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Short Communication

Efficient plant regeneration for rapid *in vitro* multiplication of Indian solanum (*Solanum surattense* Burm.f)

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Indian Solanum, *Solanum surattense* Burm.f syn. *S. xanthocarpum* Schrad & Wendl, commonly known as *Baigan Kateli* (Sanskrit-*Kantakari*), is a traditional ayurvedic medicinal herb. The plant is known to have multiple medicinal properties. Roots, fruits and occasionally the whole plant are used in medicine. The important formulations using the drug are "*Kantakari ghrtham, Putikaranjasvam, Surandhileham* etc. The species is used in ayurvedic medicine as *Dasamula* for chest pains, cough, asthma, fever etc., and fruit juice in ear ache. The plant is used in preparation of ayurvedic massage oil *Kamavirya* and also in *Bronchicyl* [1]. Owing the importance of the species in medicine, the present investigations have been carried out for rapid *in vitro* multiplication using floral bud culture in *S. surattense*.

A large number of floral buds (1.0-1.5 cms) of *S. surattense* were collected in polythene bags from the plants grown in the field. The floral buds were first washed thoroughly under running tap water (15-30 min.) followed by a detergent (Teepol). Then they were treated with 0.1% (w/v) mercuric chloride for 4-5 minutes and rinsed thoroughly with sterile distilled water. Further, the surface water was blotted with the sterilized tissue papers. The explants were placed on modified MS medium [2] (devoid of $CaCl_2$. $2H_2O$ & KI) containing 6.0 % (w/v) sucrose and solidified with 0.8% (w/v) agar and supplemented with various concentrations of growth regulators *viz.*, BAP, Kn, IAA + BAP/Kn, NAA + BAP/Kn. The pH was adjusted to 5.8 before autoclaving. Micro-shoots, well elongated (4-6 cms long) were transferred to root induction MS medium comprising 3% (w/v) sucrose with 0.5, 1.0, 1.5 mg/l IAA. Individually, the explants were cultured in culture tubes (25 × 150 mm) and subsequently sub cultured in 250 ml Erlenmeyer conical flasks. The cultures were maintained under 16 h photo period at 25 ± 0.5°C under cool white fluorescent light (light intensity 50 µmol $m^{-2}s^{-1}$) and 55-60% relative humidity. The regenerated plantlets were transferred to poly cups containing sterile compost and soil (1:1) mix for 3 weeks for acclimatization. Subsequently these were shifted to the greenhouse. For statistical analysis, means were based on 25 replicates for each treatment. Data were collected after 6 weeks beginning of the experiments. The floral buds were cultured on various concentrations and combinations of plant growth regulators such as cytokinins (6-benzyl amino purine-BAP; Kinetin-Kn) alone and also in combination with auxins (Indole acetic acid-IAA; Naphthalene acetic acid-NAA).

Within the first week, most of the inoculated flower buds enlarged. After 2 weeks of culture they opened followed by the enlargement of the ovary. Adventitious shoot buds were induced from the ovary region after 3 weeks of culture. Direct shoots were formed from the explants on modified MS medium amended with cytokinins alone and also cytokinins + auxins tested. These were developed without intervening callus phase. The floral buds cultured on MS modified medium supplemented with 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 mg/l BAP/Kn showed maximum percentage (68%) of responding cultures at 3.0 mg/l BAP/Kn. At higher concentration of BAP/Kn (8mg/l) the percentage of response was reduced gradually upto 28% and 20% respectively. Likewise maximum number of multiple shoot buds/explant was found at 3.0 mg/l BAP/Kn when it was added alone to the medium, but highest percentage of responding cultures and more number of shoots (212±0.32) were recorded at 3.0 mg/1 BAP in comparison to Kn (120±0.25). As the concentration of cytokinin increased upto 3 mg/l BAP/Kn the frequency of number of shoots induction was found to be increased (23 ± 0.12/15 ± 0.09, 44 ± 0.13/32 ± 0.03 shoots at 1.0, 2.0 mg/l BAP/Kn). Whereas at high concentrations the percentage of responding cultures and shoot bud induction were decreased gradually (121 ± 0.11/80 ± 0.13, 92 ± 0.13/65 ± 0.04, 34 ± 0.14/21 ± 0.09, shoots at 4.0, 5.0, 6.0 mg/l BAP/Kn). At 8.0 mg/l BAP/Kn very less number of shoots (22 ± 0.25/17 ± 0.35) were developed compared to other concentrations of BAP/Kn individually.

Modified MS medium containing 0.5 mg/l IAA in combination with BAP/Kn (1.0-8.0 mg/l) showed the enhanced efficiency in inducing the adventitious shoot buds from the explant as compared to media with cytokinin alone. IAA (0.5 mg/l) in combination with 3.0 mg/l BAP/Kn produced maximum number of shoots

(323 ± 0.35/157 ± 0.45) with highest responding frequency (63%) compared to other concentrations of BAP/Kn. As the concentration of BAP/Kn increased in the medium showed the less response and decreased number of shoots gradually from 4.0 mg/l BAP/Kn + 0.5mg/l IAA combination onwards (160 ± 0.13/78 ± 0.07, 75 ± 0.09/63 ± 0.13, 48 ± 0.17/27 ± 0.08, 15 ± 0.11/8.0 ± 0.01 shoots at 4.0, 5.0, 6.0 and 8.0 mg/l BAP/Kn + 0.5 mg/l IAA) Fig. 1.

Direct shoot bud proliferation was also found in all the concentrations of BAP/Kn with 0.1 mg/l NAA added to the MS modified medium. More number of adventitious shoots per explant was recorded at 0.1 mg/l NAA + 5.0 mg/l BAP/Kn compared to all other concentrations of cytokinins used. Low concentration of BAP/Kn induced less number of shoots/explant (25 ± 0.01/21 ± 0.03 shoots at 1.0 mg/l) but gradually the shoot bud induction was found to be increased up to 5.0 mg/l BAP/Kn + 0.1mg/l NAA (55.0 ± 0.12/50.0 ± 0.06, 135 ± 0.15/127 ± 0.08, 159 ± 0.17/146 ± 0.03 shoots at 2.0, 3.0 and 4.0 mg/l) and at high, concentrations the shoot bud proliferation from floral bud cultures was reduced (79 ± 0.11/68 ± 0.03, 35 ± 0.13/29 ± 0.18 shoots at 6.0 and 8.0 mg/l BAP/Kn + 0.1 mg/l NAA). High percentage (85%) of responding cultures and maximum number of shoots (342 ± 0.11) were developed on MS modified medium supplemented with 0.1 mg/l NAA + 5.0 mg/l BAP compared to all other combinations and concentrations studied (Fig. 1).

The microshoots were excised and individually transferred to MS medium augmented with 0.5 to 1.5 mg/l IAA for root induction. Root initiation was profuse with 8-10 roots in the medium containing 1.0 mg/l IAA as compared to 0.5 mg/l (4 roots) and 1.5 mg/l (6 roots) IAA. High percentage of rooting efficiency (90%) was observed at 1.5 mg/l IAA followed by 1.0 mg/l (80%) and 0.5 mg/l (70%) IAA from *in vitro* regenerated shoots. These *in vitro* regenerated plants were shifted to the greenhouse after acclimatization in the culture room. The survival percentage of *in vitro* regenerated plants was 70. These plants were normal, healthy and similar to donor plants.

In the present investigations, direct shoot regeneration from floral buds was obtained in all the concentrations and combinations of plant growth regulators used. The cytokinin BAP had shown superiority over Kn in all the concentrations and combinations studied for inducing multiple shoots in *S. surattense*. Maximum efficiency of shoots formation per explant was observed on MS modified medium comprising 0.1 mg/l NAA and 5.0 mg/l BAP/Kn followed by 0.5 mg/l IAA and BAP/Kn. When auxin (IAA/NAA) was added in combination with cytokinins showed the high induction efficiency during the present studies.

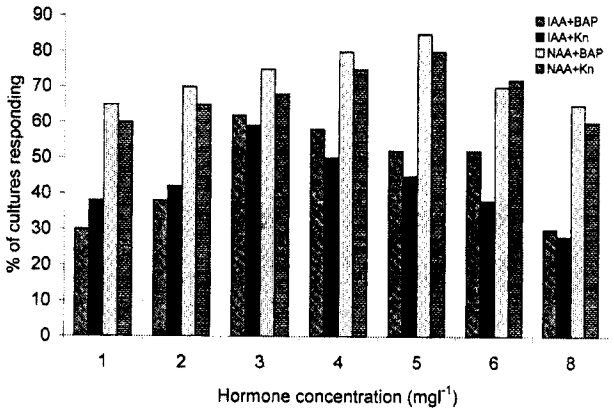

Fig. 1. Effect of IAA (0.5 mgl^{-1}) + BAP/Kn and NAA (0.1 mgl^{-1}) + BAP/Kn on induction of multiple shoots from floral bud cultures of *S. surattense*

Adventitious shoot regeneration in *S. surattense* has been reported with 213 and 148 shoot buds per explant from leaf and shoot tip cultures respectively [5] and also 25.8 shoots from leaf and 23.6 shoots from nodal explants [4]. Where as during the present investigation the number of shoots developed directly from the explant was 342. When the same was subcultured in a conical flask containing the fresh medium, induced in thousands of shoots per explant. Hence, the present study shows that the floral bud culture is amenable to high frequency regeneration in medicinally important herb *S. surattense*, which could be easily adopted for large scale multiplication of the species. Thus, it opens up the possibility of using this plant in genetic manipulation for introducing genes of interest using particle gun bombardment or *Agrobacterium tumefaciens*

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