DETECTION OF β-OXALYL DIAMINO PROPIONIC ACID IN CALLUS CULTURES OF *LATHYRUS SATIVUS*

S. BADRI NARAYAN, D. P. BHATT, P. N. BHATT and A. R. MEHTA
Department of Botany, Faculty of Science, The M S. University of Baroda, Vadodara 390 002, India.

ABSTRACT

Seeds of *Lathyrus sativus* contain a non-protein amino acid β-N-oxalyl-L-α-β diaminopropionic acid (BOAA) which has neurotoxic property. Callus cultures were initiated from germinating seeds of *L. sativus* on basal modified B_5 medium supplemented with 2,4-dichlorophenoxyacetic acid and kinetin. BOAA was detected by TLC in ethanolic extract of the callus cultures. The UV spectrum of BOAA extracted from cultured tissues could be superimposed on that of standard BOAA. Quantitative analysis revealed that the BOAA content of cultured tissue was less than that of the embryo axis of intact dry seeds.

INTRODUCTION

LATHYRUS SATIVUS L. is a drought-resistant legume containing several toxic principles in its seeds[1]. Of all the toxic compounds, the non-protein amino acid β-N-oxalyl-L-α-β-diaminopropionic acid (BOAA) has been implicated as neurotoxic to human and other mammals[2]. The concentration of BOAA is greatest in the seeds, ranging between 0.1 and 2.5% per dry weight[3], and present in lower amounts in other plant parts. We are interested to study the biosynthesis of BOAA in this plant as there is not much information available on the origin of diaminopropionic acid (DAPA), the precursor of BOAA.

Glycine, serine and asparagine are proposed as precursors in several pathways proposed for biosynthesis of DAPA[4]. Bell[5] suggested that BOAA constitutes a highly concentrated reserve in the plant immediately available to the embryo on germination. Its presence in plants is considered as a defence mechanism against predators[6]. Compared to the whole plant, callus and cell suspension culture systems provide many advantages as the experimental system to study the biosynthesis of DAPA. Various nutritional factors and environmental parameters can be controlled and manipulated more uniformly at a cell and callus culture level than at whole plant level in such a study. In this communication we report the presence of BOAA in callus cultures of several varieties of *L. sativus* and some preliminary studies on factors affecting its content.

MATERIALS AND METHODS

The cultivars LSD-1, RL-18 and Bharuch Local were obtained from the Pulse Breeding Station, Kanpur. Seeds of these cultivars were germinated under aseptic conditions on 1% agar. Callus was initiated from hypocotyl of germinating seeds on 0.8% agar based MB 5–2 medium[7] supplemented with 1 μM 2-4-dichlorophenoxy-acetic acid and 0.1 μM kinetin as growth regulators. The callus was subcultured every four weeks.

Detection and estimation of BOAA in seedlings and callus tissues

About one gram fresh weight of callus was homogenized in 4 ml of 10% aqueous trichloroacetic acid (TCA). The homogenate was spun at 6000 rpm for 15 min. To the supernatant, concentrated aqueous solution of potassium hydroxide was added to bring its final concentration to 3 N. Alkaline hydrolysis was carried out for 30 min at 80°C in a water bath. This liberates DAPA from BOAA. The concentration of DAPA was determined using *o*-pthaldehyde (OPT) reagent according to the method suggested by Rao[8]. Identification of liberated DAPA was checked by TLC. The hydrolysed extract was evaporated to dryness and the residue was dissolved in 95% ethanol. It was spotted on silica gel G coated on glass plate along with standard DAPA. The TLC plate was developed using methoxy ethanol:propionic acid:water (70:15:15:v/v/v) as the solvent system. The plate was sprayed with 0.5% ninhydrin in acetone and heated at 80°C for 5 min.

Effect of some media components on BOAA accumulation in callus cultures

(i) Three different concentrations of sucrose were

tested in the MB5 medium, viz. 2, 3 and 5% w/v of the medium. Callus that were maintained on 3% sucrose level were subcultured twice on these media, each for about 15 days. At the end of second subculture, they were analysed for BOAA content.

(ii) The basal MB5 medium contains the following compounds as the source of reduced nitrogen (mg/l); glycine, 20; glutamic acid, 7.5; glutamine, 60; aspartic acid, 7.5; asparagine, 10; urea, 45; adenine sulphate, 2.5. Two types of media were made, in one full strength of reduced nitrogenous compounds were added and in the second, they were added at half strength. This means that each of the component was reduced to one half the level in final concentration. Callus of varieties LSD-1 and RL-18 were transferred on these media and were allowed to grow for a month. They were then harvested to determine BOAA content.

RESULTS AND DISCUSSION

Identification of DAPA in the seedlings and callus tissues

Free DAPA was not detected in the dry seeds, seedlings or callus tissues. This is in accordance with the findings of Bell[9]. It was released only after alkali hydrolysis. Our identity of DAPA is based on three different criteria: (i) its ability to react with OPT to give a yellow chromophore with absorption maximum at 420 nm, (ii) its chromatographic behaviour identical to that of standard DAPA on silica gel plate (figure 1), and (iii) similarity of its UV absorption spectrum to that of standard DAPA. Since BOAA is the only known derivative of DAPA and free DAPA is not present in *L. sativus*, value of DAPA can be considered as the values representing that of BOAA.

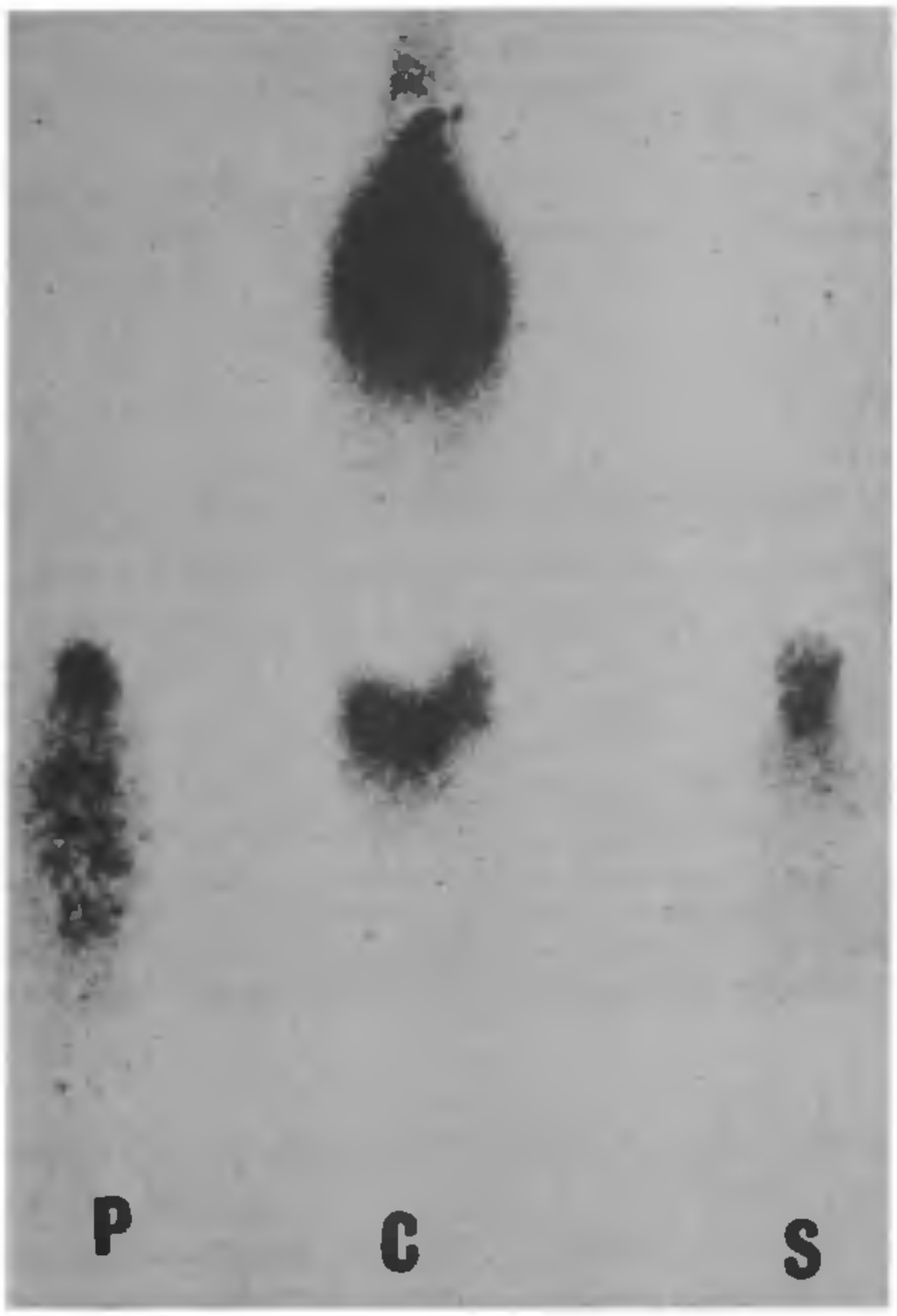

Figure 1. Identification of DAPA in the seedling (P), callus tissue (C) and authentic standard (S).

BOAA in L. sativus seedling

Dry seed of *L. sativus* var. Bharuch contains about 38 μmol of BOAA on per gram fresh weight basis. The radical emerges from imbibed seeds after 48 h. Determination of BOAA in the whole seedling was carried out till 120 h after imbibition. The BOAA content in the seeds decreased to about 9 fold in the first 24 h after imbibition but then gradually increases (table 1). However the value of BOAA in 5-day-old seedling was less than 50% to that of dry seeds.

Table 1 *Changes in BOAA in L. sativus seeds during germination and early seedling development*

Time after imbibition (h)	BOAA concentration (μmol/g fresh weight ± SD)
0	38.0
24	4.3 ± 0.47
48	6.3 ± 0.35
72	6.3 ± 0.35
96	8.1 ± 0.14
120	17.0 ± 0.04

BOAA in callus cultures

The BOAA content of callus of var. Bharuch grown on MB5 media containing 2, 3 and 5% sucrose is shown in table 2. Stimulatory effect of sucrose is greater on the accumulation of BOAA than on the accumulation of dry weight of callus. Callus tissues of two varieties were used to study the effects of reduced nitrogenous compounds of media on the

Table 2 *Effects of some media factors on the accumulation of BOAA in callus cultures*

a. Effect of sucrose:

Sucrose in media (% w v)	Dry weight of callus (mg)	BOAA content of callus (μmol/g fresh weight)
2	212	4 8
3	222	6.1
5	237	6.9

b. Effect of reduced nitrogenous compounds of media:

Variety	Reduced nitrogenous compounds level in the media*	BOAA content of callus (μmol/g fresh weight)
LSD-1	Half strength	0.62
	Full strength	1.27
RL-18	Half strength	0.85
	Full strength	1.32

*Full strength indicates full amount of each nitrogenous compound in the medium. Half strength indicates that each component was reduced to one half of the full amount.

accumulation of BOAA. With reduction of nitrogenous compounds of media to half strength, BOAA content was reduced to 50% to that present in the full strength medium (table 2). Detailed analysis of effects of nitrogenous source, osmotic stress and genotypic variation will be presented elsewhere.

Our major objective was to study the origin of DAPA in *L. sativus*. The presence of this compound in callus culture is a significant step towards establishing a useful experimental system. This basic knowledge can subsequently be used to eliminate the antinutritional neurotoxic amino acid from this drought-resistant, protein-rich pulse crop of Indian subcontinent.

ACKNOWLEDGEMENT

One of the authors (DPB) thanks DST, New Delhi, for financial assistance.

1. Rao, S. L. N., Adiga, P. R. and Sarma, P. S., *Biochemistry*, 1964, 3, 432.
2. Mani, K. S., Sriramachari, S., Rao, S. L. N. and Sarma, P. S., *Indian J. Med. Res.*, 1971, **59**, 880.
3. Roy, D. N., Rao, B. S. N., *Curr. Sci.*, 1968, 37, 395.
4. Fowden, L., Lea, P. J. and Bell, E. A., *Adv. Enzymol.*, 1979, **50**.
5. Bell, E. A. and Przybylska, J., *Biochem. J.*, 1965, **94**, 35.
6. Alton Meister, *Adv. Enzymol.*, 1979, **50**.
7. Mante, S. and Bell, W., *Can. J. Bot.*, 1975, **53**, 1542.
8. Rao, S. L. N., *Anal. Biochem.*, 1978, **86**, 386.
9. Bell, E. A., *Biochem. J.*, 1962, **83**, 225.

ANNOUNCEMENT

ANTISENSE RNA AND DNA

Antisense RNA and DNA are emerging techniques for the highly specific manipulation of gene activity, using an "antisense" sequence complementary to the coding strand to block specifically expression of the gene. The power of this technique lies in its broad applicability, combined with selectivity of action, since it is possible in principle to reduce or even block the expression of any chosen gene—with enormous implications. The meeting to be held on 29 and 30 March 1989, at the St. John's College, Cambridge will explore use of this technique in the treatment of cancer and viral infections including AIDS, as well as the control of genes of trypanosomes and the manipulation of agriculturally important processes in plants. Examples will be drawn from across the biological spectrum, and so will be of interest to all involved in biotechnology. For details contact: Dr Renata Duke, IBC Technical Services Ltd., Bath House, 56 Holborn Viaduct, London EC1A 2EX.