both MDA and H_2O_2 content indicate the extent of lipid peroxidation in the plants. The MDA content was significantly more in susceptible lines (2.60 $\pm$ 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_2O_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_2O_2 than tolerant lines (Table 2). Increased lipid peroxidation was observed in both tolerant and susceptible lines whenever plants underwent stress

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

RILs	*A.I.I.*	*Category*	*RILs*	*A.I.I.*	*Category*
RIL-6	1.1	Tolerant	RIL-81	5.0	Highly susceptible
RIL-14	1.1	Tolerant	RIL-114	5.0	Highly susceptible
RIL-18	1.1	Tolerant	RIL-152	5.0	Highly susceptible
RIL-34	1.2	Tolerant	RIL-201	5.0	Highly susceptible
RIL-218	1.3	Tolerant	RIL-210	5.0	Highly susceptible
RIL-222	1.2	Tolerant	RIL-250	4.5	Highly susceptible
RIL-235	1.5	Tolerant	RIL-322	5.0	Highly susceptible
RIL-351	1.2	Tolerant	RIL-358	5.0	Highly susceptible
EC-523368-2	1.3	Tolerant	CO-1	5.0	Highly susceptible

RIL- Recombinant Inbred Line, A.I.I- Aphid Infestation Index

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

Nature of RILs	*Mean + SEm*		*p<=0.05*	*% Change*	*ANOVA*			
	Aphid free	*Aphid infested*			*Source*	*F*	*df*	*p<=0.05*
Malondialdehyde (MDA) (nmols g^{-1} fresh weight)								
Tolerant	1.87 + 0.14	2.38 + 0.25	0.05	27.0	Tolerance	50.6	17,35	<0.0001
					Aphid infestation	2854	1,35	<0.0001
Susceptible	1.94 + 0.15	2.60 + 0.19	0.04	34.0	Tolerance x Aphid infestation	33.5	17,35	<0.0001
Hydrogen peroxide (H_2O_2) (nmols g^{-1} fresh weight)								
Tolerant	3725 + 640	4435 + 1035	0.0008	19.0	Tolerance	37.3	17,35	<0.0001
					Aphid infestation	119	1,35	<0.0001
Susceptible	4372 + 850	4908 + 1287	0.004	12.25	Tolerance x Aphid infestation	36.6	17,35	<0.0001

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

Nature of RILs	*Mean + SEm*		*p <=0.05*	*% Change*	*ANOVA*			
	Aphid free	*Aphid Infested*			*Source*	*F*	*df*	*p<=0.05*
Superoxide Dismutase (SOD) (nmols g^{-1} fresh weight)								
Tolerant	52.79 + 0.35	53.63 + 0.29	0.002	1.6	Tolerance	318	17,35	<0.0001
					Aphid infestation	792	1,35	<0.0001
Susceptible	49.58 + 0.31	43.29 + 0.64	0.004	12.7	Tolerance x Aphid infestation	126	17,35	<0.0001
Catalase (CAT) (µmols g^{-1} fresh weight)								
Tolerant	28.93+3.82	33.73+3.3	0.001	16.59	Tolerance	40.46	17,35	<0.0001
					Aphid infestation	130.19	1,35	<0.0001
Susceptible	18.72 + 2.73	23.66+1.96	0.00001	26.38	Tolerance x Aphid infestation	2.95	17,35	<0.003
Peroxidase (POX) (nmols min^{-1} g^{-1} fresh weight)								
Tolerant	92.77+9.5	163.36+38.0	0.0009	175.0	Tolerance	2664	17,35	<0.0001
					Aphid infestation	85127	1,35	<0.0001
Susceptible	107.8+21.6	328.25+66.46	<0.0001	204.5	Tolerance x Aphid infestation	2102	17,35	<0.0001
Ascorbate Peroxidase (APX) (nmols min^{-1} g^{-1} fresh weight)								
Tolerant	112.98+33.9	721.67+179.6	<0.0001	538.7	Tolerance	121	17,35	<0.0001
					Aphid infestation	21071	1,35	<0.0001
Susceptible	144.48+36.58	633.67+132	<0.0001	339.5	Tolerance x Aphid infestation	109	17,35	<0.0001

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^-), peroxy radical (ROO^-) 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_2O_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 lines after aphid infestation. This showed that catalase effectively scavenged H_2O_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 H_2O_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 H_2O_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 H_2O_2 from plant cells. Primary purpose of APX is to scavenge H_2O_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 sativ*a L. due to feeding by *Acyrthosiphon pisum* (Sylwia *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.

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

Nature of RILs	*Mean + SEm*		*p<=0.05*	*% Change*	*ANOVA*			
	Aphid free	*Aphid Infested*			*Source*	*F*	*df*	*p<=0.05*
Total phenols (µg g^{-1} Gallic Acid Equivalent)								
Tolerant	156.97+26.3	169.60+16.49	0.04	8.04	Tolerance	61.8	17,35	<0.0001
Susceptible	157.00+11.9	147.84+20.84	0.01	5.83	Aphid infestation	3.1	1,35	0.0891
					Tolerance x Aphid infestation	36.5	17,35	<0.0001
Total Glutathione (nmols g^{-1} fresh weight)								
Tolerant	5960+1256	10030+1896	<0.0001	69.0	Tolerant	7075	17,35	<0.0001
Susceptible	6825+1194	11497+1287	<0.0001	68.4	Aphid infestation	44491	1,35	<0.0001
					Tolerance x Aphid infestation	6445	17,35	<0.0001

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

Nature of RILs	*Mean + SEm*		*p<=0.05*	*% Change*	*ANOVA*			
	Aphid free	*Aphid Infested*			*Source*	*F*	*df*	*p<=0.05*
Total chlorophyll (mg L^{-1})								
Tolerant	9.92 + 0.6	8.45 + 0.91	0.03	11.0	Tolerance	20.2	17,35	<0.0001
Susceptible	9.88 + 0.78	6.65 + 1.34	0.01	33.0	Aphid infestation	563	1,35	<0.0001
					Tolerance x Aphid infestation	9.46	17,35	<0.0001
Net Photosynthesis (Pn) (µmole CO_2 m^{-2} sec^{-1})								
Tolerant	31.32 + 3.09	28.9 + 3.31	0.085	7.7	Tolerant	16.74	17,35	<0.0001
Susceptible	27.06 + 2.62	18.5 + 0.07	0.023	32.0	Aphid infestation	81.33	1,35	<0.0001
					Tolerance x Aphid infestation	3.74	17,35	0.0005
Net Assimilation Rate (NAR) (µmole CO_2 cm^{-2} leaf area)								
Tolerant	0.31 + 0.03	0.289 + 0.033	0.05	6.7	Tolerant	16.74	17,35	<0.0001
Susceptible	0.27 + 0.026	0.185 + 0.07	0.01	31.0	Aphid infestation	81.33	1,35	<0.0001
					Tolerance x Aphid infestation	3.74	17,35	0.0005
Intrinsic Water Use Efficiency (iWUE) (µmole CO_2 mol_{-1} H_2O)								
Tolerant	0.41 + 0.05	0.35 + 0.05	0.018	14.6	Tolerant	16.74	17,35	<0.0001
Susceptible	0.34 + 0.05	0.16 + 0.07	0.05	52.9	Aphid infestation	81.33	1,35	<0.0001
					Tolerance x Aphid infestation	3.74	17,35	0.0005

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_2 mol^{-1} H_2O) compared to susceptible lines (0.16 µmole CO_2 mol^{-1} H_2O) 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_2O_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.

References

Ainsworth, E.A., & Gillespie, K, M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using folin- ciocalteu reagent. Nat. Protoc, 2, 875-877.

Aldea, M., Hamilton, J.G., Resti, J.P., Zangerl, A.R., Berenbaum, M.R., & Delucia, E.H. (2005). Indirect effects of insect herbivory on leaf gas exchange in soybean. Plant Cell Environ. 28(3), 402-411.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373-399.

Arnon, D.I. (1949). Copper in isolated chloroplast polyphenol oxidase in Beta vulgaris L. Plant Physiol. 24, 1-5.

Asada K, Takahashi, M., & Nagate, M. (1974). Assay and inhibitors of spinach superoxide dismutase. Agric. Biol. Chem. 38, 471-473.

Balikai, R, A. (2000). Insect pests of safflower and their natural enemies in northern Karnataka. Karnataka Journal of

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

Beauchamp, I., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem. 44, 276-287.

Corpas, F., Gomez, J.M., Hernandez, J.A., & Luis, A.D. (1993). Metabolism of activated oxygen in leaf peroxisomes from two Pisum sativum (L.) cultivars with different sensitivity to sodium chloride. J. Plant Physiol. 141, 160-165.

Costa C, L.M., Dwyer, P. D., Stewart, D.W., Ma, L.B., & Smith, D.L. (2001). Interrelationships of applied nitrogen, SPAD, and yield of leafy and non-leafy maize genotypes. J. Plant Nutr. 24, 1173-1194.

Dhindsa, R.S. (1987). Glutathione status and protein synthesis during drought and subsequent rehydration in Tortula rurali. Plant Physiol. 83, 816-819.

Dipierro, S., & Leonardis, S.D. (1997). The ascorbate system and lipid peroxidation in stored potato (Solanum tuberosum L.) tubers. J. Exp. Bot. 48(308), 779-783.

Divya, T.C., Katageri, I.S., Jadhav, M.P., Sateesh. A., Vamadevaiah, H.M., & Nagaratna, S.O. (2017). Biochemical Constituents Imparting Resistance to Sucking Pest Aphid in Cotton (Gossypium spp.). Int. J. Curr. Microbiol. Appl. Sci. 6(12), 2749-2757.

DOR. (2012). Annual Report (2012-2013). ICAR- Directorate of Oilseeds Research. 43.

Dreyer, D.L., & Campbell, B.C. (1987). Chemical basis of host-plant resistance to aphids. Plant Cell Environ. 10, 353-361.

Giannopolitis, C.N., & Ries, S.K. (1977). Superoxide dismutases, I. Occurrence in higher plants. Plant Physiol. 59, 309-314.

Guljar, I.D., & Rajesh, S.P. (2016). Screening of Safflower Germplasm Accessions for Resistance against Safflower Aphid Uroleucon compositae (Theobald). Res. J. Agric. Sci. 7(1), 128-130.

Han, Y., Wang, Y., Bi, J.L., Yang, X.Q., Huang, Y., Zhao, X., Hu, Y., & Cai, Q.N. (2009). Constitutive and induced activities of defense-related enzymes in aphid-resistant and aphid-susceptible cultivars of wheat. J. Chem. Ecol. 35(2), 176-82.

Hanumantharaya, L., Naik, V.R, & Raju, S.G. (2007). Field Reaction of safflower genotypes to safflower aphid, Urolencon compositae (Theobald). Insect-Environment. 13(1), 39-41.

Heath, R.L., & Packer, L. (1968). Photo peroxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189-198.

Hubert, S., Pawel, C., Iwona, S., & Robert, K. (2013). Chlorophyll content of aphid infested seedling leaves of fifteen maize genotypes. Acta Biol. Cracov. Bot. 55(2), 51-60.

IIOR. (2015). Annual Report, AICRP (Safflower) (2014-2015). ICAR-Indian Institute of Oilseeds Research, Hyderabad. 139.

IIOR. (2018). Technical Compendium, AICRP (Safflower) (2018). ICAR-Indian Institute of Oilseeds Research, Hyderabad. 35-36.

Iwona, L. S., Goławska, & Wojcicka, A. (2012). Effect of Cereal Aphid Infestation on Ascorbate Content and Ascorbate Peroxidase Activity in Triticale. Pol. J. Environ. Stud. 21(6), 1937-1941.

Jat, S.L., Jat, B.L., & Choudhary, R.K. (2007). Screening of different mustard varieties for resistance against mustard aphid, Lipaphis erysimi (Kalt.). J. Oilseeds Res. 24(1), 212-214.

John, D.M., John, C.R., William, T.S., & Campbell, R.L. (2007). Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on soybean. J. Econ. Entomol. 100(5), 1657-1662.

Krieger-Liszkay, A. (2005). Singlet oxygen production in photosynthesis. J. Exp. Bot. 56: 337-346.

Kumar, G.T., Singh, G.R., Gupta, A.K., & Sandhu, J.S. (2012). Fluctuations in peroxidase and catalase activities of resistant and susceptible black gram (Vigna mungo (L.) Hepper) genotypes elicited by Bemisia tabaci (Gennadius) feeding. Plant Signal. Behav. 7(10), 1321-1329.

Kyle, G.K., Chapman, K., Louis, J., Heng-Moss, T., & Sarath, G. (2016). Plant Tolerance- A Unique Approach to Control Hemipteran Pests. Front. Plant Sci. 7, 1363.

Lawson, T., Craigon, J., Tulloch, A.M., Black, C.R. Colls, J.J., & Landon, G. (2001). Photosynthetic responses to elevated CO2 and ozone in field-grown potato (Solanum tuberosum). J. Plant Physiol. 158, 309-323.

Lu, F., Liang, X., Lu, H., Li, Q., Chen, Q., Zhang, P., Li, K., Liu, G., Yan, W., Song, J., Duan, C., & Zhang, L. (2017). Overproduction of superoxide dismutase and catalase confer cassava resistance to Tetranychus cinnabarinus. Sci. Rep. 7(1), 1-13.

Mondal, K., Sharma, N.S., Malhotra, S.P., Dhawan, K., & Singh, R. (2006). Oxidative stress and antioxidative systems in tomato fruits stored under normal and hypoxic conditions. Physiol. Mol. Biol. Plants. 12(2), 145-150.

Mukta, N., Praduman Yadav, Prasad, R.D., Srinivas, P.S., Madhuri, P., Kadirvel, P., Padmavathi, P., Murthy, I.Y.L.N., Alivelu,K., Meena, H.P., & Reddy, A.V. (2017). Technical Bulletin- Promising Trait Specific Safflower Germplasm. ICAR- Indian Institute of Oilseeds Research, Hyderabad. 11.

Nakano, Y., & Asada, K., (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.

Ni, X., & Quisenberry, S.S., (2003). Possible roles of esterase, glutathione S- transferase and superoxide dismutase activities in understanding aphid-cereal interactions. Entomol. Exp. Appl. 108(3), 187- 195.

Ni X, SS Quisenberry, T Heng-Moss, J Markwell, L Higley, B Frederick, G Sarath and R Klucas. (2002). Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. Entomol. Exp. Appl. 105(1), 43-53.

Noctor, G., & Foyer, C. (1998). Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 249-279.

Petitt, F.L., & Smilowitz, Z. (1982). Green peach aphid feeding damage to potato in various plant growth stages. J. Econ. Entomol. 75(3), 431-435.

Rabinowitch, H.D., & Fridovich, I. (1983). Superoxide radicals, superoxide dismutase and oxygen toxicity in plants. Photochem. Photobiol. 37, 674-790.

Rajput, S., Rustamani, M.A., Haider, S.A., Khanzada, M.S., Khanzada, S.R., Anwar, S., Jan, K.S., & Hayat, B. (2013). Relative resistance of safflower cultivars against black aphid, Uroleucon compositae (Theobald). Sarhad J. Agric. 29(1), 59-61.

Ram, D., Yadava, T.P., Singh, H., Rohilla, H.R., & Gupta, S.K. (1995). Effect of biochemical and anatomical traits of Indian mustard on mustard aphid, Lipaphis erysimi (Kalt.) infestation. Ann. Agric. Res. 16, 509-10.

Rao, M.V., Paliyath, G., & Ormrod, D.P. (1996). Ultraviolet-B and ozone-induced biochemical changes in antioxidant enzymes of Arabidopsis thaliana. Plant Physiol. 110, 125-136.

Ratnakumar, P., Rajendrudu, G., & Swamy, P.M. (2013). Photosynthesis and growth responses of peanut (Arachis hypogaea L.) to salinity at elevated CO2. Plant Soil Environ. 59(9), 410-416.

Sharma, D., & Rao, D.V. (2013). Biochemical analysis of cabbage (Brassica oleracea) after infestation of pest. Int. Res. J. Pharm. 4(6), 127-30.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., & Yoshimura, K. (2002). Regulation and function of

ascorbate peroxidase isoenzymes. J. Exp. Bot. 53, 1305-1319.
Singh, V. (2008). Screening of safflower germplasm accessions for resistance against safflower aphid Uroleucon compositae (Theobald). 7th International Safflower Conference, Australian Oilseeds Federation, Wagga Wagga, Australia.
Sinha, A.K. (1972). Colorimetric assay of catalase. Anal. Biochem. 47, 389-394.
Smith, I.K. (1985). Stimulation of glutathione synthesis in photo respiring plants by catalase inhibitors. Plant Physiol. 79, 1044-1047.
Srinivas, P.S., & N Mukta. (2015). Identification of resistant source of safflower against aphid, Uroleucon compositae (Theobald). National seminar on "strategic interventions to enhance oilseeds production in India", ICAR-Directorate of Rapeseed Mustard Research, Bharatpur, Rajasthan.
Sylwia, G., Robert, K., & Iwona, L. (2010). Relationship between aphid infestation and chlorophyll content in fabaceae species. Acta Biol. Cracov. Bot. 55(2), 76-80.
Van Camp, W., Capiau, K., Van Montaju, M., Inze, D., & Slooten, L. (1996). Enhancement of oxidative stress tolerance in transgenic tobacco plants over producing Fe-superoxide dismutase in chloroplasts. Plant Physiol. 12, 1703-1714.
Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., & Langebartels, C. (1997). Catalase is a sink for H2O2 and is indispensible for stress defense in C3 plants. EMBO J. 16, 4806-4816.
Wise, R.R. (1995). Chilling-enhanced photo oxidation: The production, action and study of reactive oxygen species produced during chilling in the light. Photosyn. Res. 45, 79-97.
Zimmermann, P., Heinlein, C., Orendi, G., & Zentgraf, U. (2006). Senescence specific regulation of catalase in Arabidopsis thaliana (L.) heynh. Plant Cell Environ. 29, 1049- 1060.