

Biochemical changes during plantlet regeneration in two accessions of *Mucuna pruriens*

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ABSTRACT

The genus *Mucuna* is an important medicinal herb and is extensively used in traditional Indian systems of medicine for various ailments. *In vitro* culture technique provides an alternative to plant propagation and germplasm conservation. Our aim was to study the biochemical changes occurring during regeneration of shoots (plantlets) from explants of two accessions of *Mucuna pruriens*, by monitoring the efficiency of nitrogen utilization and changes in levels of some hydrolytic enzymes. A rapid micropropagation system was developed using Murashige and Skoog's (MS) medium supplemented with BAP and IAA combined. In both the accessions, 3.0mg l⁻¹ 6-BAP, in combination with 0.2mg l⁻¹ IAA, induced shoot buds and shoot elongation; however for multiple-shoot induction, a slightly higher concentration of cytokinin, i.e., 3.5mg l⁻¹ 6-BAP, in combination with 0.2mg l⁻¹ IAA, was required. Results of the present study confirm an active growth of explants revealed by nitrate assimilation enzymes and hydrolytic enzymes. It is concluded that medium composition, growth regulator combination and culture incubation conditions are all vital in both the accessions of *Mucuna pruriens* for induction of *in vitro* plant regeneration.

Key words: *Mucuna*, *in vitro*, biochemical changes, regeneration, enzymes

INTRODUCTION

The genus *Mucuna* belongs to the family Fabaceae (Leguminoceae) and includes about 150 species of annual and perennial legumes with pan-tropical distribution. *Mucuna pruriens* L. is a well-known medicinal plant, yet, study on its pharmacological properties and corresponding compounds still continues. Importance of the genus *Mucuna* as a medicinal plant is mainly due to presence of L-Dopa. L-Dopa (3,4 dihydroxy-L-phenylalanine) is a neurotransmitter precursor used for symptomatic relief of Parkinson's disease. Further, it acts as a precursor for several neurologically important catecholamines such as the neurotransmitter dopamine and the important hormones, adrenaline and noradrenalin (Riley, 1997).

Reproducible regeneration of shoots from various explants is desirable in plant tissue culture for crop improvement (Christopher *et al*, 1991). Differentiation of structures in tissue culture is controlled by growth regulators, along with other components of the culture medium (Narender *et al*, 2011). Analysis of activities of various

enzymes provides a reasonable and promising approach to understanding the biochemical basis of developmental pathways (Singh *et al*, 2009). Therefore, there is a need to study structural and biochemical aspects underlying initiation of organized development *in vitro* (Sujatha *et al*, 2000). The present study was aimed at investigating the biochemical changes that occur during regeneration of shoots (plantlets) in explants of two accessions of *Mucuna pruriens*, viz., Accession 1 (*M. pruriens* bearing a black seed-coat) and Accession 2 (*M. pruriens* bearing a white seed-coat). This was done by monitoring the efficiency of enzymes involved in nitrogen utilization, and changes in the level of some hydrolytic enzymes.

MATERIAL AND METHODS

Plant material and preparation of explants

Seeds of both the accessions of *Mucuna pruriens* were procured from University of Agricultural Sciences, Bengaluru. The seeds were surface-sterilized with 1% mercuric chloride for 5 min, followed by washing in sterile distilled water 5-6 times to remove traces of the surface-

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sterilant. These were then germinated *in vitro* on basal MS (Murashige and Skoog, 1962) medium. Plants grown thus were used as the explant source. Explants were trimmed aseptically (1.5 to 2.0cm) and inoculated onto MS medium.

Media and culture conditions

MS medium composed of MS salts and vitamins supplemented with sucrose (30g l⁻¹), solidified with 0.8% (w/v) agar and pH adjusted to 5.8 before autoclaving at 121°C for 20 min. Cultures were maintained at 24 ± 2°C under 16h light/8h dark photoperiod using light provided by cool, white, fluorescent lamps (25µmol m⁻² s⁻¹) in a growth chamber.

Shoot induction, multiplication and rooting

MS basal medium supplemented with various concentrations (0.5, 1.0 to 5.0mg l⁻¹) of different cytokinins, viz., 6-BAP (benzyl amino purine), kinetin and 2-ip [6-(γ , γ -dimethylallylamino purine)] either singly, or in combination, with 0.2mg l⁻¹ indole-3-acetic acid (IAA) or α -naphthalene acetic acid (NAA) or no plant growth regulator to evaluate morphogenic potential of the nodal explants. All the cultures were subcultured to fresh medium of the same composition every 28 days (4 weeks). Percentage response of explants producing shoots, number of shoots produced per explants and shoot-length were recorded at weekly intervals.

Rooting of shoots was done on half-strength MS medium supplemented with different concentrations of IAA and NAA (each singly at 0.5mg l⁻¹ to 4mg l⁻¹) or in combination with 0.1% activated charcoal.

Enzyme extraction and assay

Enzyme extraction and assays were performed as described below, with slight modifications when necessary, for the present investigation. For Nitrate/ Ammonia assimilating enzymes, extraction for nitrate reductase was carried out as per Altaf Ahmad and Abdin (1999), and enzyme activity was assayed as per Campbell and Smarrelli (1978). Extraction and assay for glutamine synthetase were done as per Philippe Lenee and Yves Chupeau (1989). The same extraction procedure was adopted for glutamate dehydrogenase. Optimum conditions for enzyme activity were maintained, namely, pH, temperature, substrate and cofactor concentrations. Acid and alkaline phosphatase enzyme extraction and assay were carried as per Angosto *et al* (1988). For invertase, the method of Yolanda Cuadrado *et al* (2001) was used for extraction, and the activity was determined using the method of Miller and Ranwala (1994). Extraction and assay of α -amylase was

carried out as per Sadasivam and Manickam (2008). Peroxidase extraction was done as per Lorenza M. Bellani *et al* (2002) and its activity was assayed as per Oskar Sanchez *et al* (1989).

Statistical analysis

All the experiments were conducted in three replicates. Data were subjected to statistical analysis using Microsoft Excel (MS Office, 2003) and are presented as Mean ± SE.

RESULTS AND DISCUSSION

Shoot induction and rooting in nodal explants

Organogenesis was observed in nodal segments cultured on MS medium supplemented with each of the concentrations of BAP/ kinetin/ 2-ip (alone, or in combination) with 0.2mg l⁻¹ IAA/ NAA in both the accessions of *Mucuna*. Morphogenic response observed was better with the aminopurine class of cytokinins (BAP and 2-ip) than with the furfuryl amine class of cytokinins (kinetin), with BAP showing a better response among the former. Therefore, for further studies, only BAP was used as the cytokinin of choice. Optimum growth of shoot occurred on medium containing 3mg l⁻¹ BAP in combination with 0.2mg l⁻¹ IAA (Fig. 1a and 1b) in both the accessions

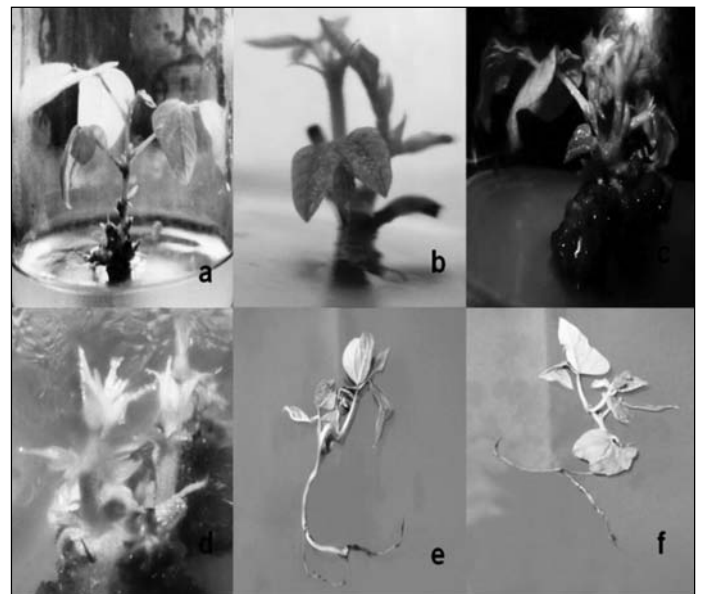


Fig. 1. Micropropagation in two accessions of *Mucuna pruriens* using nodal explants a. Shoot elongation in Accession 1 on MS + BAP (3.0mg l⁻¹) and IAA (0.2mg l⁻¹) b. Shoot elongation in Accession 2 on MS + BAP (3.0mg l⁻¹) and IAA (0.2mg l⁻¹) c. Multiple-shoot induction in Accession 1 on MS + BAP (3.5mg l⁻¹) and IAA (0.2mg l⁻¹) d. Multiple-shoot induction in Accession 2 on MS + BAP (3.5mg l⁻¹) and IAA (0.2mg l⁻¹) e. Rooting in Accession 1 f. Rooting in Accession 2.

(Table 1). A concentration of 3.5mg l⁻¹ BAP, in combination with 0.2mg l⁻¹ IAA, induced multiple shoots (Fig. 1c and Fig. 1d) in both the accessions. Average number of shoots per culture in Accession 1 was 14.25±2.46, with a survival of 97%. In Accession 2, the average number of shoots per culture was 11.50±2.61, and the survival percentage was 89 (Table 1).

After three subcultures (28-day subculture cycle), individual shoots attained nearly 5-6cm length. Successful root establishment (Fig. 1e and 1f) was achieved in individual shoots on MS medium (half-strength) supplemented with NAA (0.5mg l⁻¹) and IAA (0.5mg l⁻¹), individually, in the presence of 0.1% activated charcoal at 30 days of incubation. Of the two auxins studied, NAA was more effective in induction of rooting than IAA in the case of both the accessions of *Mucuna* (Table 2). Roots induced on NAA were thicker and survival percentage of the plants regenerated was also better (98% for Accession 1, and 92% for Accession 2) compared to IAA (69% for Accession 1, and 64% for Accession 2).

Shoot regeneration was achieved in nodal explants of Accessions 1 and 2 when cultured on MS medium supplemented with 3mg l⁻¹ 6-BAP in combination with 0.2mg l⁻¹ IAA. Similarly, organogenesis (in the form of root

emergence) was also observed in MS medium fortified with NAA/ IAA at 0.5mg l⁻¹. Morphological changes occurring in explants during the course of their proliferation on a suitable medium were monitored by determining some biochemical changes, viz., nitrate/ ammonia utilizing enzymes during shoot regeneration from nodal/ leaf explants, and changes in hydrolytic enzymes during organogenesis.

Changes in nitrate reductase (NR) activity:

Nitrate reductase (NR) is one of the key enzymes involved in the first step of nitrate assimilation in plants (Altaf Ahmed & Abdin, 1999). Table 3 shows the pattern of changes in nitrate reductase activity in both the accessions monitored from the day of inoculation up to the 30th day, at 5-day intervals.

In regenerating nodal explants of Accession 1, the activity peaked on Day 20. Thereafter, it remained the same until Day 30. Whereas, in Accession 2, two peaks of activity were observed on the 10th and 25th day (Table 3).

Changes in GS and GDH activity:

Glutamine synthetase (GS) and Glutamine dehydrogenase (GDH) are the other key enzymes involved in nitrate and ammonia assimilation in plants. In Accession 1, GS activity was found to be higher between the 10th and

Table 1. Effect of cytokinin–auxin combination in MS basal medium on shoot regeneration from nodal explants in two accessions of *Mucuna pruriens*

BAP (mg l ⁻¹)	NAA (mg l ⁻¹)	IAA (mg l ⁻¹)	Average no. of shoots per culture		Average shoot-length (cms)		Per cent survival	
			Accession 1	Accession 2	Accession 1	Accession 2	Accession 1	Accession 2
0.0	0.2	0.0	0	0	0	0		
0.5	0.2	0.0	1.83 ± 0.30	1.10 ± 0.35	0.82 ± 0.23	0.80 ± 0.20	14.42 ± 0.87	10.92 ± 0.51
1.0	0.2	0.0	1.50 ± 0.34	1.33 ± 0.31	1.33 ± 0.19	1.23 ± 0.13	23.50 ± 1.92	17.67 ± 1.24
1.5	0.2	0.0	1.50 ± 0.34	1.83 ± 0.37	1.83 ± 0.21	1.73 ± 0.21	40.75 ± 2.33	30.42 ± 1.61
2.0	0.2	0.0	1.75 ± 1.33	1.50 ± 0.38	2.25 ± 0.28	2.15 ± 0.18	52.00 ± 1.65	39.42 ± 1.76
2.5	0.2	0.0	1.75 ± 1.33	1.83 ± 0.34	3.08 ± 0.34	3.18 ± 0.34	58.17 ± 1.65	49.08 ± 3.55
3.0	0.2	0.0	2.83 ± 40.23	2.67 ± 1.37	6.42 ± 0.62	5.42 ± 0.42	94.00 ± 1.11	87.33 ± 1.44
3.5	0.2	0.0	12.75 ± 1.66	10.17 ± 2.90	4.83 ± 0.53	4.33 ± 0.23	86.33 ± 1.81	84.42 ± 2.88
4.0	0.2	0.0	1.17 ± 0.23	1.17 ± 0.47	3.58 ± 0.45	2.58 ± 0.25	64.58 ± 0.82	64.25 ± 2.04
4.5	0.2	0.0	1.50 ± 5.05	1.75 ± 0.51	2.00 ± 0.35	2.01 ± 0.35	46.17 ± 1.11	62.92 ± 1.78
5.0	0.2	0.0	1.80 ± 0.30	1.73 ± 0.35	1.83 ± 0.21	0.83 ± 0.12	40.15 ± 1.53	39.32 ± 1.56
0.0	0.0	0.2	0	0	0	0		
0.5	0.0	0.2	1.00 ± 0.33	1.67 ± 0.31	0.82 ± 0.23	0.82 ± 0.20	15.47 ± 0.67	11.12 ± 0.53
1.0	0.0	0.2	1.83 ± 0.41	1.92 ± 0.23	1.33 ± 0.19	1.24 ± 0.13	25.53 ± 1.97	18.68 ± 1.26
1.5	0.0	0.2	1.92 ± 0.29	1.50 ± 0.29	1.83 ± 0.21	1.83 ± 0.23	46.85 ± 2.53	31.41 ± 1.62
2.0	0.0	0.2	1.27 ± 0.51	1.08 ± 0.90	2.25 ± 0.28	2.25 ± 0.28	57.00 ± 1.53	39.72 ± 1.46
2.5	0.0	0.2	1.17 ± 0.97	1.17 ± 0.38	3.28 ± 0.36	3.38 ± 0.24	62.17 ± 1.56	51.13 ± 1.25
3.0	0.0	0.2	3.42 ± 1.69	2.42 ± 2.64	7.42 ± 0.61	5.74 ± 0.42	97.00 ± 1.09	89.13 ± 1.14
3.5	0.0	0.2	14.25 ± 2.46	11.50 ± 2.61	5.83 ± 0.53	4.23 ± 0.23	89.09 ± 1.80	84.02 ± 1.18
4.0	0.0	0.2	1.33 ± 0.66	1.42 ± 0.07	3.58 ± 0.45	2.78 ± 0.52	64.18 ± 0.32	65.24 ± 2.14
4.5	0.0	0.2	1.42 ± 0.01	1.92 ± 0.05	2.00 ± 0.35	2.03 ± 0.30	52.17 ± 1.17	61.82 ± 1.29

(Data represent Mean + S.E.)

Table 2. Effect of different auxins (in half-strength MS medium supplemented with 0.1% activated charcoal) on root induction in two accessions of *Mucuna pruriens*

Auxin (mg l ⁻¹)	Rooted shoots (%)		Mean no. of roots/shoot		Mean root-length (cm)		Plant survival (%)	
	Accession 1	Accession 2	Accession 1	Accession 2	Accession 1	Accession 2	Accession 1	Accession 2
No auxin	07.2 + 0.95	07.2+ 0.95	1.4 + 0.07	1.4 + 0.07	0.8 + 0.02	0.8 + 0.02	16.1 + 0.78	16.1+ 0.78
IAA								
0.2	34.2 + 1.28	32.4 +1.28	5.6 + 0.12	3.5 + 0.12	1.4 + 0.01	1.2 + 0.01	35.4 + 1.35	32.4+ 1.31
0.3	48.4 + 1.14	42.4+ 1.12	7.6 + 0.07	6.7 + 0.06	2.7 + 0.02	2.3 + 0.02	41.6 + 1.19	38.6+ 1.13
0.4	76.5 + 2.12	72.5+ 2.02	9.6 + 0.10	8.6 + 0.01	3.3 + 0.28	3.0 + 0.21	53.7 + 1.82	49.7+ 1.72
0.5	94.8 + 2.96	92.8+ 2.86	11.9 + 0.1	10.9+ 0.1	4.1 + 0.03	3.6 + 0.02	69.2 + 2.73	64.2+ 1.72
1.0	72.2 + 2.08	68.2+ 1.67	5.2 + 0.06	4.8 + 0.05	2.8 + 0.28	2.2 + 0.18	22.1 + 1.89	18.1+ 1.74
NAA								
0.2	63.5 + 4.72	61.5+ 3.62	6.9 + 0.08	5.6 + 0.04	2.7 + 0.28	2.3 + 0.25	34.2 + 0.48	32.4+ 0.34
0.3	78.6 + 3.26	72.4+ 2.36	7.7 + 0.08	7.4 + 0.07	3.1 + 0.22	2.9 + 0.12	60.2 + 0.94	58.2+ 0.84
0.4	89.2 + 2.33	85.2+ 2.13	9.9 + 0.09	9.4 + 0.08	4.0 + 0.32	3.8 + 0.26	76.2 + 0.96	72.4+ 0.86
0.5	96.2 + 6.63	94.2+ 5.52	12.4 + 0.24	12.2+ 0.23	4.4 + 0.38	4.1 + 0.28	98.6 + 0.96	92.6+ 0.76
1.0	46.3 + 2.88	43.4+ 2.68	5.6 + 0.06	5.2 + 0.05	2.9 + 0.22	2.7+ 0.21	30.2 + 0.33	28.2+ 0.23

(Data represent Mean + S.E.)

20th day, and decreased thereafter. Accession 2 had higher GS activity between the 5th and 20th day, and decreased thereafter (Table 3).

In Accession 1, it was observed that activity of both the isoforms of GDH (NAD⁺ and NADH isoforms) from Day 0 and Day 5 remained the same; but, there was an increase in activity on Day 10, and it peaked on Day 15, decreasing thereafter. But, the activity was greater in the NADH isoform on Day 10 compared to the NAD isoform (Table 3). In Accession 2, there was a gradual increase in the activity of both the isoforms of glutamate dehydrogenase (NAD⁺ and NADH isoforms) up to Day 10 and Day 15 in NADH and NAD isoforms, respectively, and decreased thereafter (Table 3).

Acid and alkaline phosphatases

Activity of acid phosphatase in regenerating shoots in Accession 2 was found to increase gradually from Day 0 to Day 30, whereas, in Accession 1, the activity of this enzyme increased from Day 0 to Day 5, and showed a dip on day '10' and, thereafter, a gradual increase until Day 30 (Table 3).

Activity of alkaline phosphatase in regenerating shoots in Accession 1 increased progressively from Day 0 to Day 30, whereas, in Accession 2, there was a reduction until Day 10, and thereon, the activity increased and peaked on Day 25 (Table 3).

Invertases

Invertases exist in at least two isoforms, such as the soluble (extracellular) and wall-bound form; and, acid and

alkali isoforms. In the present study, both acid (pH 5) and alkali (pH 7.5) isoforms were studied in organ-forming and non-organ-forming regenerating shoot cultures.

Wall-bound invertase

Activity of the wall-bound invertases in Accession 1 is presented in Table 3. Acid invertase peaked on Day 15. In the case of alkaline invertase, there was no increase in activity at all; rather, there was a gradual decrease in its activity from Day 0 to Day 30.

The activity of wall-bound invertase of Accession 2 peaked on Day 5, and, gradually decreased from Day 10 to Day 30 (Table 3); whereas alkaline invertase showed a little increase in activity on Day 5, and decreased thereafter.

Extracellular invertase

Activity of acid isoforms in Accession 1 peaked on Day 10, and gradually decreased thereon. Alkaline isoforms also showed maximum activity on Day 10 (Table 3). The activity in Accession 2 showed a gradual increase from Day 0 to Day 10 and remained constant up to Day 15, decreasing thereafter; whereas, the activity of alkaline isoforms peaked on Day 10.

α-amylase

Activity of α-amylase remained the same on Day 0 and Day 5 in both the accessions, and gradually increased from Day 10 to Day 30 (Table 3).

Peroxidase

Peroxidase activity in Accession 1 peaked on Day 25, and decreased thereafter; whereas, in Accession 2, peak activity was observed on Day 20.

Table 3. Changes in nitrate/ammonia utilizing enzymes and some hydrolytic enzymes during plantlet regeneration in two accessions of *Mucuna pruriens*

Enzyme	Accession	Days of incubation						
		0	5	10	15	20	25	30
Nitrate reductase($\mu\text{mole NO}_2 \text{ g}^{-1} \text{ min}^{-1}$)	Accession 1	25.24	25.24	25.24	49.4	73.95	73.95	73.95
	Accession 2	49.4	49.4	73.95	49.4	49.4	73.95	73.95
Glutamine synthetase (GS)(n moles of γ glutamate formed $\text{min}^{-1} \text{ g}^{-1}$ protein)	Accession 1	230	250	250	250	250	150	140
	Accession 2	200	230	230	230	230	140	130
Glutamate dehydrogenase (NADH-GDH)($\mu\text{mole NADH g}^{-1}$ F.W.)	Accession 1	100	100	240	260	120	150	100
	Accession 2	100	140	200	180	140	100	100
Glutamate dehydrogenase (NAD ⁺ -GDH)($\mu\text{mole NAD}^+ \text{ g}^{-1}$ F.W.)	Accession 1	100	100	160	260	100	80	100
	Accession 2	100	100	120	180	60	100	100
Acid phosphatase(n moles of PNP released $\text{min}^{-1} \text{ g}^{-1}$ F.W.)	Accession 1	508	508	434	579	650	675	711
	Accession 2	394	394	394	431	468	662	712
Alkaline phosphatase(n moles of PNP released $\text{min}^{-1} \text{ g}^{-1}$ F.W.)	Accession 1	468	468	394	434	468	662	712
	Accession 2	418	418	431	418	468	529	712
Wall-bound invertase(μg of reducing sugar released $\text{min}^{-1} \text{ g}^{-1}$ F.W.)	pH 5	3.05	3.05	8.76	12.8	0.876	0.292	0.292
	pH 7	2.3	2.3	2.00	1.75	0.876	0.292	0.292
Wall-bound invertase(μg of reducing sugar released $\text{min}^{-1} \text{ g}^{-1}$ F.W.)	pH 5	3.000	3.504	4.080	3.504	0.379	0.292	0.292
	pH 7.5	2.000	2.300	1.750	0.876	0.876	0.292	0.292
Extracellular invertase(μg of reducing sugar released $\text{min}^{-1} \text{ g}^{-1}$ F.W.)	pH 5	1.460	1.460	2.920	1.168	0.584	0.000	0.000
	pH 7.5	0.876	0.876	5.548	3.500	2.920	0.584	0.000
Extracellular invertase(μg of reducing sugar released $\text{min}^{-1} \text{ g}^{-1}$ F.W.)	pH 5	2.336	2.336	3.050	0.876	0.292	0.000	0.000
	pH 7.5	2.62	2.62	3.504	3.050	0.292	0.000	0.000
α -Amylase(μg maltose released $\text{min}^{-1} \text{ g}^{-1}$ F.W.)	Accession1	30	30	32	36	38	42	46
	Accession2	28	28	30	34	36	38	40
Peroxidase(Units $\text{ml}^{-1} \text{ min}^{-1}$)	Accession1	400	480	720	360	600	840	780
	Accession2	300	360	400	400	600	450	660

Mean \pm SE of 3 observations is not indicated due to the large amount of data

Nitrate uptake system in a plant must be versatile and robust, because, plants need to transport adequate amount of nitrate to satisfy the demand in the face of external nitrate concentration that can vary by five orders of magnitude (Crawford, 1995). Nitrate supplement in the medium must be converted into NH_4^+ in plants before the nitrogen can enter amino acids and other nitrogen compounds. Nitrate reductase has been studied intensively because its activity often controls protein synthesis rate in plants absorbing NO_3^- as a major nitrogen-source (Srivastava, 1980; Naik *et al*, 1982). Genes for this have been cloned from several plants and mutants, and transgenic lines are available too (Lam *et al*, 1996; Lochab *et al*, 2007). Results of the present investigation clearly suggest that there is a synergy that operates among enzymes for nitrate and ammonia assimilation when nitrate concentrations in the

medium are high ($\sim 30\text{mM}$ in MS medium). This induces production of nitrate reductase, and subsequently GS, as nitrate is converted into ammonia.

Activity of nitrate reductase persists continuously even after cultures enter the stationary phase; whereas, production of GS is directly or indirectly dependent on NR activity. From the results above, it can be concluded that decrease in GS activity when cultures enter the stationary phase may be attributed to decrease in the activity of nitrite reductase; also, this could be due to exhaustion of sucrose in the medium. Results of the present investigation are supported (in other plant species) by Philippe Lenee & Yves Chupeau (1989) and Suzuki *et al* (1987).

In the present study, lower levels of phosphatases during the initial culture-period may be because of the high

inorganic-phosphate levels in the medium. Pronounced increases in the activity of phosphatases before manifestation of a visible morphogenic event (observed to happen prominently on Day 20), i.e., on Day 15 in both the accessions, suggests that these enzymes may have a role in biochemical degradation of plasmodesmata. Such degradation may facilitate penetration of roots/ elongation of shoots (Naidu & Kavi Kishor, 1995; Kumar, 1998). Together with this, peroxidase activity was found to peak on Day 20 in Accession 2, and Day 25 in Accession 1, with gradual and progressive increase witnessed from Day 0. Peroxidases are a large group of enzymes involved in a number of biological processes such as lignification (Lagrimini *et al*, 1997a), cross-linking of cell wall proteins (Bradley *et al*, 1992) and auxin catabolism (Lagrimini *et al*, 1997b). Perhaps, an increase in enzyme-level indicates a role of these enzymes in tissue proliferation and differentiation. On examining involvement of acid phosphatases in initiation and formation of adventitious roots in *Impatiens* spp., Malik and Kumari (1977) attributed these roles to the enzymes. It was speculated that glycosidases may cleave wall-linkages and facilitate growth.

Increased activity of hydrolytic enzymes seen in the present investigation indicates that different compounds degrade in tissues, and this is concurrent with a high synthetic activity occurring during organogenesis. Results of the present investigation are supported by previous findings of Brown & Thorpe (1980), Kavi Kishor & Mehta (1988), Naidu & Kavi Kishor (1995), and Kumar (1998), in other plant species.

CONCLUSION

With a view to confirm whether nutrients or, growth regulators added to the medium and conditions like of source light and temperature at which cultures were incubated, affected growth of plants *in vitro*, biochemical studies were undertaken. In higher plants, nitrate-assimilating and hydrolytic enzymes are regulated by light, hormones, sugars, and, carbon and nitrogen metabolites. Results of the present study confirm active growth of explants revealed by the activity of nitrate assimilating enzymes and hydrolytic enzymes (excepting GDH, the activity of which decreased, as, it is a stress induced enzyme). Thus, it may be concluded that medium composition, growth regulator combination and culture incubation conditions are cited for optimal growth *in vitro* in both the accessions of *Mucuna pruriens* for plant regeneration.

REFERENCES

- Altaf Ahmad and Abdin, M.Z. 1999. NADH: nitrate reductase and NAD(P)H: nitrate reductase activities in mustard seedlings. *Pl. Sci.*, **143**:1-8
- Angosto, T., Gonzalez, F. and Matilla, A. 1988. Partial purification and some biochemical properties of acid phosphatase in germinating chick pea (*Cicer arietinum* L.) seeds. *Physiol. Pl.*, **74**:715-719
- Bradley, D., Kjellborn, P. and Lamb, C. 1992. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. *Cell.*, **70**:21-30
- Brown, D.C.W. and Thorpe, T.A. 1980. Adenosine phosphate and nicotinamide adenine dinucleotide pool sizes during shoot initiation in tobacco callus. *Pl. Physiol.*, **65**:587-590
- Campbell, W.H. and Smarrelli, Jr. J. 1978. Purification and kinetics of higher plant nitrate reductase. *Pl. Physiol.*, **61**:611-616
- Christopher, T., Prolaram, B. and Subhash, K. 1991. Differential *in vitro* morphogenetic response in hypocotyl segments of *Capsicum annum*. *Indian J. Expt'l. Biol.*, **29**:68-69
- Kavi Kishor, P.B. and Mehta, A.R. 1988. Changes in some enzyme activities during growth and organogenesis in dark-grown tobacco callus cultures. *Pl. Cell Physiol.*, **29**:255-259
- Kumar, V. 1998. Biochemical and *in vitro* plantlet regeneration studies in fig (*Ficus carica* L. cv. Gular). M. Phil. dissertation submitted to Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India
- Lagrimini, L.M., Gingas, V., Finger, F., Rothstein, S. and Liu, T-T.Y. 1997a. Characterization of antisense transformed plants deficient in the tobacco anionic peroxidase. *Pl. Physiol.*, **114**:1187-1196
- Lagrimini, L.M., Joly, R.J., Dunlap, J.R., and Liu, T-T.Y. 1997b. The consequence of peroxidase over-expression in transgenic plants on root growth and development. *Pl. Mol. Biol.*, **33**:887-895
- Lam, H.M., Coshigano, K., Oliveira, I., Melo-Oliveira, R. and Coruzzi, G. 1996. The molecular genetics of nitrogen assimilation into amino acids in higher plants. *Pl. Mol. Biol.*, **47**:569-593
- Lochab, S., Pathak, R.R. and Raghuram, N. 2007. Molecular approaches for enhancement of nitrogen use efficiency in plants. In: *Agricultural Nitrogen use & its Environmental Implications* (Eds. Abrol, Y.P.,

- Raghuram, N. and Sachdev, M.S.). IK International, Delhi, India, pp. 327-350
- Lorenza M. Bellani, Massimo Guarnieri and Anna Scialabba. 2002. Differences in the activity and distribution of peroxidases from three different portions of germinating *Brassica oleracea* seeds. *Physiol. Pl.*, **114**:102-108
- Malik, C.P. and Kumari, U. 1977. Histochemical studies on the localization of metabolic reserves and enzymes during the initiation and formation of adventitious roots in *Impatiens balsamina* L. *New Bot.*, **4**:113-115
- Miller, W.B. and Ranwala, A.P. 1994. Characterization and localization of three soluble forms of invertase from *Lilium longiflorum* flower buds. *Physiol. Pl.*, **92**:247-253
- Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Pl.*, **15**:473-497
- Naidu, K.R. and Kavi Kishor, P.B. 1995. Activities of hydrolytic enzymes in callus cultures of tobacco during organogenesis. *J. Biosci.*, **20**:629-636
- Naik, M.S., Abrol, Y.P., Nair, T.V.R., and Ramaras, C.S. 1982. Nitrate assimilation – its regulation and relationship to reduced nitrogen in higher plants. *Phytochem.*, **21**:495-504
- Narender Singh, Kuldeep Yadav, Suman Kumari, and Renu. 2011. Metabolic changes during differentiation in callus cultures of *Stevia rebaudiana* (Bertoni). *J. Phytol.*, **3**:63-67
- Crawford, N.M. 1995. Nitrate: Nutrient and signal for plant growth. *Pl. Cell*, **7**:859-868
- Oskar J. Sanchez, Alberto Pan, Gregorio Nicolas and Emilia Labrador. 1989. Relation of cell wall peroxidase activity with growth in epicotyls of *Cicer arietinum*. *Physiol. Pl.*, **75**:275-279
- Philippe Lenee and Yves Chupeau. 1989. Development of nitrogen assimilating enzymes during growth of cells derived from protoplasts of sunflower and tobacco. *Pl. Sci.*, **59**:109-117
- Riley, P.A. 1997. Melanin. *Int'l. J. Biochem. Cell Biol.*, **29**:1235-1239
- Sadasivam, S. and Manickam, A. 2008. Biochemical Methods. New Age International (P) Limited Publishers, New Delhi, India, pp. 137-139
- Singh, S.R., Singh, R. and Dhawan, A.K. 2009. Biochemical changes related to shoot differentiation in callus cultures of *Tylophora indica* Wight and Arn. *J. Indian Bot. Soc.*, **88**:49-53
- Srivastava, H.S. 1980. Regulation of nitrate reductase activity in higher plants. *Phytochem.*, **19**:725-733
- Sujatha, M., Sivaraj, N. and Satya Prasad, M. 2000. Biochemical and histological changes during *in vitro* organogenesis in *Jatropha integerrima*. *Biologia Plant.*, **40**:167-171
- Suzuki, A., Audet, C. and Oaks, A. 1987. Influence of light on the ferredoxin-dependent glutamate synthase in maize leaves. *Pl. Physiol.*, **95**:384-389
- Yolanda Cuadrado, Hilario Guerra, Ana Belen Martin, Piedad Gallego, Oscar Hita, Ana Dorado and Nieves Villalobos. 2001. Differences in invertase activity in embryogenic and non-embryogenic calli from *Medicago arborea*. *Pl. Cell Tiss. Org. Cult.*, **67**: 145-151

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