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INFLUENCE OF EXOGENOUS POLYAMINES ON DIRECT SOMATIC EMBRYOGENESIS OF *SCILLA INDICA* AND *URGINEA INDICA*: A COMPARATIVE STUDY

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ABSTRACT

The effect of polyamines (putrescine, spermidine, and spermine) was examined for somatic embryogenesis of two bulbus medicinal plants *Scilla indica* and *Urginea indica* Among the three polyamines tested, Putrescine at low level (0.40 mg l^{-1}) promoted somatic embryogenesis (3.06±0.04) and at low level of Spermine (0.25 mg l^{-1}) promoted shoot growth (19.15±0.17 mm.) but the growth is not significant in *Scilla* comparing with the control on day 30. On the other hand low level of Putrescine (0.25 mg l^{-1}) promoted shoot growth significantly (14.40±1.48 mm.) in *Urginia* after 30 days. Spermidine induced S3 stage whereas spermine induced S2 stage in *Scilla*. Callusing was found in almost all the treatments in *Urginea* but not detected in *Scilla*. In both the cases root development is insignificant.

KEYWORDS: somatic embryogenesis, polyamines, *Scilla indica*, *Urginea indica*.

INTRODUCTION:

Scilla indica and *Urginea indica* are the two medicinally important bulbous plants belongs to the family Liliaceae, commonly known as Indian squill. These plants are become rare in natural habitat due it's over exploitation and habitat destruction. *Scilla indica* is one of the most important medicinal crops and is in great demand in the present time. This plant grows wildly in the forests of Madhya Pradesh, Bundelkhand, Gwalior, Bihar, Mahabaleswar and

all districts of the Tamil Nadu state, except the west coast, up to 4000 feet. Various plant parts have been used for regeneration of plants *in vitro*. Whereas *Urginea indica* found widely distributed in the Mediterranean Sea regions, at the same time it also found in the forests of western parts of West Bengal. This plant contains Proscillaridin A and scillarin A, the bufadienolides in the bulbs (Jha *et al*. 1990). Bufadienolide is well known cardiotonic, has stimulating and diuretic properties. Indian squill is beneficial in removing any obstruction to secretion or excretion by opening the natural passages or pores of the body. It promotes the removal of catarrhal matter. It helps to remove phlegm from the bronchial tubes in asthma, bronchial catarrh and in chronic bronchitis. On the other hand *Scilla indica* contains Scillarin A and B. The bulb is used as anthelmentic, cardiac stimulant, digestive, diuretic, emmenagogue and expectorant. It is also used in asthma, cough and bronchitis, paralytic attacks, ailments of the heart, calculus affections, and rheumatism and skin diseases (Rao and Rangaswami 1967). Both the plants produced somatic embryos profusely under *in vitro* condition. In our present study the exogenous influence of polyamines are assessed among the two plants and their growth rate is compared.

METHODOLOGY:

The plants were collected from different forest areas of West Midnapore and Ayodhya Hills of Purulia district, West Bengal. Freshly collected plants were always used for the study. For *in vitro* culture, half strength modified Murashige and Skoog's (MS) basal medium (Murashige and Skoog 1962) was used. The pH of the medium was adjusted to 5.6-5.8 prior to autoclaving. For solidification of the medium 0.8% (w/v) agar (Merck) powder was added and mixed in the medium by gentle boiling. The media were poured into 250 ml conical flask (Borosil) (100 ml/flask). Finally, the flask were plugged with non-absorbent cotton, wrapped with brown paper and autoclaved at 1.02 kg /cm2 (121ºC)

for 20 min. Leaves of *Scilla indica* and scale leaves of *Urginea indica* were washed with liquid soap for 5 min and surface sterilized with 90% ethanol for 2 min followed by treatment with 0.1% w/v mercuric chloride solution for 5-6 min and 7% bleaching solution for 15 min and finally washed repeatedly with sterile distilled water. Explants (1cm2 pieces of scale leaf or young leaves) were inoculated into the conical flasks containing 100 ml MS medium (half strength), with various concentrations of Putrescine (diamine), Spermine (tetramine) and Spermidine (triamine) (0.2, 0.4 and 1.0 mg l- 1.) and the media were solidified with 0.8% agar. 30 replicates were used for each treatment. Cultures were incubated in 37.5 µmol /sqm /sec light intensity at 22± 2º C under 10h of photoperiod for 60 days and responses were recorded.

OBSERVATION:

In the preliminary experiment, various levels of auxins and cytokinins were used to examine their effect on somatic embryo regeneration. After thirty days of inoculation, about 70-80% healthy cultures were established (Table 1.1 to 1.6). In *Scilla indica*, white embryo like structures were formed after 7 days from the cut ends of the explants along with white friable, loosely attached callus mass induced from the leaf segments directly. The white embryo- like structures could be easily detached from the tissue mass. From the histological study, it was revealed that these globular white structures are bipolar in nature and ensheathed with one cell layer thick covering which is a characteristic feature of somatic embryos in general (Fig.4.1 I). Two small leaf primordial covering the meristematic shoot apex became visible in the advanced stages of development of such somatic embryos. At this stage distinct vascular connection is established (Fig.4.1 I). After 15 days of culture, S2 stage (somatic embryos with emerging leaves) (Fig.4.1B and C) was achieved, which subsequently entered into the S3 stage (somatic embryos with two leaves and root) after 6 to 8 weeks (Fig.4.1D).

In *Urginea indica*, scale leaves of the underground fleshy bulbs are used as explant for somatic embryogenesis and callus formation. It is interesting to note that somatic embryos could not be developed directly on freshly inoculated segments of scale leaves, rather the explants directly developed creamy white friable callus in all the treatments. Some of the treatments developed green nodular compact callus. The somatic embryos are indirectly originated on the callus mass. These somatic embryos also follow three developmental stages as has been detected in *Scilla indica*.

RESULT:

In *Scilla indica* response was nearly 100% in all the treatments of polyamines. Among the three polyamines tested, putrescine at low level (0.40 mg l-1) promoted somatic embryogenesis (3.06±0.04) and at high level (1.00 mg l-1) promoted shoot growth (19.00±0.18 mm.). Callusing was not detected in any of the polyamine treatments. Root induction is also insignificant. Spermidine induced S3 stage whereas spermine induced S2 stage (Fig.1).

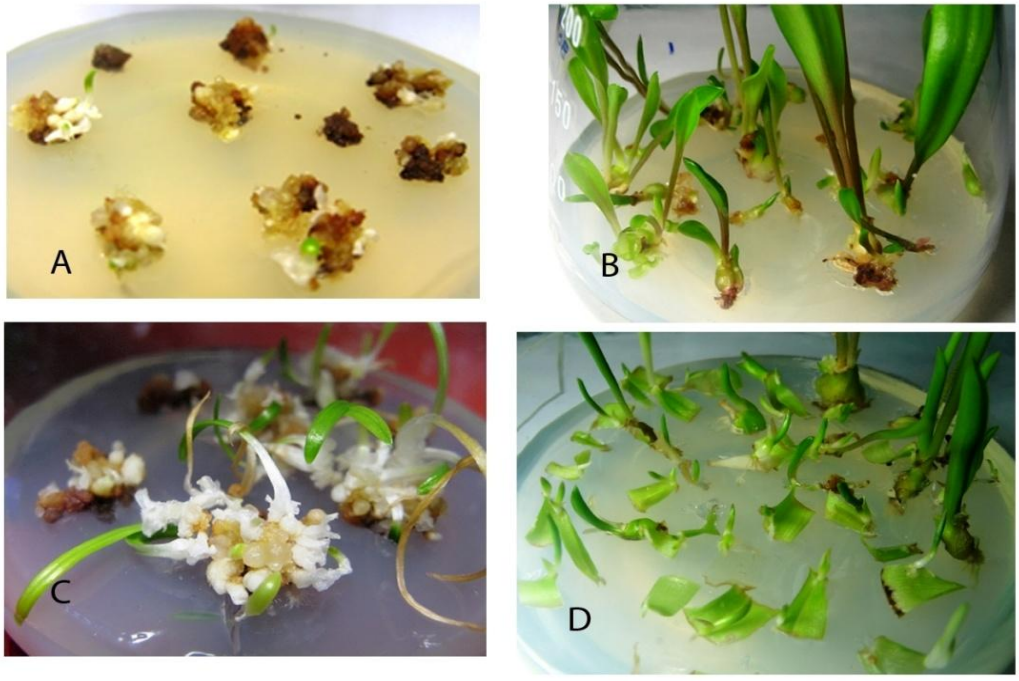


Figure-1: A. Effect of Spermidine on *Urginea*, B. Effect of Spermidine on *Scilla;* C. somatic embryo formation in *Urginea*: effect of Spermine; D. Effect of Putrescine on *Scilla* .

Table-1: Effect of the polyamines on direct somatic embryogenesis from leaf of *Scilla indica*.

Treatments	Percentage of Response	Avg number of Somatic Embryos	Avg number of roots	Percentage of Callus	Frequency of Stage1	Frequency of Stage2	Frequency of Stage3	Avg shoot length (mm.)
CONTROL	100	2.11 ± 0.91^{ab}	2.11 ± 1.04^{a}	0	$15.00\pm1.03^{a-e}$	$25.00\pm0.41^{a-f}$	$60.00\pm2.09^{a-e}$	$22.13\pm1.03^{a-d}$
SD1	100	$1.06\pm0.03^{a-h}$	$0.82\pm0.08^{a-f}$	0	$0.04\pm0.02^{a-i}$	$22.22\pm4.21^{a-g}$	77.78 ± 0.12^{a}	16.18 ± 0.02^{abc}
SD2	100	$1.33\pm0.05^{a-f}$	1.44 ± 0.12^{abc}	0	20.83 ± 0.09^{ab}	$4.17\pm1.21^{a-j}$	75.00 ± 0.18^{abc}	$11.67\pm0.19^{a-d}$
SD3	100	$1.20\pm0.18^{a-h}$	$1.13\pm0.05^{a-e}$	0	$10.42\pm2.07^{a-g}$	$13.19\pm0.07^{a-i}$	76.39 ± 1.09^{ab}	$13.92\pm3.29^{a-d}$
P1	100	$1.26\pm0.13^{a-h}$	$0.94\pm0.03^{a-f}$	0	$11.79\pm0.50^{a-f}$	$25.43\pm0.04^{a-e}$	$62.79\pm0.04^{a-f}$	$3.79\pm0.92^{a-d}$
P2	100	3.06 ± 0.04^{a}	$1.63\pm0.12^{a-d}$	0	18.37 ± 0.15^{abc}	53.06 ± 1.29^{abc}	$28.57\pm1.31^{a-i}$	$17.91\pm0.27^{a-d}$
P3	100	$1.49\pm.19^{a-e}$	1.70 ± 0.32^{ab}	0	$17.48\pm0.19^{a-d}$	$12.50\pm0.28^{a-h}$	$68.24\pm4.26^{a-d}$	19.00 ± 0.18^{a}
SP1	100	$1.25\pm0.06^{a-g}$	$0.40\pm0.07^{a-g}$	0	0.00	76.00 ± 2.07^{ab}	$28.00\pm1.02^{a-h}$	$19.15\pm0.17^{a-d}$
SP2	100	$1.59\pm0.23^{a-d}$	$0.96\pm0.03^{a-e}$	0	29.63 ± 1.03^{a}	$33.33\pm0.78^{a-d}$	$37.04\pm0.29^{a-g}$	16.38 ± 0.07^{ab}
SP3	100	1.53 ± 0.42^{abc}	$0.40\pm0.06^{a-g}$	0	$04.35\pm0.07^{a-h}$	78.26 ± 0.58^{a}	$13.04\pm0.46^{a-j}$	$10.27\pm0.28^{a-d}$

In *Urginea indica* necrosis was found in almost all the treatments which ranged from 10-20% (Table. 2.5). Spermine at higher concentration (1.00 mg l-1) promoted somatic embryogenesis (9.5±2.15) and callusing (100%). On the contrary, putrescine at lower concentration (0.2 mg l-1) promoted shoot growth (14.40±1.48 mm.). All the three stages are formed in almost all the treatments (Fig.1A and C).

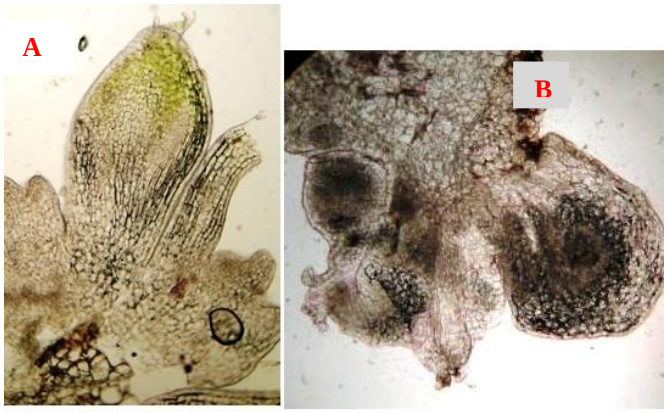
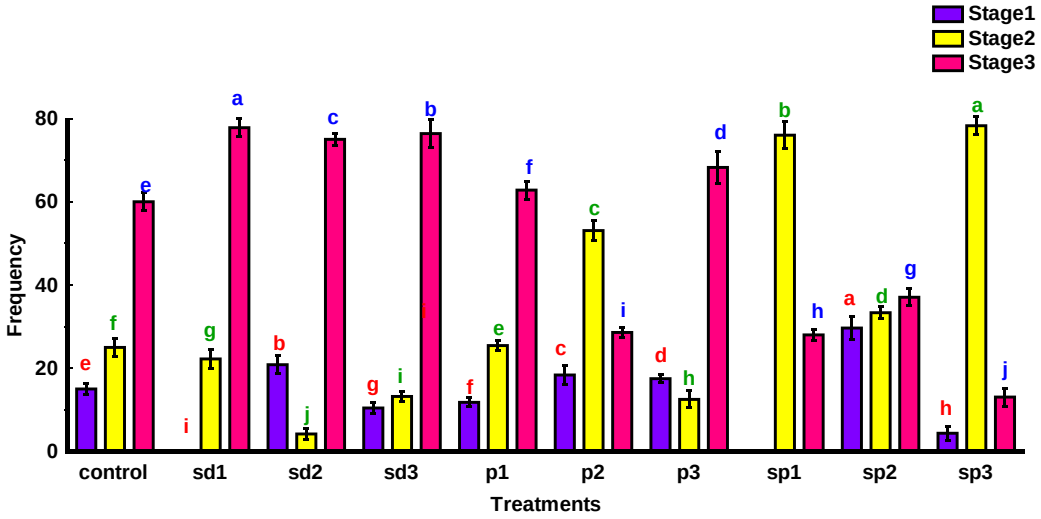


Figure-2: A. Histology of *Scilla indica* and B. Histology of *Urginea indica*.

Table- 2: Effect of the combinations of Polyamines on direct somatic embryogenesis from scale leaf of *Urginea indica.*

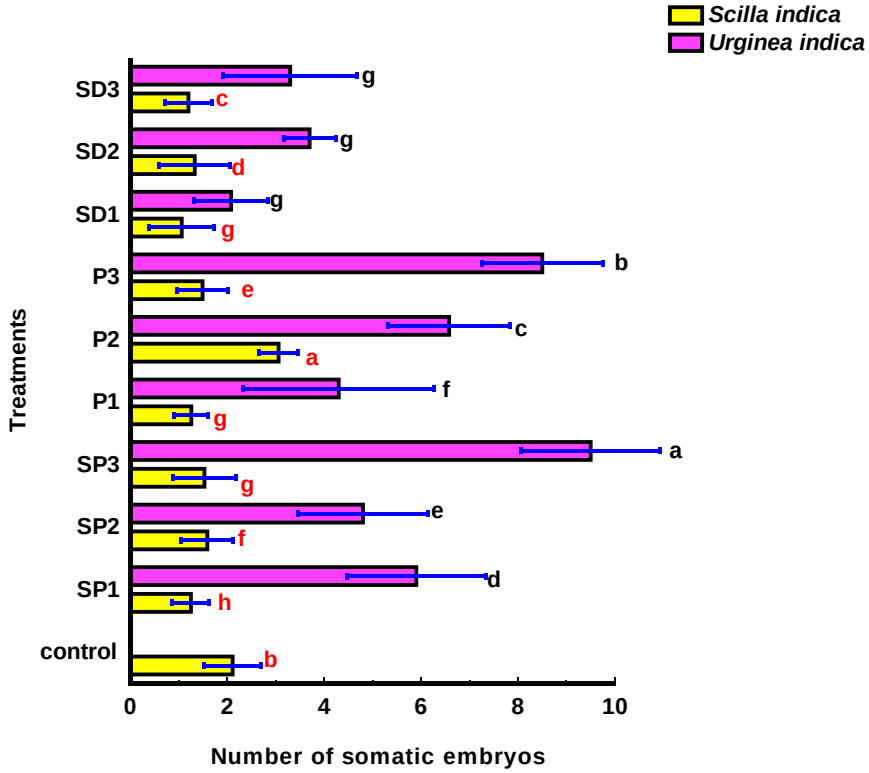
Treatments	Number of Somatic Embryos	Percentage of Callus	Length of Somatic Embryos	Frequency of S1	Frequency of S2	Frequency of S3	Percentage of response	Percentage of necrosis
SP1	$5.90\pm1.45^{a-d}$	100.00 ± 0^{a}	10.10 ± 1.22^{abc}	0.63 ± 0.05^{ab}	$0.14\pm0.001^{a-f}$	0.04 ± 0.002^{abc}	100.00 ± 0^{a}	0.00±0
SP2	$4.80\pm0.96^{a-e}$	$50.00\pm1.46^{a-f}$	$9.00\pm1.05^{a-d}$	$0.54\pm0.09^{a-d}$	$0.33\pm0.012^{a-e}$	0.13 ± 0.001^{a}	100.00 ± 0^{a}	0.00±0
SP3	9.50 ± 2.15^{a}	100.00 ± 0^{a}	13.30 ± 2.75^{ab}	0.69 ± 0.011^{a}	$0.27\pm0.009^{a-f}$	0.04 ± 0.0012^{abc}	100.00 ± 0^{a}	0.00±0
P1	$4.30\pm1.12^{a-f}$	$40.00\pm2.18^{a-g}$	14.40 ± 1.48^{a}	$0.23\pm0.038^{a-f}$	0.43 ± 0.003^{a}	0.12 ± 0.003^{ab}	80.00 ± 2.14^{abc}	20.00 ± 2.35^{a}
P2	6.58 ± 1.04^{abc}	68.18 ± 1.25^{abc}	$7.82\pm0.46^{a-f}$	$0.48\pm0.01^{a-e}$	0.38 ± 0.014^{abc}	0.04 ± 0.0013^{abc}	90.00 ± 1.26^{ab}	10.00 ± 1.08^{ab}
P3	8.50 ± 2.67^{ab}	80.00 ± 0.98^{ab}	$8.10\pm2.38^{a-e}$	$0.39\pm0.031^{a-f}$	$0.37\pm0.011^{a-d}$	0.04 ± 0.003^{abc}	80.00 ± 2.33^{abc}	20.00 ± 1.22^{a}
SD1	$2.08\pm0.96^{a-g}$	$66.67\pm2.04^{a-d}$	$5.42\pm1.54^{a-h}$	$0.32\pm0.002^{a-f}$	0.39 ± 0.051^{ab}	0.13 ± 0.007^{a}	90.00 ± 2.96^{ab}	10.00 ± 1.62^{ab}
SD2	$3.70\pm0.16^{a-g}$	80.00 ± 2.16^{ab}	$2.10\pm0.32^{a-i}$	0.51 ± 0.017^{abc}	$0.26\pm0.032^{a-f}$	0.02 ± 0.004^{abc}	80.00 ± 4.01^{abc}	20.00 ± 2.53^{a}
SD3	$3.30\pm0.28^{a-g}$	$60.00\pm0.39^{a-e}$	$6.60\pm1.88^{a-g}$	$0.38\pm0.041^{a-f}$	$0.29\pm0.002^{a-f}$	0.02 ± 0.001^{abc}	80.00 ± 2.99^{abc}	20.00 ± 2.38^{ab}

Figure 1.1: Effect of polyamines on the direct somatic embryogenesis in *Scilla indica*



***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

Figure 1.2: Effect of Polyamines on the somatic embryogenesis, P1: 0.2 mgl-1, P2:0.4 mg l-1, P3:1.0 mg l-1, SP1: 0.2 mg l-1, SP2:0.4 mg l-1, SP3:1.0 mg l-1; SD1: 0.2 mg l-1, SD2:0.4 mg l-1, SD3:1.0 mg l-1.



***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

It was found that number of somatic embryo formation is greater in *Urginea indica* than *Scilla indica*. Spermine 1.0 mgl^{-1} induces the maximum numbers of somatic embryos in *Urginea indica*.Whereas in *Scilla indica* putrescine at 0.5 mg l^{-1} shows the significant increase in the number of somatic embryos formation.

Discussion on the effect of polyamines on somatic embryogenesis:

Polyamines (PAs) are aliphatic nitrogen-containing compounds of low molecular weight and polycationic in nature. PA levels in plants depend on their biosynthesis, conjugation, degradation, transport, and conversion to other metabolites, such as alkaloids (Tiburcio *et al.* 2003). Biogenic amines putrescine, spermidine and spermine are ubiquitous in nature and have interested researchers because those are essential for cell division and viability (Handa and Mattoo 2010). Distribution of biogenic amines—the diamine putrescine (Put), triamine spermidine (Spd), and tetraamine spermine (Spm)—differ between species with Put and Spd being particularly abundant and Spm the least abundant in plant cells. These amines are important for cell viability and their intracellular levels are tightly regulated, which have made it difficult to characterize individual effects of Put, Spd and Spm on plant

growth and developmental processes (Mattoo *et al.* 2009). When the growth regulator 2iPA was omitted from the medium of *Syringa*, putrescine could partly compensate for the 2iPA-stimulated stem elongation. In *Acacia*, an interaction between the IBA and putrescine was established. Optimum concentration of putrescine in the media was in the range 0.1–1.0 µM (Scholten 1998). These reports resemble our present observations where 0.4 mg l^{-1} and 1.0 mg l^{-1} putrescine induced significant somatic embryogenesis and their development respectively in *Scilla indica* (Table. 1.12). According to Scholten (1998) spermine had a negative effect. Contradicting this observation spermine at 0.4-1.0 mg l^{-1} level showed the significant response in somatic embryogenesis and callus induction in *Urginea indica*. Putrescine influences somatic embryogenesis in the commercially important Indian pine *Pinus gerardiana*. Mature zygotic embryos produced the highest percentage of embryogenic tissue on half strength MS basal medium in response to putrescine (Malabadi and Nataraja 2007).

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