International Journal of Engineering, Science and Mathematics
Vol. 9 Issue 05, May 2020
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

IN-VITRO STUDY OF SCILLA INDICA (AN INDIAN MEDICINAL PLANT): THE EFFECT OF DIFFERENT PGRS ON DIRECT SOMATIC EMBRYOGENESIS

Dr. Poulami Jana
Sponsored Teachers' Training College
Deshbandhu Road, Purulia, West Bengal

ABSTRACT:

Leaf explants of *Scilla indica* were cultured in vitro on modified MS medium. These explants initiated somatic embryos, which provided a sterile source of secondary somatic embryos' explants. The secondary explants responded similarly to various combinations of plant growth regulators. The number of somatic embryos initiated was increased by the addition of cytokinins like IBA. BAP, 2iPA or thidiazuron (TDZ) in combination with naphthalene acetic acid (NAA). 2iPA is the most effective cytokinin in combination with NAA for direct somatic embryogenesis and their subsequent growth in *Scilla indica*. Separate root induction medium was not required because roots are generated in the same shoot induction medium itself. The plantlets were successfully acclimatized in shade house.

KEYWORDS*:* Direct Somatic embryogenesis, leaf explants, *Silla indica*

INTRODUCTION: Plants play a vital role not only in our economy but also in our health care, improvement of the quality of life, and in many cultural aspects of our daily life. Human society has been using lots of plants as drug in curing diseases and ailments since time immemorial. Medicinal plants are growing in a wide range of habitat like forests, seashores, mountains and plains. Medicinal plants include a large number of annual and

perennial species. They also include wild and cultivated plants. The knowledge related to medicinal plants is being lost day by day due to lack of awareness and deforestation. As a result, many valuable medicinal herbs are gradually becoming rare and precious information is getting lost. The aim of this study was to develop a rapid and economical method for the micropropagation of *Scilla indica* following direct pathways of plant regulation using leaf explants. Conventional propagation of red squill from bulb scales is slow and time consuming method. Only limited number of propagules can be obtained if the bulb is cut into wedge sections (Verbiscar *et al.* 1986). *In vitro* propagation is an alternative method for speedy propagation of healthy plantlets. In order to reduce exploitation from the natural habitat, standard protocol for mass clonal propagation has been developed. In order to achieve best response under *in vitro* condition, different plant growth regulators are used in various combinations and concentrations. For the study somatic embryogenesis pathway is followed.

MATERIALS AND METHODS:

Preparation of plant material for initiation of culture:

The explants used in the study were leaf segments of *Scilla indica* (Roxb.) Baker. Leaves of *Scilla 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.

Culture media and environment:

Explants were inoculated into culture tubes, each containing 20 ml, or conical flask containing 100 ml of MS basal medium (Murashige and Skoog 1962) solidified with 0.8% (w/v) agar and supplemented with various concentrations and combinations of plant growth regulators (BAP, Kin, 2,4- D, NAA, IBA, 2-iPA, TDZ). Three conical flasks each containing 10 replicates were used per treatment. All these experimental procedures

including sterilization and inoculation of explant were performed aseptically in front of a Laminar Air Flow Cabinet (Klenzaids, India).

Inoculation of explants:

For Direct Somatic embryogenesis healthy leaves of *Scilla indica* were excised into 1cm^2 pieces and were inoculated in the MS basal media (half strength) with various concentrations of NAA or IBA (0.25 -1.00 mg l^{-1}), combined with various concentrations of BAP, Kin, TDZ, 2-iPA (0.25 -4.00 mg l^{-1}), 3% sucrose and finally solidified with 0.8% agar powder. These PGRs were also used singly. Cultures were incubated in 37.5 µmol /sqm /sec light intensity at 22 ± 2º C under 10h of photoperiod for 60 days and growth responses were recorded.

Transfer of *in vitro* plants to the field:

The *in vitro* regenerated plants with well developed root systems were washed carefully under running tap water to completely remove the agar adhered to the roots and subsequently the plantlets were kept in half strength liquid MS medium for 3-5 days in septic condition keeping the cotton plug open .

When vigorous growth of the plantlets was resumed those plants were transferred to plastic cups containing sterile sand- soil mixture (1:1) with adequate water and little amount of MS macro salt solution. These plants were then transferred to the Net house and incubated in shady condition for further growth.

Surviving plants were finally transplanted to the earthen pots containing soil with fertilizer and water. The process of transfer and gradual acclimatization to the natural conditions were carefully monitored.

Histological studies:

Histological studies of the tissues bearing somatic embryos and the multiple shoot buds were carried out to confirm the pattern of morphogenesis. The serial sections of the tissue were made by free hand sectioning, stained with dilute solution of 1% safranin and observed under Olympus CH-30 research microscope and finally suitable region were photographed using 4X objective lens.

RESULTS AND DISCUSSION:

The analysis of the data revealed that the treatment with NAA (1.0 mgl^{-1}) and 2iPA (0.5 mg l^{-1}) were most effective in indirect induction of somatic embryos. These somatic

embryos pass through three developmental stages; viz. white dentate stage (Stage 1), green leaf primordial stage (stage 2), and rooted stage (stage 3) (Fig. 1A, B and D). The stages appeared as follows:

1. White dentate stage appeared after 5-7 days of inoculation;
2. Somatic embryos with two leaf primordia after 10-15 days;
3. Rooted plantlet stage after 4 weeks of inoculation.

Effect of NAA-BAP on *in vitro* growth and development:

All the treatments of NAA and BAP showed almost 100% responses. Lower concentrations of auxin and cytokinin (0.25 mg l^{-1} each of NAA and BAP) induced significant root growth (8.7±2.4) while higher concentrations of NAA and BAP (1.0 mg l^{-1} NAA and 4.00 mg l^{-1} BAP) induced maximum somatic embryogenesis (16.80±1.82). However, in NAA (1.0 mg l^{-1}) and BAP (0.50 mg l^{-1}) shoot development has been achieved (38.89±2.9 mm.) (Table: 1.1). Among the three stages of somatic embryo, stage 2 was found maximum in number in all the treatments (Fig.1B).

Effect of NAA-Kinetin on *in vitro* growth and development:

Responses were 100% in all the treatments with NAA and Kin. Lower concentrations of NAA (0.5 mg l^{-1}) and Kin (1.00 mg l^{-1}) promoted shoot (67.37±1.6 mm.) and root development (10.13±2.5). Lower concentration of NAA (1.0 mg l^{-1}) and higher concentration of Kin (4.00 mg l^{-1}) promoted significant somatic embryogenesis (10.41±2.8) (Table. 1.2). Of the three stages, maximum number of somatic embryos were restricted at stage 2 in all the treatments (Fig. 1E).

Effect of NAA-TDZ on *in vitro* growth and development:

In the experiment with NAA and TDZ, Lower concentration of auxin and higher concentration of cytokinin (0.25 mgl^{-1}NAA and 2.0 mgl^{-1} TDZ) induced somatic embryogenesis (3.30±2.15). Higher concentration of auxin and lower concentration of

cytokinin (1.0 mg l-^{1}NAA and 0.5 mg l^{-1} TDZ) promoted shoot growth significantly (2.43±1.17 mm.) where root development also increased (23.50±2.09). Low level of NAA (0.25 mg l^{-1}) and high level of TDZ (2.00 -4.00 mg l^{-1}) induced callusing. Necrosis of the tissues occurs in some of the treatments. (Table.1.4). The globular staged embryos are formed at the lower concentrations of NAA (0.25 mg l^{-1}) and TDZ (0.25 -0.50 mg l^{-1}). Occurrence of stage 2 of embryogenesis is found to be the maximum in number in all the treatments (Fig. 1F).

Fig. 1: Effect of growth regulators on direct somatic embryogenesis of *Scilla indica*. A. Somatic embryo stage 1 in IBA and BAP; B. Somatic embryo stage 2 in NAA and BAP; C. Somatic embryo stage 2 in IBA and Kin; D. Somatic embryo stage 3 in NAA and BAP; E. Somatic embryo stage 3 in NAA and Kin;

F. Somatic embryo stage 3 in NAA and TDZ; G. Somatic embryo stage 3 in NAA and 2iPA; H. Secondary Somatic embryo stage 2 ; I. Histology of somatic embryo.

Effect of NAA-2iPA on *in vitro* growth and development:

Lower concentration of auxin and higher concentration of cytokinin (0.25 mg l^{-1} NAA and 1.00 mg l^{-1} 2-iPA) promoted shoot development significantly (68.98±1.5 mm.) and (0.5 mg l^{-1} NAA and 2.00 mg l^{-1} 2-iPA) exhibited root growth (11.20±1.8) (Table-1.3). Higher concentration of auxin and lower concentration of cytokinin (1.0 mg l^{-1} NAA and 0.5 mg l^{-1} 2-iPA) induced somatic embryogenesis (19.94±8.77). In all the treatments, maximum number of somatic embryos achieved stage 3 (Fig. 1G).

Effect of IBA-BAP on *in vitro* growth and development:

Higher concentration of auxin and cytokinin (1.0 mg l^{-1} IBA and 2.00 mg l^{-1} BAP) promoted somatic embryogenesis (16.53±3.28). 1.0 mg l^{-1} IBA and 0.25 mg l^{-1} BAP induced both root (4.31±1.04) and shoot (7.47±2.03 mm.) development. Higher concentrations of IBA and BAP showed nearly 100% responses whereas lower concentration of the PGR combinations failed to exhibit 100% response. Callusing occurred only at lower combinations of IBA (0.25 mg l^{-1}) and BAP (0.25- 0.50 mg l^{-1}) (Table.1.5). In all the treatments somatic embryos are restricted to S1 stage (Fig.1A).

Effect of IBA-Kinetin on *in vitro* growth and development:

Lower concentrations (0.25 mg l^{-1} IBA and 0.5 mg l^{-1} Kin) promoted characteristic somatic embryogenesis (7.33±2.39), root (8.65±2.39) and shoot development (27.71±1.82 mm.). IBA-Kin combinations failed to induce callus (Table. 1.6). At the lower concentrations of both the PGRs S1 was found to dominate, whereas higher levels of both the PGRs are required to attain S3 stage. In this study also nearly 100% response is found in all the treatments (Fig. 1C).

Treatments	Percentage of Response	Percentage of Callus	Avg. number of Somatic Embryos	Avg. number of roots	Avg. shoot length (mm)
N1B1	100 ± 0.00^{a}	0	9.20 ± 2.09^{a-h}	8.70 ± 2.39^{a}	32.15 ± 3.27^{abc}
N1B2	100 ± 0.00^{a}	0	8.03 ± 1.9^{a-k}	2.85 ± 1.02^{a-h}	33.13 ± 2.79^{ab}
N1B3	100 ± 0.00^{a}	0	10.00 ± 2.3^{a-d}	2.15 ± 0.38^{a-j}	20.09 ± 4.2^{a-l}
N1B4	100 ± 0.00^{a}	0	9.80 ± 1.29^{a-e}	3.50 ± 1.72^{a-f}	30.80 ± 3.09^{a-d}
N1B5	100 ± 0.00^{a}	0	12.95 ± 2.09^{ab}	0.13 ± 0.03^{a-l}	16.48 ± 2.02^{a-m}
N2B1	100 ± 0.00^{a}	0	6.73 ± 0.52^{a-l}	7.28 ± 2.38^{ab}	17.67 ± 2.93^{a-m}
N2B2	100 ± 0.00^{a}	0	6.78 ± 0.98^{a-l}	6.28 ± 1.28^{abc}	21.69 ± 2.3^{a-k}
N2B3	100 ± 0.00^{a}	0	6.71 ± 1.02^{a-l}	4.57 ± 1.67^{a-d}	22.04 ± 1.58^{a-j}
N2B4	100 ± 0.00^{a}	0	7.60 ± 0.34^{a-l}	3.45 ± 1.56^{a-g}	24.18 ± 3.5 $^{a-g}$
N2B5	100 ± 0.00^{a}	0	8.56 ± 0.37^{a-i}	0.01 ± 0.002^{a-l}	26.44 ± 2.35^{a-e}
N3B1	100 ± 0.00^{a}	82.5 ± 2.9^{a}	8.18 ± 0.28^{a-j}	3.75 ± 1.28^{a-e}	22.88 ± 4.29^{a-i}
N3B2	100 ± 0.00^{a}	55 ± 3.28^{ab}	9.38 ± 1.65^{a-g}	1.85 ± 0.83^{a-k}	38.89 ± 2.93^{a}
N3B3	100 ± 0.00^{a}	50 ± 2.89^{abc}	9.61 ± 1.29^{a-f}	2.83 ± 1.02^{a-i}	20.08 ± 3.54^{a-l}
N3B4	100 ± 0.00^{a}	0	12.28 ± 2.3^{abc}	0.05 ± 0.-003^{a-l}	25.28 ± 4.9^{a-f}
N3B5	100 ± 0.00^{a}	0	16.80 ± 1.82^{a}	0.00	24.13 ± 3.28^{a-h}

Table 1.1: Effect of the NAA and BAP on direct somatic embryogenesis from leaf of *Scilla indica.* Treatments: NAA (N1 0.25, N2 0.50 and N3 1.00 mg l^{-1}) and BAP (B1 0.25, B2 0.50, B3 1.00, B4 2.00 and B5 4.00 mg l^{-1})
***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

Treatments	Percentage of Response	Percentage of Callus	Avg. No. of Somatic embryos	Avg. Shoot length (mm)	Avg. No. of roots
N1K1	100±0.00^{a}	0	3.65±1.08^{a-o}	40.76±2.56 $^{a-l}$	4.32±1.56^{a-k}
N1K2	100±0.00^{a}	0	6.45± 1.56^{a-e}	43.276±1.98 $^{a-j}$	5.28±1.34^{a-e}
N1K3	100±0.00^{a}	0	5.87±1.05^{a-i}	47.04±1.89 $^{a-f}$	3.59±0.98^{a-l}
N1K4	100±0.00^{a}	0	5.53±0.35^{a-l}	38.356±2.9 $^{a-m}$	3.27±0.26^{a-m}
N1K5	100±0.00^{a}	0	8.67±1.76 ^{ab}	36.874±3.12 $^{a-n}$	2.74±0.95^{a-m}
N2K1	100±0.00^{a}	0	3.87±0.9^{a-n}	49.9±3.46 $^{a-e}$	5.63±1.45^{a-d}
N2K2	100±0.00^{a}	0	5.67±0.24^{a-k}	50.63±2.91 $^{a-d}$	5.17±1.98^{a-f}
N2K3	100±0.00^{a}	0	7.3±1.67 $^{a-d}$	67.37±1.58 ^{a}	10.13±2.46^{a}
N2K4	100±0.00^{a}	0	6.3±1.056^{abcdef}	53.8±4.36 ^{ab}	8±2.34^{ab}
N2K5	100±0.00^{a}	0	7.43±1.057^{abc}	36.1±1.27 $^{a-n}$	4.43±1.07^{a-j}
N3K1	100±0.00^{a}	100±0.00^{a}	6.1±1.58^{a-h}	42.1±3.24 $^{a-k}$	4.97±1.05^{a-g}
N3K2	100±0.00^{a}	0	6.17±1.24^{a-g}	43.5±1.26 $^{a-i}$	4.93±1.05^{a-h}
N3K3	100±0.00^{a}	100±0.00^{a}	5.13±1.06^{a-m}	45.33±2.76 $^{a-h}$	4.93±0.36^{a-i}
N3K4	100±0.00^{a}	0	5.73±0.46^{a-j}	52.53±3.27 ^{abc}	7.93±1.25^{abc}
N3K5	100±0.00^{a}	0	10.41±2.8 ^{a}	45.48±1.087 $^{a-g}$	3±0.079^{a-m}

Table 1.2: Effect of the NAA and Kinetin on direct somatic embryogenesis from leaf of *Scilla indica.* Treatments: NAA (N1 0.25, N2 0.50 and N3 1.00 mg l^{-1}) and Kinetin (K1 0.25, K2 0.50, K3 1.00, K4 2.00 and K5 4.00 mg l^{-1}).
***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

Treatments	Avg. No. of somatic embryos	Avg. No. of roots	Avg. Shoot length (mm)
N0P0	1.25± 0.45^{a-j}	0	1.06 ± 1.08^{a-j}
N1P1	9.89±0.74^{a-j}	9.87 ± 0.97^{a-g}	66.17 ± 2.03^{a-d}
N1P2	8.46±0.75^{a-j}	8.83± 0.87^{a-j}	61.93 ± 1.79^{a-g}
N1P3	8.89±0.96^{a-j}	8.83±1.07^{a-j}	68.98 ± 1.47^{a}
N1P4	7.93±1.02^{a-j}	8.7± 1.04^{a-k}	62.07 ± 3.53^{a-f}
N1P5	7.34±0.61^{a-j}	8.21± 0.58^{a-l}	65.39 ± 2.93^{a-e}
N2P1	9.04±0.53^{a-j}	10.47 ±0.64^{a-e}	60.23 ± 1.73^{a-i}
N2P2	10.63±1.44^{a-h}	10.70 ±1.37^{a-d}	61.77 ± 2.68^{a-h}
N2P3	12.76±1.36^{a-f}	10.93± 2.07^{ab}	66.6 ± 1.27^{ab}
N2P4	16.46±1.52^{abc}	11.2 ± 1.87^{a}	64.9 ± 1.65^{a-f}
N2P5	10.46±0.65^{a-i}	9.58±0.73^{a-i}	55.63 ± 2.07^{a-j}
N3P1	15.67±6.9^{a-e}	9.87±2.8^{a-g}	57.37 ± 1.06^{a-j}
N3P2	19.94±8.77^{a}	9.73 ± 3.02^{a-h}	58.83 ± 1.69^{a-j}
N3P3	16.11±7.11^{abcd}	10.77 ± 2.39^{abc}	51.37 ± 1.39^{a-j}
N3P4	17.03±7.49^{ab}	10.42 ± 2.08^{a-f}	66.18± 2.57^{abc}
N3P5	11.95±5.27^{abcdefg}	4.01 ±1.04^{a-m}	48.24 ± 1.07^{a-j}

Table 1.3: Effect of the NAA and 2iP on direct somatic embryogenesis from leaf of *Scilla indica.* Treatments: NAA (N1 0.25, N2 0.50 and N3 1.00 mg l^{-1}) and 2iP (P1 0.25, P2 0.50, P3 1.00, P4 2.00 and P5 4.00 mg l^{-1}).
***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

Treatments	Percentage of callus	Percentage of necrosis	Avg. Number of Somatic Embryos	Avg. Shoot length (mm)	Avg. Number of roots	Globular Stage
N1T1	25.00±2.19^{a-d}	8.33±3.29^{abc}	1.72±0.92^{abc}	2.28±1.02^{ab}	12.17±3.87^{a-d}	0.27±0.033^{a}
N1T2	0.00±0	0.00±0	1.13±0.18^{a-g}	1.65±0.36^{a-f}	15.42±2.18^{abc}	0.00±0
N1T3	0.00±0	25.00±2.16^{ab}	1.28±0.28^{a-g}	0.67±0.07^{a-j}	10.08±2.08^{a-f}	0.00±0
N1T4	75.00±2.15^{ab}	25.00±1.28^{ab}	3.30±2.15^{a}	0.00±0	1.38±0.25^{a-o}	0.94±0.15^{a}
N1T5	33.33±2.48^{abc}	41.67±2.94^{a}	1.39±0.26^{a-e}	0.00±0	4.42±2.16^{a-m}	0.00±0
N2T1	0.00±0	0.00±0	1.29±0.57^{a-g}	1.79±0.37^{a-e}	11.25±2.14^{a-e}	0.00±0
N2T2	100.00±0^{a}	0.00±0	1.34±0.39^{a-g}	0.26±0.067^{a-k}	9.83±1.27^{a-g}	0.32±0.076^{a}
N2T3	0.00±0	16.67±3.07^{abc}	1.45±0.75^{a-d}	0.84±0.048^{a-h}	6.83±1.12^{a-j}	0.00±0
N2T4	0.00±0	0.00±0	1.25±0.38^{a-g}	1.31±0.07^{a-g}	23.50±2.09^{a}	0.00±0
N2T5	0.00±0	0.00±0	1.09±0.03^{a-g}	0.76±0.16^{a-i}	8.00±3.29^{a-h}	0.00±0
N3T1	100.00±0^{a}	0.00±0	1.97±0.27^{ab}	0.00±0	4.50±0.28^{a-l}	0.00±0
N3T2	16.67±2.73^{a-e}	8.33±4.23^{abc}	1.02±0.76^{a-g}	2.43±1.17^{a}	7.33±0.228^{a-i}	0.00±0
N3T3	0.00±0	25.00±2.47^{ab}	0.90±0.38^{a-g}	1.92±0.76^{abc}	17.58±1.48^{ab}	0.00±0
N3T4	0.00±0	0.00±0	1.32±0.28^{a-g}	1.89±0.27^{a-d}	6.17±0.59^{a-k}	0.00±0

N3T5	33.33±3.98^{abc}	8.34±3.18^{abc}	1.38±0.41^{a-f}	0.00±0	2.92±1.24^{a-n}	0.42±0.02^{a}

Table 1.4: Effect of the NAA and TDZ on direct somatic embryogenesis from leaf of *Scilla indica.* Treatments: NAA (N1 0.25, N2 0.50 and N3 1.00 mg l^{-1}) and TDZ (T1 0.25, T2 0.50, T3 1.00, T4 2.00 and T5 4.00 mg l^{-1}).
***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

Treatments	Percentage of Response	Percentage of Callus	Avg. No. of Somatic Embryos	Avg. No. of roots	Avg. shoot length (mm)
I1B1	89.88±2.93^{a-g}	3.85±1.03^{a}	4.77±2.19^{a-k}	0.75±0.09^{abc}	7.00±2.73^{ab}
I1B2	96.35±3.52^{a-d}	0.00±0	3.43±1.52^{a-l}	0.18±0.016^{a-h}	5.41±1.39^{a-f}
I1B3	95.00±3.29^{a-e}	3.85±1.82^{a}	4.10±2.1^{a-kl}	0.47±0.04^{a-f}	6.21±2.93^{abc}
I1B4	47.50±4.29^{a-i}	0.00±0	2.05±1.24^{a-l}	0.00±0	3.10±1.92^{a-l}
I1B5	23.75±5.21^{a-i}	0.00±0	1.02±0.79^{a-l}	0.00±0	1.55±0.94^{a-n}
I2B1	85.36±3.23^{a-h}	0.00±0	7.51±2.81^{a-j}	0.68±0.038^{a-d}	2.31±1.037^{a-n}
I2B2	98.39±2.15^{ab}	0.00±0	10.76±3.27^{a-f}	0.96±0.027^{ab}	4.00±2.04^{a-j}
I2B3	93.08±4.2^{a-f}	0.00±0	11.73±3.2^{a-e}	0.61±0.012^{a-e}	5.03±2.19^{a-g}
I2B4	100.00±0^{a}	0.00±0	14.30±2.85^{abc}	0.27±0.03^{a-h}	3.68±1.9^{a-k}
I2B5	97.50±1.29^{abc}	0.00±0	15.29±3.8^{ab}	0.23±0.072^{a-h}	2.97±1.67^{a-m}
I3B1	95.00±1.06^{a-e}	0.00±0	9.64±2.59^{a-h}	4.31±1.04^{a}	7.47±2.03^{a}
I3B2	100.00±0^{a}	0.00±0	8.03±2.18^{a-i}	0.43±0.056^{a-g}	4.19±1.27^{a-i}
I3B3	100.00±0^{a}	0.00±0	10.52±3.16^{a-g}	0.61±0.042^{a-e}	5.55±0.34^{a-e}
I3B4	100.00±0^{a}	0.00±0	16.53±3.28^{a}	0.26±0.09^{a-h}	4.72±0.84^{a-h}
I3B5	100.00±0^{a}	0.00±0	12.35±2.9^{a-d}	0.20±0.083^{a-h}	5.77±1.03^{a-d}

Table 1.5: Effect of the IBA and BAP on direct somatic embryogenesis from leaf of *Scilla indica.* Treatments: IBA (I1 0.25, I2 0.50 and I3 1.00 mg l^{-1}) and BAP (B1 0.25, B2 0.50, B3 1.00, B4 2.00 and B5 4.00 mg l^{-1}).
***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

Treatments	Percentage of Response	Percentage of Callus	Avg. No. of Somatic embryos	Avg. No. of roots	Avg. shoot length (mm)
I1K1	93.91±2.04^{a-h}	0.00	2.08±0.93^{a-k}	1.57±0.92^{a-j}	7.70±2.18^{a-h}
I1K2	97.83±3.29^{ab}	0.00	7.33±2.39^{a}	1.33±0.27^{a-k}	4.67±2.11^{a-n}
I1K3	95.87±4.32^{a-f}	0.00	4.70±3.22^{a-i}	1.45±0.33^{a-k}	6.19±3.4^{a-j}
I1K4	96.85±3.80^{abc}	0.00	6.02±2.18^{abc}	1.39±0.72^{a-j}	5.43±3.05^{a-n}
I1K5	96.36±2.14^{a-e}	0.00	5.36±1.24^{a-f}	1.42±0.86^{a-k}	5.81±3.94^{a-l}
I2K1	96.60±2.19^{a-d}	0.00	5.69±1.29^{a-d}	1.41±0.23^{a-k}	5.62±2.21^{a-m}
I2K2	100.00±0^{a}	0.00	3.24±1.02^{a-k}	8.65±2.39^{a}	27.71±1.82^{a}

I2K3	**95.00±3.29^{a-g}**	**0.00**	**5.45±3.29^{a-e}**	**1.79±0.34^{a-h}**	**6.13±1.29^{a-k}**
I2K4	**100.00±0^{a}**	**0.00**	**4.02±2.88^{a-j}**	**3.75±1.29^{a-f}**	**9.05±2.03^{a-f}**
I2K5	**100.00±0^{a}**	**0.00**	**1.90±0.49^{a-k}**	**1.74±0.21^{a-i}**	**8.49±3.8^{a-g}**
I3K1	**100.00±0^{a}**	**0.00**	**2.71±1.92^{a-k}**	**4.44±2.37^{a-d}**	**20.65±3.27^{ab}**
I3K2	**100.00±0^{a}**	**0.00**	**5.34±2.38^{a-g}**	**2.73±1.25^{a-g}**	**14.82±1.27^{a-d}**
I3K3	**100.00±0^{a}**	**0.00**	**6.53±3.23^{ab}**	**4.24±2.22^{a-e}**	**16.05±1.33^{abc}**
I3K4	**100.00±0^{a}**	**0.00**	**3.16±1.17^{a-k}**	**5.62±3.27^{ab}**	**10.55±2.34^{a-e}**
I3K5	**100.00±0^{a}**	**0.00**	**5.00±2.19^{a-h}**	**4.46±2.31^{abc}**	**7.31±2.83^{a-i}**

Table 1.6: Effect of the IBA and Kinetin on direct somatic embryogenesis from leaf of *Scilla indica.* Treatments: IBA (I1 0.25, I2 0.50 and I3 1.00 mg l^{-1}) and Kinetin(K1 0.25, K2 0.50, K3 1.00, K4 2.00 and K5 4.00 mg l^{-1}).
***Mean values followed by same letter (s) are not significantly different at 0.05 level (DMRT).**

Explants cultured on media containing NAA and BAP showed 100% bulblet regeneration in *Lilium ledebourii* (Bakhshaie *et al*. 2010). This result is consistent with that of Azadi and Khosh-Khui (2007) who also reported similar results for the *L. ledebourii* in winter-harvested bulbs. Such kind of consistency in growth response is also maintained in *Scilla indica* (Table. 1.1). A comparative study to determine the efficiency of different cytokinins with respect to NAA in somatic embryogenesis of *Scilla indica* has been made.

In *Scilla indica* among the four cytokinins used, combinations of TDZ and 2iPA with NAA gave the best response in somatic embryogenesis (Fig. 1F & G). Initial pre-culturing for 40 days on medium with a higher concentration of TDZ (20.0 μM) followed by transferring in lower concentration (5.0 μM TDZ) significantly enhanced direct shoot regeneration from the leaves of *Arnebia euchroma* (Royle) Johnston (Malik *et al*. 2010). In rice, preculture of rice calli in presence of certain levels of 2iPA before transferring onto a normal regeneration medium significantly increased the rate of embryogenesis as well as plant regeneration (Zhu *et al.* 1996). Our results corroborate with these findings.

In the present investigation higher concentration of TDZ (2.0 mg l^{-1}) and lower concentration of 2iPA (0.5mg l^{-1}) in combination with NAA generated highest number of somatic embryos per explants in *Scilla indica* (Fig.1F & G). In *Saintpaulia ionantha* Wendl at concentration lower than 2.5 μM, TDZ induced shoot organogenesis, whereas at higher doses (5-10 μM) somatic embryos were formed (Mithila *et al* 2003). Therefore, it can be conclude that TDZ and 2iPA play a key role in induction and development of somatic embryos and shoot buds. TDZ-induced regeneration is the manifestation of a

metabolic cascade that includes an initial signaling event, accumulation, and transport of endogenous plant signals such as auxin and melatonin, a system of secondary messengers, and a concurrent stress response (Jone *et al*. 2007). However, in the present study it has been already observed that for shoot development Kin and 2iPA in combination with NAA found more effective than BAP and TDZ for *Scilla indica* (Fig.1D&F). From the above observations it is revealed that 2iPA is the most effective cytokinin in combination with NAA for direct somatic embryogenesis and their subsequent growth in *Scilla indica.*

IBA 1.0 mg l^{-1} and BAP 2.0 mg l^{-1} in generaly promote somatic embryogenesis (16.53±3.28). However, IBA 1.0 mg l^{-1} with low BAP 0.25 mg l^{-1} level induce greater root (4.31±1.04) and shoot (7.47±2.03 mm.) development in callus (Table. 1.5).

Lower concentration of IBA 0.25 mg l^{-1} and Kin 0.50 mg l^{-1} promoted somatic embryogenesis (7.33±2.39), root development (8.65±2.39) and shoot development (27.71±1.82). From these results it is revealed that in combination with IBA, BAP gave best result for somatic embryogenesis whereas Kin was more effective in root and shoot development in *Scilla indica* (Table. 1.5 and 1.6). Sen and Sharma (1991) observed that maximum number of shoots was formed when 2.5 μM IBA was added to medium containing 4.4 μM BA during initiation of shoot multiplication in *Withania somnifera.* Application of IBA helps in rooting in some plant, as is found in *Camellia sinensis.* The larger shoots were separated after multiplication and rooted on half MS medium supplemented with 11.4 μM ascorbic acid and 34.5 μM IBA in *Camellia sinensis* (Agarwal *et al.* 1992). Maximum rooting was obtained on half MS medium supplemented with 0.5 mg l^{-1} IBA in *Cotoneaster wilsonii* (Sivanesan *et al*. 2011). For the rooting process Banciu *et al*. (2010) recommended sub culturing on MS modified with the addition of 1.8 mg l^{-1} IBA and 0.022 mg l^{-1} Kin as other scientists recommended in literature (Banciu *et al*. 2008). However, in the present observation in *Scilla,* separate root induction medium was

not required because roots are generated in the same shoot induction medium itself. The medium supplemented with a range of different auxins was investigated and it was found that NAA and IBA did not show much difference in their effect on somatic embryogenesis in *Scilla*.

REFERENCES:

Agarwal, B., Singh, U. and Maitreyi, B. "*In vitro* clonal propagation of tea (*Camellia sinensis*)". Plant Cell Tissue Organ Cult 30: 1-5, 1992.

Azadi, P. and Khosh-Khui, M. . "Micropropagation of *Lilium ledebourii* (Baker) Boiss as affected by plant growth regulator, sucrose c... "· Electronic Journal of Biotechnology. 2007

Banciu, C., Carasan, M.E., Brezeanu, A., "Date comparative privind variaţia spectrelor izoenzimatice la Ruscus aculeatus L., in vitro şi in habitatul natural". Buletinul Societăţii Naţionale de Biologie Celulară. Risoprint Press, 36: 145, 2008.

Banciu, C., Mitoi, M., Helepciuc, F., Aldea, F. *In vitro* propagation of critically endangered species Scilla autumnalis L. – biochemical analyses of the regenerants. AnUniv. Oradea Fasc. Biol. 17: 318–328. 2010.

Malik S, Sharma S, Sharma M, Ahuja PS. "Direct shoot regeneration from intact leaves of *Arnebia euchroma* (Royle) Johnston using thidiazuron"**.** Cell Biol Int. doi: 10.1042/CBI20090372.; 34(5):537-42, 2010 Apr 8.

Mithila J, Hall J.C, Victor J.M and Saxena P.K. Plant Cell Rep. Epub 2002 Nov 12. Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl). 21(5):408-14. 2003.

Murashige T. & Skoog F. "A revised medium for rapid growth and bioassays with tobacco tissue cultures". Physiol. Plant. 15:473-97. 1962.

Sivanesan I., Song J. Y., Hwang S. J. and Jeong B. R. "Micropropagation of Cotoneaster wilsonii Nakai—a rare endemic ornamental plant". *Plant Cell Tissue Organ Cult.* 105 55–63. 10.1007/s11240-010-9841-2, 2011.

Verbiscar, A.J, Patel, J., Banigan T.F. and Schatz R.A. "Scilliroside and other *Scilla* compounds in red squill." J. Agric. Food Chem. 34:973-979. 1986.

Zhu, Y. , Ouyang, W. , Li, Y. & Chen, Z. "The effects of 2ip and 2,4-D on rice calli differentiation". *Plant Growth Regulation* volume 19, pages19–24. 1996.