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Effect of phenolic compounds on symbiotic nitrogen fixation in pigeonpea (*Cajanus cajan* (L.) Millsp.)

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Abstract

The effect of different phenolic compounds: *p*-hydroxybenzoic acid, resorcinol and chlorogenic acid (mono-, di- and polyphenol) was studied on nodulation and related metabolic processes in pigeonpea (*Cajanus cajan* L. cv. Al-15). Nitrogenease activity, leghaemoglobin and ascorbic acid content of the nodules increased with the application of phenols. Phenols increase the contents of amino acids, proteins and total soluble carbohydrates in the nodules as reserve food materials.

Introduction

Phenolic compounds came into lime light since late seventies as naturally occurring growth regulators playing a vital role as secondary metabolites in growth and developmental processes of plants (Kefeli and Kutáček 1977, Apte and Laloraya 1982, Srivastava *et al.* 1982, Kalita and Shah 1983, Mangat *et al.* 1988). These compounds, also, play an important role in the initiation and development of nodules (Dhir and Lalitha 1989). Present investigations were extended with an objective to get some information in respect of their effect on symbiotic nitrogen fixation as also the metabolic changes taking place during the process in pigeonpea.

Material and methods

The seeds of pigeonpea (*Cajanus cajan* L. cv. Al-15) were inoculated with a pure rhizobial culture (F-7) broth just before sowing. The growing conditions described elsewhere (Dhir and Lalitha 1989) were followed. One hundred pots with 3 plants of uniform size after thinning were divided into 4 lots. Three lots of plants were treated with 10^{-4} M solution of *p*-hydroxybenzoic acid, resorcinol and chlorogenic acid (mono-, di-and polyphenol, respectively) 20 d after sowing and repeatedly wetted during the course of the day. Fourth lot was kept as control. The treatments were repeated in the same manner 10 d after the first set of treatments.

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Two pots from each lot were sampled at appropriate intervals. The rate of nitrogen fixation in nodules was measured by using acetylene reduction assay (Hardy *et al.* 1973) on *Perkin Elmer GC*. The nodules were detached immediately after the sampling and their leghaemoglobin content was determined (Hartree 1955) which is based upon the conversion of hematin to pyridine hemochromogen. The data were expressed as hemin [$\mu g\ g^{-1}$(nodule fr. m.)]. The ascorbic acid content of nodules at various pigmentation stages was estimated by colorimetric method based on the reduction of dye 2,6-dichlorophenol indophenol (Bharti and Garg 1970). Alcoholic extract for esti-mation of total soluble carbohydrates (Yemm and Willis 1954) as well as free amino
acids (Lee and Takahashi 1966) was analysed using the spectrophotometric method. The absorbances of the different samples were read in U.V. double beam spectrophotometer (*Shimadzu*). The residue left after alcoholic extraction was hydrolyzed in 1N NaOH for overnight and centrifuged at 4000 rpm for 15 min. The protein was estimated from the supernatant by the method of Lowry *et al.* (1951). All the experiments were repeated at least three times and the data were statistically analyzed.

Results and discussion

Treatment of plants with phenolic compounds, resulted in a number of changes in the functioning of nodules. Nitrogenase activity increased with the application of all the phenolics used (Fig. 1A). The activity was maximum at 85 d *i.e.*, the flowering stage, after which it decreased at the nodule senescence stage. Of all the PGRs used, chlorogenic acid lead to the maximum enhancement. In this experiment, p-hydroxybenzoic acid also resulted in an enhancement of nitrogenase activity. Our observations indicate that these exists a relationship in the number and the position of the hydroxyl groups in phenolic acids an in their biological activity. Hess (1968) had, however, pointed out that two hydroxyl groups at ortho position are necessary for biological activity of phenolic compounds.

Data further reveal that leghaemoglobin content (Fig. 1B) also increases with the treatment of all these phenols and the drift was similar to nitrogenase activity at various stages. A rough parallelism between leghaemoglobin content of nodules and their nitrogen fixing efficiency has been drawn by Bisseling *et al.* (1978). The content was more at flowering stage after which it declined (Fig. 1B). Blum and Rice (1969) on the contrary, reported a reduction in leghaemoglobin content of red kidney bean with the application of gallic acid (10^{-6} -10^{-2} M). Ascorbic acid content of the nodules (Fig. 1C) showed a similar pattern at different stages of growth. It was higher at 85 d after which it started declining both in treated as well as in control plants. Swaraj and Garg (1977) observed that ascorbic acid content of nodules starts declining with the onset of nodule browning. The decline in ascorbic acid content may be due to a pertial oxidation of leghaemoglobin. Another interesting point that emerges from the current investigations was that the nodules remained pink and their greening was considerably delayed (as compared to control) with phenolic acid treatments, thus increasing the functional span of nodules by maintaining the

leghaemoglobin in its reduced functional form. Ascorbic acid has been suggested to protect nitrogenase against the denaturing effect of quinones formed as a result of oxidation of phenols (Koch *et al.* 1967).

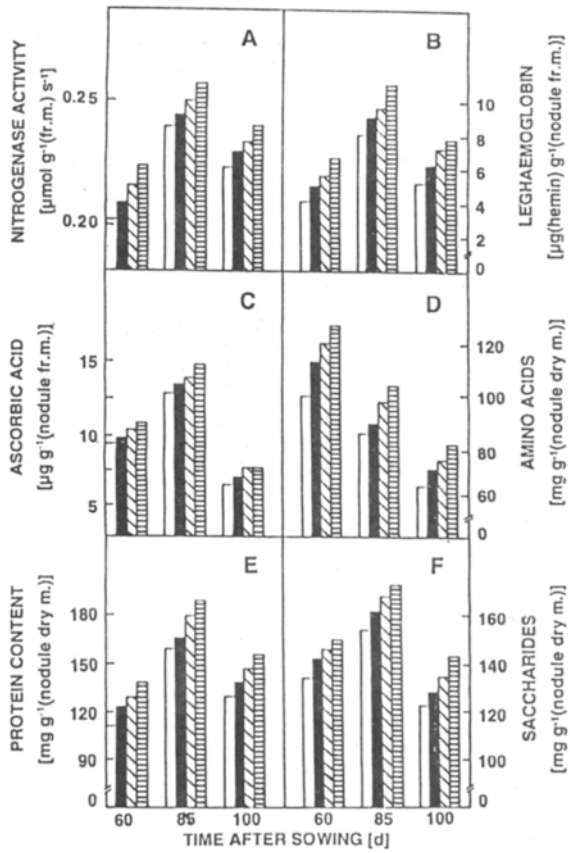

Fig. 1. The effect of different phenolic compounds on metabolic activities of nodules of pigeonpea (*Cajanus cajan* (L.) Millsp cv. AL-15): on nitrogenase activity in nodule (*A*) [µmol (C_2H_4 evolved) mg^{-1} (nodule fresh mass) s^{-1}], (CD at 5 % = 0.099), on leghaemoglobin content (*B*) [µg (hemin) g^{-1}(nodule fresh mass)], (CD at 5 % = 0.068), on ascorbic acid content (*C*) [µg g^{-1}(nodule fresh mass)], (CD at 5 % = 0.093), on amino acid content (*D*) [mg g^{-1}(nodule dry mass)], (CD at 5 % = 0.084), on protein content (*E*) [mg g^{-1}(nodule dry mass)], (CD at 5 % = 0.063), on soluble carbohydrate content (*F*) [mg g^{-1}(nodule dry mass)], (CD at 5 % = 0.077). *Open column:* control; *full column: p*-hydroxybenzoic acid; *hatched column:* resorcinol; *horizontally hatched column:* chlorogenic acids. The presented results were derived from three independent experiments and were evaluated by analysis of variance and by the *t*-test.

Saccharides, proteins and amino acids are actively involved in the initiation and development of nodules (Dhir and Rao 1987). Perusal of data (Fig. 1D) further reveals that amino-acid content was more at 60 d after which it started declining. But, protein and total soluble carbohydrate (Fig. 1E,F) content continued to increase till 85 d (flowering stage) which also declined at maturity, *i.e.*, 100 d (pod filling stage). Mangat *et al.* (1988) have reported that phenolic acids make available high levels of soluble saccharides by suppressing oxidative phosphorylation which is detoured towards biosynthesis of free amino-acids and proteins. However, in the present investigation the increased free amino acid content is not due to enhanced degradation of protein but is due to their increased synthesis and turnover. Phenolic treatments that enhance nodulation may concomitantly increase nitrogenase activity and this promotes the depletion of carbohydrate reserves. The effect of phenolic compounds seems to be primarily exerted on the development of increased uptake rate, rather than on the transport rate *per se*, thus involving changes in cell metabolic processes (Pospíšil *et al.* 1987). This would suggest that whereas amino acids were not allowed to accumulate, the soluble carbohydrates were being more efficiently utilized for nitrogen fixation in the nodules and translocation of fixed products to the aerial parts of plants. The effect of phenols may, thus be mediated hrough an increase in mobilization of reserve food materials as also reported by Datta and Nanda (1985). The reduction of plant metabolism by phenols might be through the combination of their oxidation products like quinones with either amino-acid or proteins resulting into changes in cell wall permeability (Pospíšil *et al.* 1987).

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