J. Phytol. Res. 17(2) : 171-173, 2004

EFFECT OF CATECHOL ON NITROGEN METABOLISM AND FLOWERING OF *PHASEOLUS VULGARIS*

KANANBALA SARANGTHEM
Plant Physiology Research Laboratory, Department of Life Sciences, Manipur University, Imphal- 795003, India
* e-mail:ksarangthem@yahoomail.com

Leaves of *Phaseolus vulgaris* were sprayed topically with catechol(0.0, 0.1 ,0.25, and 0.5%, v/v) and its effect on the plant nitrate reductase activity , protein level and nitrogen contents were determined in fresh secondary leaves of 30 days after germination . The effect of catechol on the flowering of *Phaseolus vulgaris* were also studied. Under the optimum dose of treatment (0.25%,v/v) the level of protein, nitrate reductase activity and nitrogen contents increased.

Keywords : Catechol; Nitrogen metabolism; *Phaseolus vulgaris*.

Introduction

Phaseolus vulgaris is an important legume crop, as it produces nutritious seeds for human consumption. Legumes are cultivated not only to produce seeds of high protein content (FAO- USPHS, 1968) but also cultivated for fixing nitrogen from the atmosphere. The protein deficient nutrition of millions of people in the regions of hot climate has become one of today's most acute problems. Solving this problem largely depends on the further expansion of areas under cultivation and to use improved technology of cultivation for increased yield of legume. In this regard, use of plant growth regulators is well documented. Though phenolics, the secondary plant products are known to exert significant effects on plant growth process when applied topically at physiological concentrations by acting as analogues of growth hormones[1],they remain an almost untapped potential for increasing crop productivity [2]. Catechol, a diphenol which is acting as a plant hormones is a secondary plant products with two adjacent hydroxyl groups[3].In the present paper , the first enzyme of nitrogen assimilation pathway , nitrate reductase activity and the soluble protein level in the leaves and pods ,nitrogen content and flowering in *Phaseolus vulgaris* have been determined following foliar topical application of catechol.

Material and Methods

The locally purchased seeds of *Phaseolus vulgaris* were grown in earthenware pots containing sandy clay loam soil. The experiments were conducted during May- July. The seeds were first washed thoroughly with tap water and were soaked for eight hours in distilled water in a petridish. The soaked seeds were sown in the earthen pots. After germination (which took about a week), five seedlings were retained in each pots for further studies. The spraying treatment of an equal volume of catechol (0.0, 0.1, 0.25, 0.5%,v/v) were given separately to the foliage of all five plants in the pots in triplicate on 15 days after germination. The pots were supplied with 500 ml of tap water daily and these were kept under natural conditions of light and temperature in the garden. Nitrate reductase activity was determined *in vivo* in fresh leaves on day just before spray treatment and continued till 30 days. The leaves were harvested from 09: 00 to 09: 15 A.M. on each experiment day. Care was taken in the selection of leaves for enzyme assay. Few leaves (1-2) from the middle portion of the shoots were selected for these studies. The leaves were cut into narrow strips and mixed uniformly, weighed freshly (250mg sample for each assay) and used immediately for *in vivo* Nitrate reductase assay according to Srivastava *et al* [4].For soluble proteins content estimation, the leaves harvesting was done as mentioned above and protein were determined by the method of Lowry *et al*[5]. All these experiments were conducted in triplicate. Nitrogen estimation were done in the leaves of both control and spray treated plants on day 30 after germination. 50mg of the dried (60°C for 12 hr) leaves were made to powder form and the estimation of nitrogen (ethanol soluble) was done by Micro-kjeldahl method [6].All the calorimetric determination were carried out by a Bauch and Lombs spectronic- 20. Flowering pattern in the control and treated plants were recorded from 24th day after germination. The data on day 24, when flowering initiation was first noticed was considered as o-day value. The number of flowers opened on 10 and 30 days were determined.

Results and Discussion

The *in vivo* nitrate reductase activity showed increase activity on all days of estimation. Maximum nitrate reductase activity (95.3) was found with 0.25%, v/v of catechol treated plants as compared to control plants as in Table1. The level of phosphate buffer soluble protein in leaves and pods (seeds) of catechol treated plant increased

Table 1. *In vivo* nitrate reductase activity (NRA) in fresh leaves of control and catechol treated *Phàseolus vulgaris* with different concentration on 15 days old plants after germination. The NRA on 0 (zero) day was 20.2 ± 0.44 µmol (NO_2^{-1}) h^{-1} g^{-1} (leaf fr. wt.).

Conc. of catechol (%, v/v)	Nitrate reductase activity (NO_2^{-1})$h^{-1}g^{-1}$ (leaf fr. Wt.)
	Time (30 days after germination)
0 (control)	57.6 ± 0.28 *
0.1	87.2 ± 0.28
0.25	95.3 ± 0.41
0.5	58.3 ± 0.12

* standard error of the mean (n = 3)

Table 2. Phosphate buffer soluble protein in fresh secondary leaves of control and catechol treated *Phaseolus vulgaris* plants.The soluble proteins on 'O' (zero) day was 50.1±0.15 mg g^{-1} (leaf fr. wt.).

Conc. of catechol (%, v/v)	Phosphate buffer soluble proteins (mg/g seed fr. wt.)	(mg/g leaf fr.wt.)
	Time (30 days after germination)	
0(control)	128.9 ± 1.5*	52.8 ± 0.19
0.1	255.1 ± 2.1	134.2 ± 0.14
0.25	258.2 ± 3.5	138.7 ± 0.14
0.5	118.2 ± 3.0	101.1 ± 0.14

* standard error of the mean (n = 3)

Table 3. Level of ethanol soluble nitrogen in fresh secondary leaves of control and catechol treated *Phaseolus vulgaris* plants.

Conc. of catechol (%, v/v)	Nitrogen (mg/g leaf dry wt.)
	Time (30 days after germination)
0 (control)	4.55 ± 0.21*
0.1	12.4 ± 0.8
0.25	12.7 ± 0.4
0.5	8.1 ± 0.12

* standard error of the mean (n = 3)

Table 4. Flowering pattern in control and catechol treated plant.The data on day 24 when flowering initiation was first noticed was considered as 0 (zero) day values.

Conc. of catechol (%, v/v)	Number of flowers opened in $plant^{-1}$ (days after initiation)		
	0	10	35
0 (control)	0	3	8
0.1	1	5	18
0.25	1	5	22
0.5	0	1	10

* The values are mean of five replicates.

(ranging from 138.7 mg/g to 258.2mg/g fr.wt.) compared to the control plant leaves (53.0 mg/g fr.wt.) and seed (128.9 mg/g fr.wt.).The optimum doses for maximum yield of protein in fresh leaves and seeds were also found to be 0.25 %,v/v of catechol treated plants as in Table 2.The level of soluble nitrogen in secondary leaves of catechol treated plants showed gradual increase up to 0.25% (12.7 mg/g) and then decrease in higher concentration as compared to those of the control plants (4.55 mg/g) (Table3) . The time taken for the plant to show first flower opening varied from 24 to 30 days after germination in both treated and control plants. Maximum number of flowers opened was observed on 30 days after flower initiation , there is a gradual increase in the number of flowers opened with 0.25 %, v/v conc. of catechol on all days of estimation (Table 4).

Phenolic compound, a diverse group of plant secondary metabolites, play an important role in the regulation of plant growth [7].The significant exertic effect of topical foliar application of salicylic acid, a natural occurring phenolic compound as a growth regulating substances is well established [8]. However, in *Phaseolus vulgaris*, topical foliar application of catechol, a secondary plant metabolites also played an important role in the morphogenetic regulation of plant growth, metabolism and development including as a flower inducing factor and thus acts as one of the potent plant growth hormones. The maximum nitrate reductase activity was obtained with 0.25%, v/v concentration of catechol treatment coinciding with the optimum dose for almost all other nitrogen metabolism. The increased level of nitrate reductase activity reveals that the nitrogen assimilation rate may be improved by the treatment of catechol. The increases in protein on all treatment may be due to the reason that phenol influences the enhancement of amino acid biosynthesis [9] and this may also be due to stimulation of the activity of nitrogen utilizing enzyme such as nitrate reductase [10].The increase in soluble nitrogen in leaves of treated plants as compared to that of the control plants indicates the activity of the phenol as analogues of growth hormones[1].The decrease of the nitrogen content with the rise of the concentration again indicates the inhibitory activity of the phenols at higher concentration and this reduced value of nitrogen content indicates their reduced efficiency to fix nitrogen . Flowering was hastened significantly by the treatment of phenol. This agrees with the earlier views that phenol play a significant role in the initiation and development of floral buds[11].Faster flowering in the treated plants than in the control plants is suggestive of the association of the phenols with enhanced transport of photosynthates and other metabolites of the reproductive sink as suggested by Nanda and Kumar[2].

To conclude, this research finding for increased yield of high quality accumulation of leaves/seed protein of *Phaseolus vulgaris* with topical spray of catechol has been found to be helpful to correct protein energy malnutrition which is one of the main serious global public health problem in most low income tropical and subtropical countries with predominantly rural populations.

References

1. Wain R L and Taylor HE 1985, Phenols as plant growth regulators. *Nature* **207** 167-169.
2. Nanda KK and Kumar S 1982, Morphogeneitic effect of phenolic compounds in relation to hormones. In : *Plant physiology – an under utilized potential for increasing crop productivity.* Bhabha Atomic Research Centre, Trombay, Mumbai, 211-255.
3. Luckner M and Schreiber K 1979, *Regulation of secondary products and plant hormon metabolism.* Pergamon Press, Oxford.
4. Srivastava RC, Mukherji D and Mathur SN 1980, *In vivo* activity of nitrate reductase in *Vigna mungo,* an assessment of the underestimation due to presence of nitrate reductase.*Ann. Bot.* **45** 717-718.
5. Lowry OH, Rossbrough NJ, Farr AL and Randel RJ 1951, Protein measurement with the Folin – Phenol reagent. *J. Biol. Chem.* **193** 265-275.
6. Lang CA 1958, Simple micro-determination of kjeldahl nitrogen in biological material. *Anal. Chem.* **30** 1692-1694.
7. Kumar S and Tayal MS 1982, Effect of Phenols and gibberellic acid on the germination and seedling growth of some legumes. *J. Ind. Bot. Soc.* **61** 125-128.
8. Asthana J S and Srivastava H S 1978, Effect of presowing treatment of maize eeds with ascorbic acid and salicylic acid on seed germination, seedling,growth and nitrate assimilation in the seedling. *Indian J. Plant Physiol.* **21** 150 – 155.
9. Kefeli VI and Kutachek M 1977, Phenolic substances and their possible role in plant growth regulation. In: *Plant growth regulation.* 181-188.
10. Johnson CB , Willington WJ and Blackwood GC, 1976, Nitrate reductase, possible predictor test for crop yield. *Nature* **262** 133-134.
11. Nanda KK , Kumar S and Sood V 1976, Effect of gibberellic acid and some phenol on flowering of *Impatiens balsamina*, a quantitative short day plant. *Physiol. Plant.* **38** 53-56.