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Studies on Biochemical Constituents of Seeds of Susceptible to Resistant Cultivars of Chickpea

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Abstract **– The present studies were undertaken for the estimation of Nitrogen, Phosphorus, Potassium, Sugar and Phenol content in seeds of six resistant to susceptible genotypes such as, JG 315 (Resistant), Vaibhav, JG 74 (Moderately resistant), ICCV 2 (Susceptible), JG 62 and L 550 (Highly susceptible). Nitrogen, Phosphorus, Sugars and Phenols content of seed had significant relation with the resistance of chickpea against wilt complex pathogens. Significantly higher percentage of Nitrogen, Phosphorus and Sugars was recorded in highly susceptible varieties (L 550 and JG 62) and it was significantly lower in resistant variety (JG 315). While, Total phenol content of seed was found maximum in resistant variety (JG 315) and minimum in highly susceptible variety (JG 62). This indicate that Nitro- - gen, Phosphorus and Sugars content of seed had direct relationship and Total phenol had inverse relationship with the resistance of host against wilt complex causing fungi.**

Keywords **– Biochemical Analysis, Phenols, Sugars, Wilt, Chickpea, Root Rot, Collar Rot.**

I. INTRODUCTION

On global basis chickpea (*Cicer arietinum* L.) is the third important pulse crop after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). Pulses, including chickpeas are increasingly being used in health-conscious diet to promote well-being and reduced the risk of illness. Nutritionally chickpea is low in sodium and fats (4.5%), cholesterol free and overall an excellent source of both soluble and insoluble fibre, complex carbohydrates (61.5%), vitamins (especially B vitamins) and minerals (especially potassium, phosphorus, calcium, magnesium, copper, iron and Zinc). Therefore, chickpeas are an excellent heart healthy food that may be beneficial to the prevention of coronary and cardiovascular disease and by reducing blood lipids also help some serious complications of diabetes (Anonymous, 2004). Chickpeas are an inexpensive and high quality source of protein (20-25%) i.e. two to three times more than cereals and also cheaper than meat. Hence they are referred as 'Poor man meat' in developing country like India.

Prolonged saprophytic survival ability of the pathogen in soil makes chemical control and crop rotation ineffective. Resistant cultivars is the most practicable, feasible, and economical approach for the management of chickpea wilt complex fungi, but only a few sources with low level of genetic resistance are available, so there is a need to identify the resistant sources in chickpea. Biochemical analysis is important to understand the resistant mechanism and to isolate the resistance sources.

II. MATERIALS AND METHODS

These studies included estimation of Nitrogen, Phosphorus, Potassium, Sugars and Phenols from the seeds of susceptible to resistant variety of chickpea namely JG 62 and L 550 (Highly susceptible), ICCV 2 (Susceptible), JG 74 and Vaibhav (Moderately resistant) and JG 315 (Resistant) for understanding the relationship with wilt complex disease. The seeds of each variety were grinded and obtained in powder form for the estimation of the above mentioned constituents.

Estimation of Nitrogen

Plant samples (grain) 0.25 g have been weighed in digestion tube. Salt mixture (K_2SO_4 and $CuSO_4.5H_2O$ in ratio of 10: 1) was added in the tube. Five ml of concentrated H_2SO_4 acid was added in the same tube. The material was digested at 350^0C (KEL-PLUS, KES-20L), till the material become colourless. Then the tube was fitted to Distyl EM where appropriate NaOH (40%) was added automatically. The end of the system was having boric acid in conical flask. The distillate was titrated against sulphamic acid ($NH_2.SO_3.H$), till the colour was changed from green to rose pink. Control served without sample used as a blank reading. Five replications were maintained for each variety.

Estimation of Phosphorus

Plant samples (grain) of 0.5 g were weighed in digestion tube. Then 10 ml of tri-acid (Concentrated HNO_3, H_2SO_4 and $HCLO_4$ in 9:4: 1) was added. The material was digested for 3 hours in KEL-PLUS, KES-20L, till the material become colourless and temperature was increased in increasing order *viz.*, 50, 100, 150, 200, 250^0C. The material was washed with distilled water and the volume was made up to 100 ml. An aliquot of 10 ml was taken. Eight ml of yellow reagent was added. Volume was made up to 50 ml. After 1/2 hour, reading was taken by spectrophotometer (UV-VIS 119). Each variety replicated five times and control served without sample used as blank.

Estimation of Potassium

Potassium content was determined by flame photometer as described by Chapman and Pratt (1961). Remaining aliquot from Phosphorus estimation was used for the Potassium estimation.

Estimation of Sugars

1. *Estimation of Total sugar*

Plant sample of (grain) 0.1 g was taken for the extraction with the 5 ml phosphate buffer 7.2 pH in 0.05 N with the help of mortar and pestle at room temperature under dark conditions. The extracted content was centrifuged at 3000 rpm for 15 minutes. Collect the supernatant and diluted to 50 ml of volumetric flask.

The reaction mixture was prepared as given below

Plant extract	0.2 ml
Distilled water	1.0 ml
Anthrone reagent	2.8 ml

The above 4 ml of the each plant reaction mixture was kept in hot water bath (80^0C) concerned with glass bulb for 10 minutes. Cooled the glass tubes and take reading on streptophotometer at 625 nm. Blank sample containing all the reaction mixture except plant extract was used for reference. Standard curve was prepared using standard glucose solution (20 mg/100 ml). Total sugar was expressed as g per 100 g of seed sample. Five reading taken for each sample.

2. *Estimation of Reducing and Non reducing sugar*

Reducing sugar of seed sample was estimated by Dinitrosalicylic acid method. Plant sample of (grain) 0.1 g was taken for extraction in 5 ml of hot 80 per cent ethanol twice. Collect the supernatant and evaporate it by keeping it on water bath at 80^0C. Add 10 ml of distilled water to dissolve sugars. The reaction mixture contained the following chemical composition.

Plant extract	0.5 ml
Distilled water	2.5 ml
Dinitrosalicylic acid reagent	3 ml

The above 6 ml of the each plant reaction mixture was heated in a boiling water concerned with glass tube for 5 minutes. When the content of tubes are still warm, add 1 ml of Rochelle salt solution (40%). Cooled and read the intensity of dark red colour on streptophotometer at 510 nm. Blank sample as given elsewhere was used as a reference. Standard curve was prepared by using standard glucose solution (10 mg/100 ml). Reducing sugar was expressed as g per 100 g of seed sample. Five sets were maintained for each sample. Non reducing sugar was analysed by using formula

Non reducing sugar = Total sugar – Reducing sugar.

Estimation of Phenols

1. *Extraction Procedure*

Plant sample of (grain) 0.1 g was taken for the extraction in 7 ml 80 per cent alcohol borate buffer (0.2 M, pH 7.6) with the help of mortar and pestle at room temperature under dark conditions. The entire extracted content was centrifuge at 3000 rpm for 15 minutes and repeated the centrifugation. The extracted sample was finally made to 7 ml with borate buffer and from this fresh aliquot sample was used for the estimation.

2. *Estimation of Total Phenol*

Total phenolic content was estimated by the method of Swain and Hills (1959). The reaction mixture was prepared as given below.

Plant extract	0.1 ml
Distilled water	0.8 ml
Folin reagent (1N)	0.1 ml
Sodium carbonate	2.0 ml

The above 3 ml of the each plant reaction mixture was incubated for 45 minutes in order to develop the blue colour and the absorbance of which was measured at 660 nm. Blank sample containing all the reaction mixture except plant extract was used for reference. Standard curve was prepared by using different concentrations of chlorogenic acid stock solution (1 x 10^{-3} g ml^{-1}) for estimation. Phenolic content was expressed as mg Total phenol per g of seed sample. Five readings were taken for each sample.

3. *Estimation of Monophenol*

Monophenol was determined by the method of Emerson (1943). The reaction mixture was prepared as given below:

Plant extract	0.1 ml
Distilled water	0.4 ml
Sodium hydroxide (0.5N)	0.4 ml
4-Aminopyrine (0.6%)	0.5 ml
Sodium bicarbonate (9.5M)	0.6 ml
Potassium ferricyanide (2.4%)	0.5 ml

The above reaction mixture (2.5 ml) was incubated for 45 minutes (till the pink colour developed) and per cent absorbance was measured at 520 nm using spectrophoto- -meter. Blank sample containing all the reaction mixture except plant extract was used for reference. Standard curve was prepared by using different concentrations of phenol stock solution (1 x 10^{-3} g ml^{-1}). The phenolic content of the test sample was expressed as mg Monophenol per g of seed sample. Five readings were taken for each test sample.

4. *Estimation of Diphenol (O-Dihydroxy benzene)*

The Diphenol content was assayed by the method given by Mahadevan (1975). The reaction mixture contained the following chemical composition.

Plant extract	0.1 ml
Distilled water	0.4 ml
Hydrochloric acid (0.5N)	1.0 ml
Arrow reagent	0.5 ml
Sodium hydroxide (1N)	1.0 ml

The above reaction mixture of 3 ml volume was incubated for 45 minutes (till the pink colour developed) and absorbance was measured at 525 nm. Blank sample as given elsewhere was used as a reference. Standard curve was prepared by using different concentrations of cate- -chcol (O-dihydroxy benzene) stock solution (1 x 10^{-3} g ml^{-1}). The phenolic content was expressed as mg Diphenol per g of seed sample. Five replications were maintained for each of the variety.

III. Results and Discussion

Biochemical Constituents of the Seed and their Influence

Biochemical constituents were reported to play an important role in resistance of the host against pathogen infection. Therefore, present studies were undertaken for

the estimation of Nitrogen, Phosphorus, Potassium, Sugar and Phenol content in seeds of six resistant to susceptible genotypes such as, JG 315 (Resistant), Vaibhav, JG 74 (Moderately resistant), ICCV 2 (Susceptible), JG 62 and L 550 (Highly susceptible).

Role of Nitrogen, Phosphorus and Potassium

It is evident from the data presented in Table 1 that, significant difference were recorded in Nitrogen, Phospho--rus and Potassium per cent in six evaluated varieties. But Potassium could not give any relation with the resistance of the host against pathogen. Resistant variety JG 315, Highly susceptible varieties JG 62 and L 550 contain 0.85, 0.87 and 0.84 per cent potassium, which was at par with each other.

Significantly higher percentage of Nitrogen and Phosphorus was recorded in highly susceptible varieties L 550 (1.73% N and 0.51% P) and JG 62 (1.65% N and 0.50% P), while significantly lower percentage was recorded in resistant variety JG 315 (1.29% N and 0.25% P). Susceptible variety ICCV 2 (1.52% N and 0.33 % P), moderately resistant varieties Vaibhav (1.57% and 0.35% P) and JG 74 (1.55% N and 0.38% P) were at par with each other, but recorded significantly higher percentage over resistant variety JG 315 and significantly lower percentage over highly susceptible varieties JG 62 and L 550. This clearly revealed that Nitrogen and Phosphorus content of seed had importance in resistance to the host against wilt complex pathogens. There are no literature available on N, P and K content of seed of chickpea in respect of resistant against test pathogens. Pandey (1984) showed that, Nitrogen, Phosphorus, Carbon and Sulfur deficient Asthana and Hawker's medium had reduced the dry weight of mycelium of *F. oxysporum* f.sp. *ciceri*, *R. bataticola* and *S. rolfsii*. Sugha *et al.*, (1994) reported that increase in P and K concentration did not influence the development of chickpea wilt but it was favoured by an increase in N and organic carbon. This reflects that N favours fungal development and also imparts susceptibili--ty in crop plants.

Role of Sugars

Total sugar, Reducing sugar and Non reducing sugar per cent in six different evaluated varieties were presented in Table 1. Significant different was observed in the Total, Reducing and Non reducing sugar content in seeds of resistant, moderately resistant and susceptible varieties. The resistant variety JG 315 showed lowest content of Total (4.70%), Reducing (1.11%) and Non reducing sugar (3.60%) followed by moderately resistant varieties JG 74 (5.30, 1.18 and 4.12%, respectively) and Vaibhav (6.20, 1.38 and 4.82%, respectively). The highly susceptible variety L 550 showed highest per cent of Total (7.74%), Reducing (2.11%) and Non reducing sugar (5.64%) followed by highly susceptible JG 62 (7.49, 1.85 and 5.64 %, respectively) and susceptible variety ICCV 2 (7.35, 1.74 and 5.62%, respectively). In general, seeds of all the resistant varieties had lower amount of Total, Reducing and Non reducing sugar as compared to susceptible varieties indicating that more sugar content of seed resulting in the susceptibility against the pathogens. Geda *et al.* (2003) found that chickpea cultivar ICCV 2 had more sugar than JG 74. The present findings were also in agreement with this report that a susceptible cultivar had more sugar than moderately resistant cultivar. Root exudate of susceptible cultivar Giza 5 had higher sugar and amino acid than Local 235 resistant cultivar against *F. oxysporum* f.sp. *ciceri* and *S. rolfsii* (El-Moneem *et al.*, 2003).

Role of Phenols

Data presented in Table 1 showed significant difference in Total phenol content by seeds of different 6 varieties. Resistant variety JG 315 contained significantly higher amount of Total phenol (3.70mg g^{-1}) over moderately resistant varieties, Vaibhav (3.58mg g^{-1}) and JG 74 (3.33mg g^{-1}), whereas Vaibhav and JG 74 had significant--ly higher amount of Total phenol than susceptible variety ICCV 2 (3.01mg g^{-1}). Lowest Total phenol was recorded in seed of highly susceptible variety JG 62 (2.94 mg g^{-1}). It is clearly indicated that, all the resistant varieties had more Total phenol as compared to susceptible varieties. Similar findings were also given by Geda *et al.* (2003) and Singh *et al.* (2003). Ellil *et al.* (1998) studied role of phenols in host resistance against *S. rolfsii*, *R. solani* and *F. solani* and concluded that highest reduction in disease index was associated with an increase in phenol content. Total phenolics and gallic acid in chickpea plant parts worked as a biochemical barrier against *S. rolfsii* infection (Sarma *et al.* 2002).

Quantities of Monophenol and Diphenol were very low as compared to Total phenols. The resistant variety JG 315 (0.131mg g^{-1}) has significantly higher amount Mono--phenol over highly susceptible variety JG 62 (0.108mg g^{-1}), while other varieties were at par with the resistant variety in Monophenol content. The highest amount of Diphenol was recorded in moderately resistant variety Vaibhav (0.251mg g^{-1}) followed by resistant variety JG 315 (0.245mg g^{-1}) which were at par with Diphenol content in highly susceptible variety JG 62 (0.215mg g^{-1}), but significantly higher than the Diphenol content of susceptible ICCV 2 (0.164 mg g^{-1}) and highly susceptible variety L 550 (0.185mg g^{-1}) (Table 1). This indicated that Monophenol and Diphenol were not always higher in resistant varieties than susceptible varieties. The results clearly revealed that Total phenol is playing major role in resistance to the host against the wilt complex causing pathogens as compared to Monophenol and Diphenol.

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Table 1. Biochemical constituents in the seeds of resistant to susceptible genotype of chickpea to wilt complex.

Varieties	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sugar (%)			Phenol (mg g^{-1})		
				Total	Reducing	Non-reducing	Total	Mono-	Di-
JG-315	1.29 (6.523)	0.25 (2.853)	0.85 (5.286)	4.70 (12.517)	1.11 (6.039)	3.60 (10.929)	3.70	0.131	0.245
Vaibhav	1.57 (7.185)	0.35 (3.389)	0.90 (5.441)	6.20 (14.411)	1.38 (6.742)	4.82 (12.675)	3.58	0.125	0.251
JG-74	1.55 (7.14)	0.38 (3.530)	0.83 (5.224)	5.30 (13.303)	1.18 (6.226)	4.12 (11.708)	3.33	0.127	0.227
ICCV-2	1.52 (7.07)	0.33 (3.271)	0.95 (5.591)	7.35 (15.719)	1.74 (7.572)	5.62 (13.705)	3.01	0.116	0.164
JG-62	1.65 (7.372)	0.50 (4.051)	0.87 (5.355)	7.49 (15.876)	1.85 (7.809)	5.64 (13.734)	2.94	0.108	0.215
L-550	1.73 (7.546)	0.51 (4.092)	0.84 (5.256)	7.74 (16.147)	2.11 (8.344)	5.64 (13.968)	3.14	0.119	0.185
SEm±	**0.0366**	**0.0515**	**0.0442**	**0.0789**	**0.0583**	**0.1275**	**0.0616**	**0.0056**	**0.0126**
CD (5%)	**0.11**	**0.15**	**0.13**	**0.23**	**0.17**	**0.37**	**0.179**	**0.016**	**0.036**

Figures in parenthesis are Arcsine transformed values; Average of five replication; % - g per 100 g of seeds.