2.5 h. Flasks were cooled and their contents were extracted thrice with hexane. Hexane was made acid-free by passing it through a mixture of anhydrous sodium sulfate and sodium bicarbonate. The hexane was then partitioned with acetonitrile and assayed as described. A similar technique was adopted for the study of the metabolism of endosulfan derivatives, with the difference that the time of exposure was 24 h.

Results and discussion. Analysis of endosulfan I- and II-treated bees revealed the presence of endosulfan ether, hydroxyether, lactone, endosulfan I, endosulfan II and two unknown compounds (table). The rate of metabolism of endosulfan was maximal in the first 2 h of treatment, in which 45–50% of the applied dose was metabolized. After 24 h 15–17% of the unmetabolized endosulfan could be extracted from tissues and 10–20% from excreta.

The products of endosulfan metabolism indicated both oxidative and hydrolytic pathways (fig. 2). The two isomers of endosulfan were found to be interconvertible, with the equilibrium of the reaction towards endosulfan I. The quantity of endosulfan sulfate obtained from endosulfan II-treated bees was twice that obtained from the endosulfan I-treated bees. These results support our earlier findings, that piperonyl butoxide synergized endosulfan II more than it synergized endosulfan I in bees[5]. Analysis (GLC and TLC) of endosulfan sulfate-treated bees indicated the presence of endosulfan I and II. These results are enigmatic, as earlier endosulfan sulfate could not be synergized in bees with Piperonyl butoxide[5]. Chopra and Mahfouz reported the presence of endosulfan I in endosulfan-sulfate treated tobacco leaves[8].

Endosulfan ether, diol and hydroxyether applied to bees separately broke up into the other two metabolites, showing their interconversion. In endosulfan lactone-treated bees, no metabolite was observed. However, the recovery of lactone was very poor (25%) after 24 h of treatment.

The conjugates of endosulfan diol, hydroxyether and lactone were significant in excreta and nonsignificant in tissues. It could be that the metabolites after conjugation are immediately excreted, or that the conjugation of metabolites takes place in the alimentary canal of bees rich in sugars.

The data on the rate of metabolism of endosulfan isomers in honey bees do not indicate any correlation with bee toxicity: nevertheless, the high rate of excretion of topical endosulfan suggests the possibility of its residues finding their way into honey through the honey stomach. The presence of endosulfan and its metabolites suggests that the feces could also contaminate wax in the hive and endanger the relatively susceptible brood. This important aspect needs further investigation. When residues of endosulfan in honey are estimated, the presence of its toxic metabolites produced by bees, namely endosulfan sulfate, ether and lactone, should also be taken into consideration.

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Pyridoxine application enhances nitrate reductase activity and productivity of *Vigna radiata*

Samiullah, S.A. Ansari[1], M.M.R.K. Afridi and M. Akhtar

Department of Botany, Aligarh Muslim University, Aligarh (India), 19 April 1984

Summary. Pyridoxine, applied to seeds before sowing or by the spraying of a standing crop of moong *(Vigna radiata)* significantly enhanced leaf nitrate reductase activity (NRA). Leaf NRA levels, being significantly correlated with seed yield, can be utilized for predicting crop productivity and for adopting corrective measures, if necessary, at an early growth stage.

Key words. Vigna radiata; leaf nitrate reductase; seed yield; crop productivity.

Leguminous crops constitute a major source of vegetable proteins in tropical and subtropical countries. Simultaneously, they furnish to the soil inexpensive and nonpolluting nitrogen by symbiotic fixation. However, these crops have not received the attention they deserve in underdeveloped countries where protein malnutrition prevails on a large scale. To remedy the situation, it is desirable to increase the productivity of grain legumes in these countries and to improve the quality of their seeds.

Earlier studies in the authors' laboratory have established that pyridoxine (vitamin B_6) is effective as well as economical in increasing the yield of barley[2,3]. Pyridoxine has long been known to promote the growth of excised organs of various plant species[4–7]. Its presence in the culture medium promotes the uptake of glucose, nitrate and phosphate in excised roots of grasses[8]. Cotton seedlings have been reported to possess higher concentrations of nitrogen and phosphorus as a result of treatment of the seeds with pyridoxine[9].

Surprisingly, information on the effectiveness of pyridoxine application on the growth and yield of crops grown under field conditions is meagre[2,3,10]. However, all previous in vitro, pot culture and field studies indicate that pyridoxine invariably enhances root growth of the plants studied so far. This led us to suggest that, if this vitamin promoted root growth in legumes also, the absorption of nitrate and other nutrients would be enhanced by its application. Simultaneously, a larger surface area would be provided for *Rhizobium* infection. The consequent increase in the number of root nodules would be expected ultimately to benefit the host through enhanced nitrogen fixation. This could be of considerable practical significance for farmers as it would result in better growth and higher productivity of these hitherto neglected crops.

To test this hypothesis, two field experiments were conducted to study the effect of pyridoxine on nitrate reductase (E.C. 1.6.6.1) activity (NRA), growth and yield of *Vigna radiata* (L.) Wilczek var. K-851 (moong). The vitamin significantly enhanced NRA measured in leaves according to the method of Jaworski[11]. In addition, root growth, root nodule number and seed yield were also found to be significantly increased (table 1).

Table 1. Effect of pyridoxine treatment on root length, root nodule number and seed yield of *Vigna radiata* var. K-851

Experiment	Growth	Parameter	Pyridoxine concentration						SE of treatments; significant at $p < 0.01$ (F-test)
No.	stage		0.0%	0.1%	0.2%	0.3%	0.4%	0.5%	
			(Means of three replicates)						
1	20 days	Root length (cm)	6.05	6.22	6.83	6.38	6.39	5.83	± 0.09
(seed soaking)		Root nodule No.	–	–	–	–	–	–	Root nodules not conspicuous
	30 days	Root length (cm)	8.47	9.44	9.60	11.58	10.04	9.87	± 0.27
		Root nodule No.	8.00	8.88	12.48	19.52	16.00	11.52	± 0.65
	40 days	Root length (cm)	6.94	7.33	7.72	7.83	8.34	7.80	± 0.24
		Root nodule No.	10.47	10.99	11.56	13.10	10.32	9.95	± 0.23
	50 days	Root length (cm)	6.13	6.50	7.11	7.33	7.67	7.28	± 0.30
		Root nodule No.	3.28	3.97	4.08	4.11	3.47	3.32	± 0.21
	Harvest	seed yield (q/ha)	8.92	8.66	10.40	13.82	11.56	9.02	± 0.49
2	45 days	Root length (cm)*	9.39	8.89	8.11	8.72	8.33	7.75	Nonsignificant
(Foliar spray)		Root nodule No.*	7.02	8.27	7.28	6.36	6.43	5.51	± 0.16
	55 days	Root length (cm)*	8.87	9.72	7.39	6.78	7.11	7.32	± 0.28
		Root length (cm)**	7.25	8.56	10.41	7.75	7.55	6.78	± 0.28
		Root nodule No.*	–	–	–	–	–	–	Root nodules degenerated
		Root nodule No.**	–	–	–	–	–	–	
	Harvest	seed yield (q/ha)*	9.49	12.69	11.23	10.98	10.43	10.61	± 0.32
		seed yield (q/ha)**	9.62	12.01	12.16	11.53	10.72	10.67	± 0.32

* Plants sprayed at 35 days. ** Plants sprayed at 45 days.

In experiment 1, seeds of moong were soaked for 4 h in aqueous pyridoxine solutions of different concentrations. NRA was estimated at 20, 30, 40 and 50 days after sowing. Soaking in 0.3% pyridoxine solution increased NRA by 29.7, 7.1, 11.8 and 15.6% over the respective water-soaked controls at these intervals (fig. 1). There was significant correlation between pyridoxine soaking and NRA at 40 and 50 days only ($r = +0.857$ and $+0.762$ respectively; $n = 6$). The computed values of the correlation coefficients at 20 and 30 days were not found to be significant. Similarly, NRA at 20 and 50 days was significantly correlated with seed yield ($r = +0.838$ and $+0.810$, respectively; $n = 6$). However, there was no significant correlation between NRA levels and seed yield at 30 or 40 days.

In experiment 2, the same concentrations of pyridoxine solutions were applied by foliar spray either at the pre-flowering (35 days) or at the post-flowering (45 days) stage. NRA was measured 10 days after each spraying (45 and 55 days after sowing). The spraying of 0.1% pyridoxine solution at the pre-flowering and of 0.2% at the post-flowering stage proved to be optimal for NRA which, when estimated at 45 and 55 days, was found to be increased by 49.2 and 29.6% respectively over the water-sprayed controls (fig. 2). A significant correlation was noted between the spraying of pyridoxine at the pre-flowering stage and NRA estimated at 45 days ($r = +0.989$; $n = 6$). However, spraying at the later stage (45 days) was not significantly correlated with the enzyme activity estimated at 55 days. The most noteworthy finding of practical importance was that NRA estimated at 55 days (albeit not at 45 days) was significantly correlated with seed yield ($r = +0.692$; $n = 12$), thus confirming that a positive correlation between NRA and yield could be obtained in *V. radiata* as in barley[12] and lentil[13].

Vitamins (thiamine, riboflavin and niacin) have been reported to

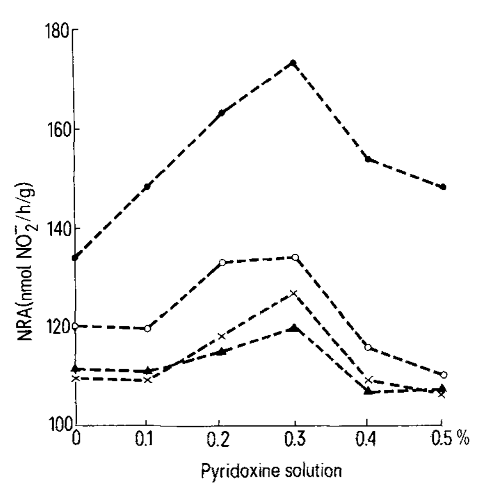

Figure 1. Nitrate reductase activity (mean of three replicates) in leaves of moong (*Vigna radiata* var. K-851) as related to soaking of seeds in aqueous pyridoxine solutions, at various intervals after sowing under field conditions: ● 20 days (SE = ± 2.91), ▲ 30 days (SE = ± 1.21), ○ 40 days (SE = ± 1.75) and × 50 days (SE = ± 1.74). Data are significant at $p < 0.01$ (F-test).

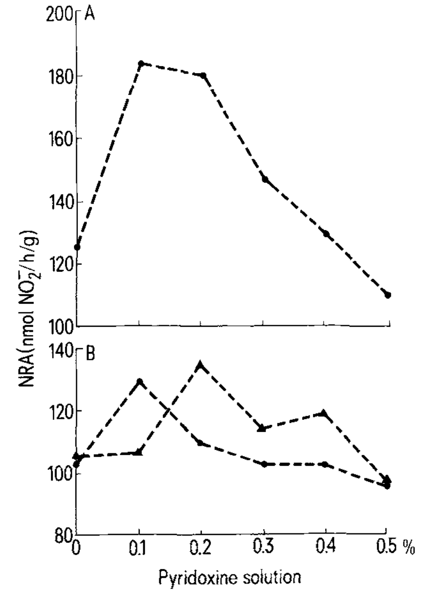

Figure 2. *A* nitrate reductase activity (SE = ± 2.55) estimated at 45 days in leaves of moong (*Vigna radiata* var. K-851) as influenced by spraying of aqueous pyridoxine solution at 35 days (pre-flowering) (●); *B* nitrate reductase activity at 55 days (SE = ± 1.22), ● spray at 35 days (pre-flowering) and ▲ spray at 45 days (post-flowering). Data represent means of three replicates and are significant at $p < 0.01$ (F-test).

enhance the activities of carbonic anhydrase and catalase in oats and beans[14]. However, the effect of pyridoxine on enzymes, including nitrate reductase (which occupies a key position in the nitrogen metabolism of plants), does not seem to have been studied so far. Our observation, that pyridoxine significantly increases leaf NRA levels in moong, assumes greater importance in the light of the positive correlation with seed yield noted in both experiments. As pyridoxine was also found to increase root growth and root nodule number (table 1), it may be argued that this effect facilitated a greater availability of nitrate[8], which is the inducer and stabilizer of nitrate reductase[15,16].

Comparison of the yield data of experiments 1 and 2 (table 2) clearly indicates the superiority of the soaking of seeds in pyridoxine solution, as it is more effective than spraying. Seed treatment also requires a smaller quantity of the vitamin for treating the same population of plants. Estimation of NRA is, moreover, a rapid and reliable technique for predicting crop productivity[12,13]. It is well established that yields may be augmented by appropriate remedial measures, including spraying of nutrients[17], if their deficiency is detected early enough; N deficiency, for example, may be indicated by NRA estimation. Seed treatment with very dilute pyridoxine solutions may, therefore, be exploited commercially as a simple, convenient and economical farm practice for ensuring higher productivity.

Table 2. Balance sheet for alternative methods of pyridoxine application in relation to seed yield of *Vigna radiata* var. K-851

Experimente No.		Remarks	Seed yield (q/ha)	Quantity of pyridoxine required (kg/ha)
1	I)	Optimum seed treatment (0.3% pyridoxine)	13.82	0.09
	II)	Water-soaked control	8.92	–
	III)	Increase over control I–II	4.90	–
2	IV)	Optimum spray treatment (0.1% pyridoxine)	12.69	2.00
	V)	Water-sprayed control	9.49	–
	VI)	Increase over control IV–V	3.20	–

Economics of seed treatment versus spray treatment

a)	Increase in seed yield III–VI	1.70 q/ha
b)	Price of additional yield at $40/ha	$68.00
c)	Saving on pyridoxine IV–I	1.91 kg/ha
d)	Price of pyridoxine saved at $80/kg	$152.80
e)	Net profit b + d	$220.80/ha

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Comparison of the effects of different isomers of bicuculline infused in the preoptic area on male rat sexual behavior

A. Fernández-Guasti, K. Larsson[1] and C. Beyer[2]

Department of Psychology, University of Göteborg, Box 14158, S-40020 Göteborg (Sweden), and Centro de Investigación en Reproducción Animal, CINVESTAV-UAT, Ap. Postal 62, Tlaxcala 90-000 (México), 25 October 1984

Summary. Intracerebral infusion of (+) bicuculline methiodide, but not of its (–) isomer, in the preoptic area, stimulated masculine sexual behavior in rat as evidenced by a decrease in the number of intromissions preceding ejaculation and a shortening of the ejaculation latency and postejaculatory interval. Data suggest a role of the GABAergic system in mediating masculine sexual behavior.

Key words. (+) and (–) bicuculline methiodide; masculine sexual behavior; preoptic-anterior hypothalamic area; rat.

The masculine sexual behavior in the rat is an innate, stereotyped behavior pattern: the male repeatedly mounts and penetrates the receptive female until ejaculation is achieved; he then becomes unresponsive to sexual stimulation for a period which becomes progressively longer with each successive ejaculation[3]. Only little is known about the neuroendocrine processes underlying this behavior. The medial preoptic-anterior hypothalamic continuum (MPOA) is of critical importance since lesions in this area abolish sexual behavior[4] whereas electrical stimulation of it facilitates the elicitation of copulation[5,6]. Efforts have been made to study the neurotransmitters involved in determining this behavior, the interest mainly focussed on the role of brain monoamines[7]. It is generally agreed that depression of central 5-hydroxytryptamine (5-HT) activity results in a facilitation of the masculine sexual behavior in rats while central 5-HT stimulation has an opposite effect[8]. The importance of dopamine and noradrenaline for sexual activity is less well established[7]. Recent evidence also indicates a role of the cholinergic system in male rat sexual behavior[9].

Little attention has been given to a possible role of amino acids in the mediation of sexual behavior, although in quantitative terms, amino acids now appear to be the major neurotransmitters in the mammalian central nervous system[10]. As a inhibitory neurotransmitter, gamma aminobutyric acid (GABA) ap-