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EFFECT OF EXOGENOUS TREATMENT WITH SOME PHENOLIC COMPOUNDS ON NITROGEN FIXATION, GROWTH AND YIELD IN *CICER ARIETINUM* L. (CHICKPEA)

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PHENOLIC compounds, which are generally the secondary plant products, have recently come to limelight as regulators of many physiological processes. However, reports of the effect of phenolic compounds on symbiotic nitrogen fixation are scanty. It was therefore, considered worthwhile, to initiate these studies on the effect of some phenolic substances on symbiotic nitrogen fixation in *Cicer arietinum* L. (Chickpea) cv. C-235 (seeds procured from the Haryana Agricultural University, Hisar). Two hundred earthenware pots (25 cm dia.) were filled with a mixture of thoroughly powdered garden loam, sand and farm yard manure in the proportion of 3:1:1 by volume. Ten seeds were sown in each pot. The seedlings were inoculated with appropriate rhizobial strains. After thinning, three plants were left in each pot. The pots were divided into five equal lots. The plants in each lot were treated as follows: (i) a monophenol, vanillic acid; (ii) monophenol, salicylic acid; (iii) a diphenol, caffeic acid; (iv) a polyphenol, chlorogenic acid, and (v) distilled water to serve as untreated control. Aqueous suspensions of each of the phenols at a concentration of 10^{-4} M were made afresh during treatment. The concentrations used, were selected on the basis of preliminary trials made earlier. The plants were treated on the basis of dispensing five drops each of the above solutions on each cotton wad placed at the apices of the plants. The applications with the various solutions were made thrice during a day. Two successive treatments were made in the same manner, the first at 40 days after sowing and the second, 10 days after the first set of treatments. The effects of phenolic compounds on some important enzymes namely IAA oxidase, peroxidase and polyphenol oxidase were studied. The IAA oxidase assay was carried out on nodule extracts using the colour reaction by modified salkowski reagent[1]. Peroxidase activity was measured[2] by using *o*-dianisidine in the presence of H_2O_2. The activity of polyphenol oxidase was estimated by using catechol in 80% alcohol as substrate[3]. The rate of nitrogen fixation was determined by the acetylene reduction bioassay[4]. The effect of various phenolic treatments on the number and dry weight of nodules was studied to express their effect on plant growth.

With the application of two monophenols, vanillic acid and salicylic acid, there was a well marked reduction in the number and dry weight of nodules (table 1), which became more pronounced with time. The application of diphenol, caffeic acid and polyphenol, chlorogenic acid resulted in an increase in the number and dry weight of nodules. Chlorogenic acid application was the most promotive showing 30% increase after 80 days and 100% after 110 days.

The rate of nitrogen fixation (table 2) showed considerable reduction with the application of two monophenols, while the other phenols, especially chlorogenic acid, resulted in an enhancement of this activity.

Phenolic compounds released from root exudates, leaf leachate and decaying organic matter have been

Table 1 *Effect of different phenolic compounds on nodule numbers/dry weight (g/plant) in chickpea*

Days after sowing	Treatments				
	DW	VAN	SAL	CAF	CHLOR
80	76/0.10	66/0.09	61/0.09	88/0.11	84/0.13
110	85/0.12	49/0.08	47/0.08	105/0.15	115/0.25

DW, Distilled water; VAN, Vanillic acid; SAL, Salicylic acid; CAF, Caffeic acid, and CHLOR, Chlorogenic acid.

Table 2 *Effect of different phenolic compounds on rate of nitrogen fixation (m.molesethylene/mg nodule dry wt./h) in the nodules of chickpea*

Days after sowing	Treatments				
	DW	VAN	SAL	CAF	CHLOR
95	66	54	41	116	114
125	23	15	18	34	38

DW, Distilled water; VAN, Vanillic acid; SAL, Salicylic acid; CAF, Caffeic acid, and CHLOR, Chlorogenic acid.

reported to cause inhibition of rhizobial growth, nodule production and nitrogen fixation[5-10]. Such studies, however, do not directly explain the inhibition of nitrogen fixation by phenolics. Blum and Rice[11] reported significant inhibition of nodulation with tannic acid and gallic acid application through rooting medium at concentrations of 10^{-10} M to 10^{-4} M. On treatment with 10^{-2} M tannic acid, the plants died. However, they observed that 10^{-2} M gallic acid application resulted in increased nodule number per plant. The nitrogen fixing efficiency of these nodules was not estimated by these workers. The promotion of nodulation and nitrogen fixation by chlorogenic acid has not been reported so far. A direct correlation between endogenous phenolic content of the nodules as a result of phenolic treatments and their effect on the rate of nitrogen fixation by polyphenol, must therefore, involve other factors. The numbers and the position of the hydroxyl groups in the molecules of the phenolic compounds are considered important factors in their mode of action.

The IAA oxidase activity in the nodules (table 3) increased on treatment with all the phenols including monophenols. In fact it was generally higher with diphenol and polyphenol treatments. Similarly, peroxidase activity (table 3) was also the highest in the nodules of plants treated with caffeic acid and chlorogenic acid. It would, therefore, appear that the auxin content of the nodules does not determine their function. Similar increase in peroxidase activity with chlorogenic acid does not explain the promotion of nodulation and nitrogen fixation by this phenol. It may perhaps act through efficient removal of H_2O_2 by peroxidase activities.

Total dry weight of the plant, seed weight per plant and hundred seed weight (table 4) during harvest, were reduced in comparison with control, by treatments with two monophenols and considerably increased with caffeic acid and chlorogenic acid treatments. The maximum promotion of growth occurred by chlorogenic acid treatment. Growth and the harvest data (table 4) parallelled the effects of these phenols, particularly chlorogenic acid, on nodulation and nitrogen fixation. The harvest index was also the highest by chlorogenic acid treatment. The increase in the weight of hundred seeds, notably with chlorogenic acid treatment is of great significance especially when it is accompanied by an increase in other yield characters. The seeds appeared bolder even to the naked eye. The promotion of growth and yield by chlorogenic acid appears to be so promising that large-scale trials if made, with chlorogenic acid treatments may perhaps turn out to be practically exploitable.

Table 3 *Effect of different phenolic compounds on IAA oxidase (μg IAA oxidised/mg fresh wt./h) peroxidase activities (units/g fresh wt./h) in the nodules of chickpea 110 days after sowing*

Enzyme activity	Treatments				
	DW	VAN	SAL	CAF	CHLOR
IAA oxidase	108 4	134 4	190.6	162.9	205.6
Peroxidases	16.0	21 0	19.0	20 0	26.0

DW, Distilled water; VAN, Vanillic acid, SAL, Salicylic acid; CAF, Caffeic acid, and CHLOR, Chlorogenic acid.

Table 4 *Effect of different phenolic compounds on seed weight plant, 100 seed weight and harvest index in chickpea*

Treatments	Total plant dry weight	Seed wt./ plant	100 seed weight	Harvest index
DW	3 62	1 41	10 89	38
VAN	2.81	1 07	9 64	38
SAL	2 87	1 02	9 46	35
CAF	4.66	2 02	12.30	43
CHLOR	5 71	2.92	13 10	51

DW, Distilled water, VAN, Vanillic acid, SAL, Salicylic acid; CAF, Caffeic acid, and CHLOR, Chlorogenic acid

10 August 1987; Revised 18 June 1988

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