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EFFECT OF PHENOLS ON NITRATE REDUCTASE ACTIVITY AND PROTEINS IN DEVELOPING FRUITS OF *VIGNA MUNGO* (L). HEPPER

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Different phenolic compounds, salicylic acid (monophenol), resorcinol (diphenol) and tannic acid (polyphenol) of 10 ppm concentration were applied exogenously on *Vigna mungo* plants at seedling and flowering stages (15 and 65 days after sowing respectively), to determine their effect on the time course changing pattern of nitrate reductase activity and proteins, during fruit development. All the three phenolic compounds tested, significantly increased enzyme activity and protein content over the water sprayed control plants. Resorcinol treatment recorded highest nitrate reductase activity and protein content.

Keywords: Salicylic acid; Resorcinol; Tannic acid; Nitrate reductase; Proteins, *Vigna mungo*.

Introduction

Mashbean (*Vigna mungo* (L.) Hepper) is an important food legume and produces highly nutritious seed for human consumption. Compared with cereals, its yield is very low. The biochemical changes accompanying fruit and seed development are of particular interest since they may lead to identification of factors involved in the control of yield and composition of the harvested seed. In this context, nitrate reductase is a very important enzyme which plays a key role in the nitrogen metabolism of the plant. It is also present in fruit wall and plays an important role in seed development as the fruit wall photosynthates have been reported to contribute about 25% in seed development (Shantha Kumari *et al.*, 1975).

Phenols are secondary plant products which exert significant effects on growth processes when applied at physiological concentrations (Henderson and Nitsch, 1962), by acting as analogues of growth substances (Wain and Taylor, 1965). Their diverse chemical structure makes it unlikely that all phenolics act in the same way to regulate plant growth and development. Though their effect on biochemical changes

during fruit development has not been investigated, many other physiological processes are shown to be regulated by them, viz, flower induction (Cleland and Azami 1974), stomatal closure (Bhatia *et al.*, 1986), dormancy (Phillips, 1961), seed germination (Malik *et al.*, 1986), and yield (Chander *et al.* 1988). The present Investigation aimed to study the effect of different phenols on time course changes in nitrate reductase activity and soluble protein content in developing mashbean fruit.

Materials and Methods

Mash bean (*Vigna mungo* var. 1-1) crop was sown on July 10, 1985 in three replicates in the experimental area of the Department of Botany, Punjab Agricultural University, Ludhiana. The plants were sprayed twice at seedling stage (15 days after sowing) and flowering stage (65 days after sowing) with 10 ppm concentration of salicylic acid-monophenol, resorcinol-a diphenol and tannic acid-a polyphenol. Controls were sprayed with water. Fifty plants from each replicate were selected for sampling. Individual flowers were tagged at anthesis. Samples were collected at anthesis and then at four days intervals until 24 days after anthesis (DAA) when more than half part of fruit wall turned black. Each replicate was treated as separate sample. The analysis of fruit wall and seeds were done from zero and 8 days stage respectively. Five fruit walls and ten seeds from each replicate were selected randomly for analysis.

Nitrate reductase activity was measured in triplicate using the anaerobic *in vivo* method (Jaworski, 1971). The endogenous nitrate present at zero time of incubation was used to correct the assay for endogenous nitrite in the tissue. Enzyme activities were calculated from a standard curve for KNO_2 and expressed as μg nitrite produced per organ fresh weight per hour.

Total soluble proteins were estimated by the method of Lowry *et al.* (1951).

Result

Significant increase was observed (Table 1) in nitrate reductase activity by all the phenolic treatments tested, upto 12 Days after anthesis (DAA). Thereafter, the activity declined continuously upto 20 DAA followed by an increase at maturation stage. Maximum enzyme activity during 0-4 DAA was recorded with tannic acid treatment whereas this treatment lowered the enzyme activity during 20 DAA stage compared to control. Maximum enzyme activity was discernible in fruit wall with resorcinol treatment during 12 DAA stage.

Table 1. Effect of phenols on Nitrate reductase activity (μg/organ/hr) in fruit wall of developing fruits of *Vigna mungo* (L.) Hepper

Treatments	Days after anthesis						
	0	4	8	12	16	20	24
Salicyiic acid	0.04	0.43	1.42	3.27	2.40	1.43	2.74
Resorcinol	0.03	0.35	1.22	3.53	2.48	1.26	2.23
Tannic acid	0.05	1.04	2.51	3.40	2.21	0.99	2.20
Control	0.02	0 33	1.08	2.63	2.15	1.18	1.69

C.D. at 5%→Treatments=0.025, Stages=0.081, Treatments × stages=0.163

Table 2. Effect of phenols on Nitrate reductase activity (μg/organ/*hr*) in seeds of developing fruits of *Vigna mungo* (L.) Hepper

Treatments	Days after anthesis				
	8	12	16	20	24
Salicylic acid	0.04	0.6	0.37	0.30	0.82
Resorcinol	0.02	0.94	0.72	0.36	0.68
Tannic acid	0.03	0.53	0.60	0.35	0.82
Control	0.01	0.53	0.58	0.31	0.61

C.D. at 5%→Treatments=0.008, Stages=0.026, Treatment × Stages=0.052

Table 3. Effect of phenols on protein content (μg/organ) in fruit wall of developing fruits of *Vigna mungo* (L.) Hepper

Treatments	Days after anthesis						
	0	4	8	12	16	20	24
Salicylic acid	10	270	570	2040	995	770	620
Resorcinol	30	290	810	2610	1290	850	650
Tannic acid	10	240	720	2430	860	760	650
Control	10	210	480	1880	800	720	520

C.D. at 5%→Treatments=2.127. Stages=2.687, Tteatment × Stages=5.373

Table 4. Effect of phenols on protein content (μg/organ) in seed of developing fruits of *Vigna mungo* (L.) Hepper

Treatments	Days after anthesis				
	8	12	16	20	24
Salicylic acid	4	320	1290	1590	2120
Resorcinol	3	410	1770	2220	2370
Tannic acid	4	270	1470	1710	2080
Control	3	210	1090	1620	1960

C.D. at 5%→Treatments=3.717, Stages=3.555, Treatments × stages=7.111

During different seed developmental stages, the nitrate reductase activity continued to rise upto 12 DAA stage (salicylic acid and resorcinol treatment) and 16 DAA (tannic acid), followod by a decline in activity during 20 DAA with a cocanitant increase in activity during the final stage. Out of three phenolic compounds tested, maximum enzyme activity was shown by resorcinol treatment at 12 DAA stage (Table 2).

As is evident from the data (Table 3 & 4) all the phenolics tested significantly increased the total soluble protein content over control in developing fruits. In fruit wall, the protein content increased upto 12DAA stage and then decreased in subsequent stages whereas in developing seeds, the contents were found to increase with maturity. Amongst the phenols tested, resorcinol treatment recorded maximum protein contents both in fruit wall and seed.

Discussion

Nitrate reductase (NR) is the main nitrate assimilating enzyme and its activity is generally correlated with nitrogen status of the plant. Present studies point towards the rising level of NR activity both in fruit wall and in seeds upto 12 DAA. Thereafter, the enzyme activity declined with maturity except during the last stage which shows increase in activity mainly due to the sudden loss of tissue moisture Time course changes in NR activity in the developmental stages of *Vicia faba* fruit pericarp has been demonstrated by Schlesier and Muentz (1974). The high level of NR activity both in fruit wall and in seeds in phenolics treated plants is indicative of reduction of small amount of nitrate within the seed and pod besides its reduction in leaves. The present results also get support from the findings of Sharma *et al.* (1984), who observed that caffeic acid and salicylic acid regulates NR activity in the developing leaves of *Cicer arietinum*. Jain and Srivastava (1981) demonstrated increase in NR activity by salicylic acid application in maize seedlings and correlated increase in the pool of nitrogenous substrate in the treated plants compared with controls. Kefeli and Kutacek (1977) have hypothesized that the physiological effects of exogenously applied phenolics may be due to their action at enzyme level. The phenolics induced increase in the level of NR activity in developing fruits assumes significance as Singh *et al.* (1980) have demonstrated in pigeon-pea that approximately 25% of the nitrogenous reserve materials in mature seeds were translocated from the developing pod. Concomitantly, increase in the level of soluble proteins has also been recorded in developing *Vigna mungo* fruits with the foliar application of various phenols. The promotory role of

phenols can be explained on the basis of mobilization of reserve food from vegetative to reproductive part by qualitative and quantitative changes in hydrolysing and oxidizing enzymes (Kumar and Nanda, 1981). Kalita and Shah (1983) also observed significant increase in the amount of seed protein with salicylic acid in mungbean. Therefore, the increase in nitrate reductase activity and protein content in developing fruits by the exogenous application of different phenols can play an important role in determining the grain yield and seed quality of pulses.

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