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Biochemical studies of host resistance against chickpea rust

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Abstract
The experiment was conducted at Department of plant pathology, University of agricultural sciences, Raichur during 2018-19. Biochemical basis of host resistance was studied by analysis of total sugars and total phenols. Estimation of total sugars, reducing sugars, non reducing sugars and total phenols were done in samples from both healthy and rust infected leaf tissues of Annigeri-1, Bheema, JG11, Jaki, ICCL-86111 and ICC-3137 varieties of chickpea. The results reveled that, the activity of reducing and non reducing sugar content was decreased in all the genotypes when chickpea leaves were infected with *Uromyces ciceris-arietini.* Significant increase in the activity of phenol content was recorded when chickpea leaves were infected with *U. ciceris-arietini.*

Keywords: Chickpea, rust, reducing sugar, non reducing sugar, total sugar and total phenols

Introduction
Chickpea (*Cicer arietinum* L.) is also known as Gram or Bengal gram. It is the third most important pulse crop after bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) on a world basis. Chickpea seed contains on average 17-23% protein, 54-60% total carbohydrates (47% starch, 6% soluble sugar), 4-5% fat, crude fiber 6% and 3% ash. Chickpea is also very rich in mineral content like phosphorus (340 mg/100g), calcium (190 mg/100 g), magnesium (140 mg/100 g), iron (7 mg/100 g), zinc (3 mg/100 g) and 100 g serving of cooked chickpeas provides 164 kilocalories (690 kJ) (Huisman and Van, 1994) [4]. Chickpea suffers from about 172 pathogens consisting of fungi, bacteria, viruses and nematodes. Among the several diseases rust caused by *Uromyces ciceris-arietini* is causes epidemic in the recent years. In the present study, biochemical changes in healthy and infected leaves of chickpea were estimated on the basis of important biochemical compounds like sugars and total phenols. Healthy and rust infected leaves were used for understanding biochemical mechanism of host resistance.

Materials and Methods
Estimation of total sugars (both reducing and non reducing sugars) and total phenols were done using samples from both healthy and rust infected leaf tissues of Annigeri-1, Bheema, JG11, Jaki, ICCL -86111 and ICC-3137 varieties of chickpea.

Extraction of plant tissues in alcohol
Reagent: Ethanol (80%)
One gram of tissue was weighed and made into small pieces and plunged immediately in boiling alcohol. Then it was cooled and passed through double-layered muslin cloth. The pieces of the tissue were ground thoroughly in a mortar and pestle with 10 ml alcohol, later it was passed through muslin cloth. The above step was repeated once again. The filtrates were pooled and filtered through Whatman No. 41 filter paper and made up to 10 ml with alcohol. The extract was stored in a refrigerator at 4 ^{0}C. This alcoholic extract was used further for analysis of reducing sugar, non-reducing sugar, total sugar and phenols.

Clarification of alcoholic extracts
Dark colored alcoholic extracts of the tissues create a great problem in analytical procedure. Heavy metal salts were used for clarification of alcoholic extracts. For clarification saturated solution of neutral lead acetate and saturated solution of disodium hydrogen phosphate were used.

Procedure
Two ml of saturated lead acetate solution was added drop wise to 10 ml of the colored alcoholic extract with three ml of saturated solution of di-sodium hydrogen phosphate till the precipitation is completed. The above solutions were mixed thoroughly and kept for overnight and filtered through Whatman No. 1 filter paper and made up to 15 ml with 80 per cent alcohol and stored in refrigerator at 4 ^{0}C.

Estimation of reducing sugars
The total sugar content was estimated after acid hydrolysis of non-reducing to reducing sugars by following Nelson's modification of Somogyi's method (Nelson, 1944) [11].

Reagents
Solution A
Twenty-five gram of anhydrous sodium carbonate, 25 g of sodium potassium tartrate, 20 g of sodium bicarbonate and 200 g of sodium sulphate were dissolved in 800 ml of distilled water and volume was made up to one liter.

Solution B
Fifteen gram of copper sulphate was dissolved in distilled water to which one or two drops of concentrated sulphuric acid were added and made up to 100 ml volume with distilled water. Solutions A and B were mixed in 24:1 (v/v) proportion just before use.

Arsenomolybdate reagent
1. Twenty-five gram of ammonium molybdate was dissolved in 450 ml of distilled water. 21 ml of concentrated sulphuric acid was added and mixed with above solution.
2. Three gram of sodium orthoarsenate was dissolved in 25 ml distilled water. Both the solutions (1 and 2) were mixed with stirring and placed in an incubator at 37 ^{0}C for 24-48 hrs. The reagent was stored in brown colored bottle.

Procedure
One ml of each sample (alcohol extract) was pipetted out into a test tube. To each one ml of extract, one ml of mixture of solution A and B was added. The test tubes were heated on a hot water bath for 20 min. The tubes were then cooled under a running tap water. After cooling one ml of arsenomolybdate reagent was added. The color developed after 15 min and then the above solution was diluted to 20 ml. The absorbance of the solution was measured in spectrophotometer at 520 nm. The amount of reducing sugars was determined by using standard curve prepared with glucose.

Estimation of non-reducing sugars
Non- reducing sugar was first hydrolyzed with the help of 1N hydrochloric acid. The hydrolysate was neutralized and the reducing sugar was estimated by Nelson Somogyi's method (Nelson, 1944) [11].

Estimation of total phenols
Total phenol content was determined by following Folin - Ciocalteau reagent (FCR) method (Bary and Thrope, 1954). For estimation of total phenols Folin- Ciocalteau reagent (FCR 1%) and Sodium carbonate (2%) reagents were used.

Procedure
One ml of each alcoholic extract was taken in a test tube to which 1 ml of FCR reagent will be added followed by 2 ml of sodium carbonate solution (2%). The tubes were shaken well and heated in a hot water bath for exactly one minute and then cooled under running tap water. The color developed was diluted to 25 ml with distilled water and its absorbance was recorded at 650 nm in spectrophotometer. The amount of phenols present in sample was calculated.

Results and Discussion
The activity of total sugar content was decreased in all the genotypes when chickpea leaves were infected with *U. ciceris-arietini.* Maximum total sugar observed in uninfected leaves of ICCL-86111 (15.52 mg/g) and ICC-3137 (15.52 mg/g) varieties compared with the infected leaves 13.44 mg/g and 10.96 mg/g respectively. Uninfected leaves of Annigeri-1, JG-11, Bheema and Jaki contains 13.44, 14.64, 11.28 and 12.8 mg/g of total sugars respectively. In infected leaves total sugars reduced to 10.96, 12.64, 8.72 and 13.44 mg/g respectively (Table 1).

Table 1: Biochemical studies of host resistance against chickpea rust

Varieties	Reducing sugar (mg/g)		Non reducing sugar (mg/g)		Total sugar (mg/g)		Phenols (mg/g)	
	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
A-1	5.52	4.16	7.92	6.8	13.44	10.96	10.72	12.16
Bheema	5.84	5.20	8.80	7.44	14.64	12.64	11.04	12.32
JG 11	4.56	3.60	6.72	5.12	11.28	8.72	11.36	13.44
Jaki	4.96	4.32	7.84	6.24	12.80	10.56	10.48	11.28
ICCL -86111	7.12	5.76	8.40	7.68	15.52	13.44	12.30	14.58
ICC-3137	10.00	7.60	5.52	3.36	15.52	10.96	9.60	11.20
S.Em. ±	0.17	0.14	0.20	0.16	0.37	0.30	0.29	0.33
CD at 1%	0.75	0.60	0.87	0.71	1.59	1.27	1.25	1.43

The activity of reducing and non reducing sugar content was decreased in all the genotypes when chickpea leaves were infected with *U. ciceris-arietini.* There was significant maximum reducing and non reducing sugars were present in uninfected leaves compared to diseased leaves. Uninfected leaves of Annigeri-1, JG-11, Bheema, Jaki, ICCL-86111 and ICC-3137 genotypes contains 5.52, 5.84, 4.56, 4.96, 7.12 and 10 mg/g of reducing sugar and it was reduced to 4.16, 5.20, 3.60, 4.32, 5.76 and 7.60 mg/g respectively in the infected leaves. Uninfected leaves of Annigeri-1, JG-11, Bheema, Jaki, ICCL-86111 and ICC-3137 genotypes contains 7.92, 8.80, 6.72, 7.84, 8.40 and 5.52 mg/g of non reducing sugar and it was reduced to 6.80, 7.44, 5.12, 6.24, 7.68 and 3.36 mg/g respectively in the infected leaves (Table 1).
The above results are in agreement with the earlier workers Mathar and Vidyasekaran (1978) [7], Neenamitter *et al*. (1997) [9], Neeraj and Verma (2010) [10] and Jyoti *et al.* (2018) [6], they reported that healthy leaves contains more total, reducing and non reducing sugars compared to diseased leaves. According to Mc Combs and Winstead (1964) [8], reduction in

sugar content was due to rapid utilization of sugars by developing fungus but Padmanabhan *et al*., 1974 [13], suggested that this reduction may be due to the impairment of photosynthetic activity due to infection. The depletion of sugars during host-parasite interaction might be due to increased respiration or utilization of sugars by the fungi which depends on the capability of fungi to secrete carbohydrate degrading enzyme. Similarly, Nema (1989) [12] suggested that reduction in sugars during disease development might be due to utilization of sugars probably for energy and synthetic reactions involved in multiplication of the pathogen. The sugar content was found to be more in healthy leaves compared to diseased one.

Significant increase in the activity of phenol content was recorded when chickpea leaves were infected with *U. ciceris-arietini.* Uninfected leaves of Annigeri-1, JG-11, Bheema, Jaki, ICCL-86111 and ICC-3137 genotypes contains 10.72, 11.04, 11.36, 10.48, 12.30 and 9.60 mg/g of phenol respectively whereas, the infected leaves contains 12.16, 12.32, 13.44, 11.28, 14.58 and 11.20 mg/g of phenol respectively (Table 1). Among all the genotypes ICCL-86111 contains more (14.58 mg/g) phenol content and showed maximum resistance against pathogen infection. This clearly indicates that, there was increase in phenol content in diseased leaves and phenol provides the resistance against the pathogen infection.

The above results are in agreement with the earlier workers Neenamitter *et al*. (1997) [9], Ammajamma and Patil (2008) [1], Raithak and Gachande (2012) [14] and Jyoti *et al*. (2018) [6], they reported that healthy leaves contain less phenol than that of the diseased leaves. Phenolic compounds were found to be most important group of secondary plant products that played an important role in reducing the susceptibility of a plant to pathogen (Jyosthna *et al*., 2004) [5]. During pathogen infection, deposition of phenolics into the cell wall was an important defense mechanism, either because of a hypersensitive reaction (HR) of entire cells or due to local wall reinforcement (Conceicao *et al*., 2006) [3]. Phenolic compounds such as cinnamic and ferulic acids released into plant cell walls was common and early response to fungal attack, which resulted in cell strengthening and then enhanced resistance to pathogen penetration (Stadnik and Buchenauer, 2000) [15]. This showed that when plant cells were recruited into infection, it switches from normal primary metabolism to a multitude of secondary metabolism defense pathway and activation of novel defense enzymes and genes takes place (Tan *et al*., 2004) [16]. In the present study also, the phenol content increased after infection and it was high in resistant genotypes than in the susceptible genotypes.

Conclusion

There was decrease in the activity of reducing sugar, non reducing sugar and total sugar content in all the genotypes which were infected by *U. ciceris-arietini.* The studies revealed that, the diseased leaves had less sugar content than that of the healthy leaves. The rust pathogen is sugar loving and it utilizes sugar for its growth and development. The diseased leaves had more phenols than that of the healthy leaves. Due to the infection, the total phenol content increased in all the genotypes. This clearly indicates that there was increase in phenol content in diseased leaves and it provides the resistance against the pathogen infection.

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