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Varietal reaction and biochemical basis of resistance in safflower against aphid, *Uroleucon compositae*

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Abstract
Among different varieties screened against aphids, the variety A-1 recorded the foliage drying grade 3 indicating its moderately tolerant reaction to aphid infestation which was on par with the varieties A-300 (foliage drying grade 3) and A-2 (foliage drying grade 3). The varieties PBNS-12 and Nari-6 were susceptible to aphid infestation and thus recorded the foliage drying grade of 4. Under protected condition, reducing sugars were significantly and negatively correlated with aphid population. Amino acids, total phenols and total sugars showed no significant correlation with aphid population. Total phenols correlated significantly and negatively with foliage drying grade. Under unprotected condition, amino acids correlated significantly and positively with aphid population. Reducing sugars, total sugars and total phenols correlated negatively and significantly with aphid population. Amino acids correlated significantly and positively with foliage drying grade. Reducing sugars, total sugars and total phenols correlated non-significantly with foliage drying grade. The genotypes having high concentration of amino acids *viz.*, NARI-6, PBNS-12 recorded more infestation of aphid population. The genotypes having less concentration of reducing sugars, total phenols and total sugars *viz.*, A-1, A-300 and A-1 recorded less aphid population and showed resistance / tolerance to aphids population.

Keywords: Biochemical, **c**orrelation, foliage drying grade, resistance and safflower aphid

Introduction
Safflower (*Carthamus tinctorius* L.) is an ancient crop of the family Compositae or Asteraceae, originated in the near east and has been grown for centuries in China, India and North Africa. It is a multi-purpose species with many traditional uses. In India, safflower cultivation is being done for centuries for its orange red and yellow dye (Carthamine) extracted from the florets were once used to colour food and clothing and for its oil, rich in poly unsaturated fatty acids which are considered to reduce blood cholesterol and good for heart patients. There are several causes for low productivity in Karnataka, among them biotic factors play key role.

Among the insect pests that attack safflower the aphid, *Uroleucon compositae* (Theobald) is considered as a major pest causing severe losses to the crop throughout the world. Safflower aphid, *U. compositae* is one of the most destructive pests (Akashe *et al*, 1999) [1], which alone causes 35-72 percent yield loss, during heavy infestation period (Anon., 2007) [2]. Balikai (1999) [5] recorded 46.2 percent yield loss of safflower in Karnataka. Seed and oil content losses due to this pest to the extent of 20 to 80 percent have been reported from different parts of country (Singh *et al.*, 2000) [15]. The aphids not only reduce yields of seed and oil content but also attack petals lowering the quality of the value added product of this part of the plant (Kalpana Sastry, 1997) [9]. Control of safflower aphid has been achieved by using different insecticides. This unilateral approach has provided an effective but short term remedy. The major limitations of this method are high cost of cash inputs and insecticidal hazards for plant protection. The increasing awareness on deleterious effects of using chemical insecticides and the demand for insecticide free food has prompted to give emphasize on alternative management options. Identification of resistant lines and biochemical basis of resistance are important for the development of host plant resistance. The use of resistant varieties / genotypes is a way to lower the cost of crop protection as part of integrated pest management in safflower. Thus, the present study was targeted to evaluate safflower varieties for resistance against safflower aphids and to know biochemical basis of resistance to aphids in these varieties / genotypes.

2. Materials and Methods

The varieties used for biochemical analysis included A-1, A-2, A-300, Nari-6 and PBNS-12 which were grown separately under protected and unprotected conditions. The leaf samples for biochemical analysis were collected from the experimental field at 65 days after sowing. Biochemical constituents such as total sugars, reducing sugars, total phenols and amino acids in five safflower varieties under protected and unprotected conditions were estimated from the plants.

Plant samples were washed with distilled water and air dried and then cut into small bits. Small plant bits were refluxed for 30 minutes in 25 ml of 80 percent alcohol on hot water bath. Supernatant extract was decanted into another flask and the residue was again re-extracted with small quantity of hot ethanol. Both the extracts were pooled and filtered through Whatman number 1 filter paper (Mahadevan, 1965 [10]; Jayapal and Mahadevan, 1968 [8]). The final volume was maintained at 25 ml. This extract was directly used to estimate total phenol, amino acids, total sugars and reducing sugars in the alcohol extract. The alcohol part of the extract was evaporated and the aqueous fraction was analyzed.

Total phenols were estimated from the various extracts using Folin-ciocalteau reagent (Bray and Thorpe, 1954) [7]. Reducing sugars and total sugars in the extracts were estimated by following the procedure of Nelson (1944) [14]. Amino acids were determined by employing the ninhydrin method of Moore and Stein (1958) [12].

Total numbers of aphids (2 tender twigs of 5 cm/plant) present in five plants per genotype were recorded at 75 days after sowing and foliage drying grade was recorded as mentioned in Table 1.

Table 1: Foliage drying grade due to aphid and resistance categories

Foliage drying grade	% Foliage drying	Visual symptoms	Categories
1	0 to 20	Healthy plant with normal seed yield.	Highly tolerant (HT)
2	20 to 40	Healthy plant but yellowing and drying of leaves.	Tolerant (T)
3	40 to 60	Drying of 50% leaves on the tender shoots of the plant, small to medium capitula with low seed setting.	Moderately tolerant (MT)
4	60 to 80	Drying of leaves and tender shoots withering of branches stunted growth, less number of capitula, poor seed setting.	Susceptible (S)
5	80 to100	Death of plant before maturity and no seed yield.	Highly Susceptible (HS)

The correlations were worked out between biochemical constituents and safflower aphid counts and foliage drying grade.

3. Results and Discussion

Under protected condition

Total numbers of aphids (2 tender twigs of 5 cm/plant) present in five plants per genotype recorded at 75 days after sowing and foliage drying grade along with biochemical constituents such as total sugars, reducing sugars, total phenols and total amino acids are presented in Table 2.

Table 2: Biochemical constituents present in different varieties under protected condition and unprotected conditions

S. No.	Varieties	No. of aphids on 5 cm apical twig per plant at 75 DAS	Foliage drying grade	Amino acids (mg/g)	Reducing sugars (mg/g)	Total sugars (mg/g)	Total phenols (mg/g)
Biochemical constituents in different varieties under protected condition							
1	NARI-6	55.7	4	2.833	7.10	7.602	3.281
2	A-2	37.8	3	2.725	7.18	8.579	3.640
3	A300	37.4	3	2.563	7.20	9.368	3.760
4	PBNS12	39.4	4	2.785	7.16	8.246	3.342
5	A-1	35.9	3	2.421	7.24	9.862	3.834
Biochemical constituents in different varieties under unprotected condition							
1	NARI-6	104.5	4	3.012	6.92	7.531	3.092
2	A-2	90.0	3	2.831	7.02	8.192	3.257
3	A300	87.4	3	2.734	7.09	9.146	3.328
4	PBNS12	94.8	4	2.981	7.04	8.072	3.126
5	A-1	84.9	3	2.653	7.12	9.284	3.476

DAS- Days after sowing

The total amino acids content varied from 2.421 to 2.833 mg in different varieties. The highest total amino acids were recorded in the variety Nari-6 (2.833 mg) which was followed by PBNS-12 (2.785 mg), A-2 (2.725 mg) and A-300 (2.563 mg) whereas; lowest total amino acids were recorded in the variety A-1 (2.421 mg).

Among different varieties reducing sugars varied from 7.10 to 7.24 mg. The highest reducing sugars were recorded in the variety A-1 (7.24 mg) which was followed by A-300 (7.20 mg), A-2 (7.18 mg) and PBNS-12 (7.16 mg) whereas, lowest reducing sugars were recorded in the variety Nari-6 (7.10 mg).

Among different varieties total sugars varied from 7.602 to 9.862 mg. The highest total sugars were recorded in the variety A-1 (9.862 mg) which was followed by A-300 (9.368 mg), A-2 (8.579 mg) and PBNS-12 (8.246 mg) whereas, least total sugars were recorded in the variety Nari-6 (7.602 mg).

Among different varieties total phenols varied from 3.281 to 3.834 mg. The highest total phenols (3.834 mg) were recorded in the variety A-1 which was followed by A-300 (3.760 mg), A-2 (3.640 mg) and PBNS-12 (3.342 mg) whereas, least total phenols were recorded in the variety Nari-6 (3.284 mg).

Among different varieties screened against aphids, the variety A-1 recorded the foliage drying grade 3 indicating that it is moderately tolerant to aphid infestation which was on par with the varieties A-300 (foliage drying grade 3) and A-2 (foliage drying grade 3). The varieties PBNS-12 and Nari-6 were susceptible to aphid infestation and thus recorded the foliage drying grade of 4.

Aphid population and foliage drying grade in five safflower varieties were correlated with biochemical constituents and the results are presented in Table 3.

Table 3: Correlation of biochemical constituents with aphid population and foliage drying grade under protected and unprotected conditions

Under protected condition		
Biochemical constituents	**Correlation with aphid population**	**Correlation with foliage drying grade**
Amino acids	0.66	0.77
Reducing sugars	-0.90*	-0.81
Total sugars	-0.71	-0.83
Total phenols	-0.76	-0.96*
Under unprotected condition		
Amino acids	0.91*	0.91*
Reducing sugars	-0.95*	-0.69
Total sugars	-0.92*	-0.79
Total phenols	-0.89*	-0.86

* Significant at 0.05% level

Total amino acid concentration correlated positively with aphid population (0.66). Total reducing sugars (-0.90) were negatively correlated with aphid population which was significant. Total sugars and total phenols correlated negatively with aphid population (-0.71 and -0.76, respectively).

Total amino acid concentration correlated positively with foliage drying grade (0.77). Other biochemical constituents such as total reducing sugars (-0.81), and total sugars (-0.83) correlated negatively with foliage drying grade. Total phenols (-0.96) correlated negatively and significantly with foliage drying grade.

Under unprotected condition

Among different varieties, total amino acid content varied from 2.653 to 3.012 mg in different varieties. The highest total amino acids recorded in the variety Nari-6 (3.012 mg) which was followed by PBNS-12 (2.981 mg), A-2 (2.734 mg) and A-300 (2.563 mg) whereas, lowest total amino acids were recorded in the variety A-1 (2.653 mg).

Total reducing sugars varied from 6.92 to 7.12 mg. The highest total reducing sugars were recorded in the variety A-1 (7.12 mg) which was followed by A-300 (7.09 mg), PBNS-12 (7.04 mg) and A-2 (7.02 mg) whereas, lowest total reducing sugars were noticed in the variety Nari-6 (6.92 mg).

Among different varieties, total sugars varied from 7.531 to 9.284 mg. The highest total sugars were recorded in the variety A-1 (9.284 mg) which was followed by A-300 (9.146 mg), A-2 (8.192 mg) and PBNS-12 (8.072mg) whereas, least total sugars were recorded in the variety Nari-6 (8.531 mg).

Among different varieties, total phenols varied from 3.092 to 3.476 mg. The highest total phenols (3.476 mg) were recorded in the variety A-1 which was followed by A-300 (3.328 mg), A-2 (3.257 mg) and PBNS-12 (3.126 mg) whereas, least total phenols were recorded in the variety Nari-6 (3.092 mg).

Among different varieties screened against aphids, the variety A-1 recorded the foliage drying grade 3 indicating its moderately tolerant reaction to aphid population which was on par with the varieties A-300 (foliage drying grade 3) and A-2 (foliage drying grade 3). The varieties PBNS-12 and Nari-6 were susceptible to aphids and thus recorded foliage drying grade of 4.

The total amino acids correlated positively and significantly with aphid population (0.91). Total reducing sugars (-0.95), total sugars (-0.92) and total phenols (-0.89) correlated negatively and significantly with aphid population.

Biochemical constituents such as total sugars, reducing sugars, total phenols and total amino acids in five safflower varieties were correlated with foliage drying grade. Total amino acids correlated positively and significantly with foliage drying grade (0.91). Other biochemical constituents such as total reducing sugars (-0.68), total sugars (-0.78) and total phenols (-0.86) correlated negatively with foliage drying grade.

The amino acids concentration was positively correlated with aphid infestation and foliage drying grade under protected and unprotected conditions. Total reducing sugars, total sugars, total phenols were negatively correlated with aphids population and foliage drying grade under protected and unprotected condition.

Auclair and Maltais (1950) [4], Auclair *et al.* (1957) [3] who worked on pea varieties susceptible to aphid attack, reported that presence of higher amount of amino acids and lower amount of sugars may be responsible for susceptibility of the plants against the aphids. In the present study, variation in biochemical constituents noticed in the five safflower varieties (four spiny and one non-spiny) under protected and unprotected condition with regard to total amino acids, total sugars, reducing sugars and total phenols clearly indicated their role in the resistance to the aphids. These biochemical constituents were correlated with aphid population and foliage drying grade. The tolerant varieties *viz*., A-1, A-2 and A-300 contained higher amount of reducing sugars, total sugars and total phenols with less amino acids as compared to the susceptible variety (spiny) PBNS-12 and Nari -6, which are comparable with results of Basavanagouda *et al*. (1980) [6] on safflower crop. Also similar results were reported by Auclair and Maltais (1950) [4], Auclair *et al*. (1957) [3] and Maltais and Auclair (1957) [11], who worked on similar aspects on other crops.

The genotypes having high concentration of amino acids *viz.*, NARI-6, PBNS-12 recorded more infestation of aphid population. The genotypes having less concentration of reducing sugars, total phenols and total sugars *viz.*, A-1, A-300 and A-1 recorded less aphid population and showed resistance / tolerance to aphids population. The present findings are in line with Narang *et al.* (1997) [13] who reported that biochemical constituents such as total free amino acids, total sugars and soluble proteins were found to be responsible for susceptibility of the plants, as plants having high amount of these supported more number of aphids per plant.

4. Conclusion

Under unprotected condition, amino acids correlated significantly and positively with aphid population. Reducing sugars, total sugars and total phenols correlated negatively and significantly with aphid population. Amino acids correlated significantly and positively with foliage drying grade. Reducing sugars, total sugars and total phenols correlated non-significantly with foliage drying grade. The genotypes having high concentration of amino acids *viz.*, NARI-6, PBNS-12 recorded more infestation of aphid

population. The genotypes having less concentration of reducing sugars, total phenols and total sugars *viz.*, A-1, A-300 and A-1 recorded less aphid population and showed resistance / tolerance to aphids population.

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