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RAPID MULTIPLICATION OF A RARE MEDICINAL PLANT *SOLANUM ANGUIVI* LAM.

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Abstract:
Solanum anguivi Lam. (African Eggplant) is a rare ethno-botanical plant used in **'**Dashmula**'** formulation. *In vitro* regeneration has been developed as an alternative tool for the propagation, conservation and rapid multiplication of medicinal plants which are difficult to propagate through conventional methods.The present investigation has been focused on the *in vitro* shoot multiplication by using nodal explant. MS medium supplemented with different concentrations and combinations of cytokinins (BA, Kn and TDZ) were used for study. Initial studies revealed that Kn (0.75 mg/L) induced highest number of shoots (3.00 ± 0.1 shoots/node) with highest number of shoot length approximately 1.08 ± 0.084 cm, than other concentrations tried. MS medium supplemented with Kn (0.75 mg/L) showed 100 % shoot initiation. Studies on *in vitro* root induction are in progress, using hormones IAA, IBA and NAA singly or in combination.

Keywords: *Solanum anguivi*, Multiple shoots, Kinetin, Conservation, Dashmula.

Introduction

Solanaceae (Potato family) is an important family of angiospermic plants having numerous economic important plant species distributed all over the world with great diversity in habitats, ecology, morphology and distribution. In terms of habit, it ranges from small annual herb to tree; in habitat, from desert to the wettest tropical rain forests; and in morphology, with amazing different variation in many characters of both flowers and fruits are observed [1]. It consists of approximately 98 genera with 2,700 species [2]. The vast diversity of this family is responsible for its usefulness in food, spice and medicine. Out of which two species (*Solanum anguivi* Lam. and *Solanum virginianum* L.) are used in Ayurvedic formulations. One of the most popular, polyherbal and routinely used Ayurvedic preparation is **'**Dashmula**'**.

The term **'**Dashmula**'** was first given by Acharya Sushruta. Dashmula is a combination of ten plant roots together [3]. It comprises root of five tree species i.e. brihat panchmula and root of five small herbs i.e. laghu panchmula [4]. The five tree species of Brihat Panchmula documented in Ayurveda are: *Aegle marmelos* (L.) Corr., *Premna optusifolia* R. Br., *Gmelina arborea* Roxb., *Oroxylum indicum* Vent. and *Stereospermum colais* Mabb. Plants documented in Laghu Panchmula are: *Desmodium gangeticum* (L.) DC., *Solanum anguivi* Lam., *Solanum virginianum* L., *Tribulus terrestris* L., and *Uraria picta* (Jacq.) Desv. ex DC.

Solanum anguivi Lam. (Fig.1.a) is one of the species used in Dashmula formulation belonging to the genus *Solanum.* Many *Solanum* species are used as vegetable crops and alkaloid producing plants [5]. *Solanum* species are medicinally important because, various chemical constituents have been isolated from them like alkaloids, phenolics, flavonoids, sterols Saponins, their glycosides and tannins [6]. Majority of *Solanum* species are widely used in folk medicine [7] and about 21 species and one variety in this genus are used as herbal medicines [8, 9] due to presence of secondary metabolites. Solasodine is the active principle compound of genus *Solanum,* because it has property to synthesize steroidal hormones [10, 11].

S. anguivi is a rare ethno-medicinal plant [12]. It is shrub, 60 to 250 cm in height with yellow, purple, straight or curved spines all over the plant. Young stems are greenish yellow or purple with stellate hair on them. Leaves are generally ovate with oblique base, 5 to 20 cm long and 4 to 18 cm wide. Leaf lobes are shallow or deep nearly pinnatified; the lobes are triangular with subacute or acute tips. Stems and leaves vary almost from glabrous to densely wooly. Inflorescence varies from a few flowers to many flowers. Flower (Fig. 1. b) is 8 mm to 2.5 cm across. Fruits (Fig. 1. c) are green at young stage and red at mature stage. Fruit is a berry 8 mm to 1 cm in diameter [13]. It is used as therapeutic agent for various diseases. The cough due to kapha and vata are controlled with the decoction of its roots given along with honey and ghee. Cholesterol lowering properties of saponin form *S. anguivi* has also been reported [14]. The roots are carminative and expectorant useful in coughs, catarrhal inflamation, colic, nasal ulcers, asthama, tooth ache, nervous disorder and fever [15]. It has been widely used in folk medicine as an analgesic for toothache, rhinitis and breast cancer. In Nigeria, the fruits of *Solanum anguivi* are claimed to reduce the risk of atherosclerosis which is usually associated with hypertension. It is an appetizer and beneficial to the heart. It is

the best blood purifier, hence, benevolent in blood disorders [16].

Globally, plant biotechnology and plant tissue culture (PTC) in particular have been recognized valuable in terms of their beneficial role in the production and conservation of plant-based resources [17, 18]. Now-a-days, there is a great decrease in plant resources due to human disturbances of the natural environment. Microprpagation is the most popular, advanced commercial activity [19]. It is a process in which small pieces of living tissue (explants) are isolated from plant and grown aseptically for indefinite periods on a nutrient medium under controlled conditions [20]. *In vitro* regeneration or micropropagation has been developed as an alternative tool for the propagation, conservation and rapid multiplication of medicinal plants which are difficult to propagate through conventional methods. The present investigation is focused on the micropropagation of a rare ethano-medicinal *S. anguivi*.

Materials and Methods

Collection of plant materials

S. anguivi was collected from different localities of Maharashtra (Panhala, Ajara and Vishalgad). The plants were identified at the Department of Botany, Shivaji University, Kolhapur. Voucher specimens were prepared and deposited in the Herbarium of the Department of Botany, Shivaji University, Kolhapur, (M.S.), India. Germplasm of these plants were maintained in the Botanical Garden of Botany Department, Shivaji University, Kolhapur for the further study.

Explant preparation and inoculation of *S. anguivi*

Shoot apices (20-25 cm) with leaves of *S. anguivi* (3 month old) were collected from field. The shoot apices were defoliated and segmented at 4-5 cm containing 2-3 nodes after bringing to laboratory. Stem segments were washed thoroughly under running tap water for 15 minutes for removal of excess soil particles and other superficial contaminants. Subsequently, were soaked in dettol for 5 minutes. Then all stem segments were washed with sterile distilled water for 3-4 times. A pre-treatment of 0.1% Bavistin (commercial fungicide) was given for half an hour and then washed with sterile distilled water for 3-4 times. All stem segments were kept in the laminar air flow for explant sterilization procedure.

In the laminar air flow, sterilized stem segments were placed. They were pre-treated with HgCl2 (0.1% w/v) for different time intervals (2-5 minutes). This was followed by treatment with 70% (v/v) ethanol (30 seconds). After each treatment 3-4 washes with sterile double distilled water were given in order to wash out traces of the sterilant. All stem segments were blotted on tissue papers and cut into small segments containing one axillary bud and inoculated on MS basal medium [21] with different concentration and varied combinations of cytokinins. Observed data viz. response of shoots, mean number of shoots per explant and mean length of shoots was recorded after 25 days. The data was analyzed statistically by Duncan**'**s multiple test for significance.

Results and Discussion

Sterilization of explants

The present investigation was focused on the surface sterilization of nodes with sterilant and multiple shoot induction on MS medium with different plant growth regulators and varied concentration. Shoot apices (20-25 cm) with leaves of *S. anguivi* were used for the sterilization. Aqueous Mercuric chloride (0.1% HgCl2) was used as surface sterilant with different time exposure. When nodal regions exposed to 0.1% HgCl2 with different time exposure (2 min, 3 min, 4 min and 5 min) showed varied response for growth, browning and contamination of explants (Table 1, Fig. 2). In the nodal surface sterilization studies of *S. anguivi*, 4 minute time exposure to 0.1% HgCl2 demonstrated the highest positive response (66.66%) with 20% browning and 13.33 % contamination. However lowest percentage of browning (0 %) and contamination (6.67 %) were found in 2 min. and 5 min. time exposure respectively. Highest percentage of contamination (60 %) and browning percentage (31.67 %) were found in 2 min. and 5 min. time exposure respectively. In this experiment optimum time period was found as 4 min time exposure to 0.1% HgCl2 with highest percentage (66.66 %) of response with 13.33 % contamination. Further increase in the time exposure resulted decrease in the % response as well as % contamination however their browning percentage was increased. It indicated that sterilant and different time exposure to the sterilant affect the survival as well as disinfection of explants. Surface sterilization experiment on the leaf, nodal and internodal part of stem of *Hemidesmus indicus* and *Rubia cordifolia* was successfully carried out using $HgCl_2$ [22]. Out of all the concentration of Mercuric Chloride used, 0.1% HgCl2 found most effective for both plants. Higher concentration of Mercuric Chloride suppressed the rate of contamination but at the same time it resulted into high percentage of browning, leading to death of explants [22]. In this experiment 0.1% HgCl2 was used as surface sterilant. Exposure of nodal region to different

time period is also important for the survival of nodal region on medium. From the present study it is clear that, 0.1% HgCl2 with short exposure period was not effective but at the same concentration of HgCl2 with extended (4 min.) exposure period was found effective in overall experiment conducted for surface sterilization.

Shoot multiplication

MS medium supplemented with different concentrations and combinations of cytokinins (BA, Kn and TDZ) were used for multiple shoot induction. Multiple shoots emerged from the nodal explants after 10 days of inoculation. The induction of shoot and shoot length was not found on MS basal medium which was used as control. The effect of cytokinins on shoot multiplication from nodal explants is shown in Table 2. The shoot multiplication frequency was observed on all hormones (BA, Kn and TDZ). The frequency of shoot induction (Fig.1. d) ranged from 33.33 % to 100%. Highest frequency of regeneration (100%) was found when MS medium supplemented with 0.75 mg/L Kn.

The number of shoots produced on the MS medium supplemented with BA ranged between 0.6 ± 0.06 to 2.2 ± 0.31 with highest 2.2 ± 0.31 in 0.75 mg/L BA. The number of shoots ranged between 1.00 ± 0.14 to 3.00 ± 0.10 with the highest in 0.75 Kn as 3.00 ± 0.10. When MS medium fortified with TDZ, the shoot multiplication ranged between 0.7 ± 0.08 to 2.5 ± 0.32 with highest in 0.25 mg/L TDZ as 2.5 ± 0.32. However various concentration and combinations of these hormones showed varied range of shoot multiplication between 0.5 ± 0.06 to 2.25 ± 0.38 with highest (2.25 ± 0.38) in combination of 1.5 mg/L BA and 0.75 mg/L TDZ. Shoot induction was noticed prominently in Kinetin. MS medium fortified with 0.75 mg/L Kn showed highest number of shoots (3.00 ± 0.10) with highest number of shoot length 1.08 ± 0.08 cm. Amongst all three cytokinins used on MS medium with singly or in combinations for the purpose of shoot multiplication, Kn (0.75 mg/L) showed best result (3.00 ± 0.10 shoots / node) as compared to other cytokinins.

Similar type of results were obtained in *Solanum nigrum*, where large number of shoots was produced from the nodal explants on MS medium supplemented with both BA and Kn [23]. Several growth regulators are available for shoot multiplication; BA and Kn are widely used. In the present investigation, out of three cytokinins tested, Kn was more suitable in shoot induction and proliferation than BA and TDZ for *S. anguivi*. MS medium fortified with BA and Kn in combination with other growth hormone showed callus formation at the base of explants thus less number of shoots were observed [24]. In present investigation, shoot grown on TDZ showed basal callus formation. This may probably have declined shoot number drastically as well as may also have lowered the shoot length. Cytokinins have been proved responsible for cell division, cell elongation and to induce shoots from nodal meristem of explants [24]. Shoot length (cm) variation was found in above three cytokinins. The highest (1.08 ± 0.08 cm) shoot length was found on MS medium fortified with 0.75 mg/L Kn and lowest (0.15 ± 0.02 cm) on MS medium with 2.5 mg/L TDZ.

Further investigation followed the *in vitro* root induction (Fig. 1. e) which is in progress, using hormones IAA, IBA and NAA singly or in combination.

Conclusion

This shoot rapid multiplication protocol have been standardized with BA, Kn and TDZ hormones for shoot multiplication which may help to multiply the plant rapidly and continuously give raw material for extraction of important secondary metabolite solasodine.

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Table 1: Effect of surface sterilant (HgCl2) on sterilization of the explant

Sr. No.	Explant	% of HgCl2	Time Exposure (min)	Remarks		
				% Response*	% Browning*	% Contamination*
1	Node	0.1	2	40	0	60
		0.1	3	45	8.3	46.66
		0.1	4	66.66	20	13.33
		0.1	5	61.66	31.67	6.67

* Value represents percentage of twenty replicates per treatment. All the experiments were repeated at least thrice.

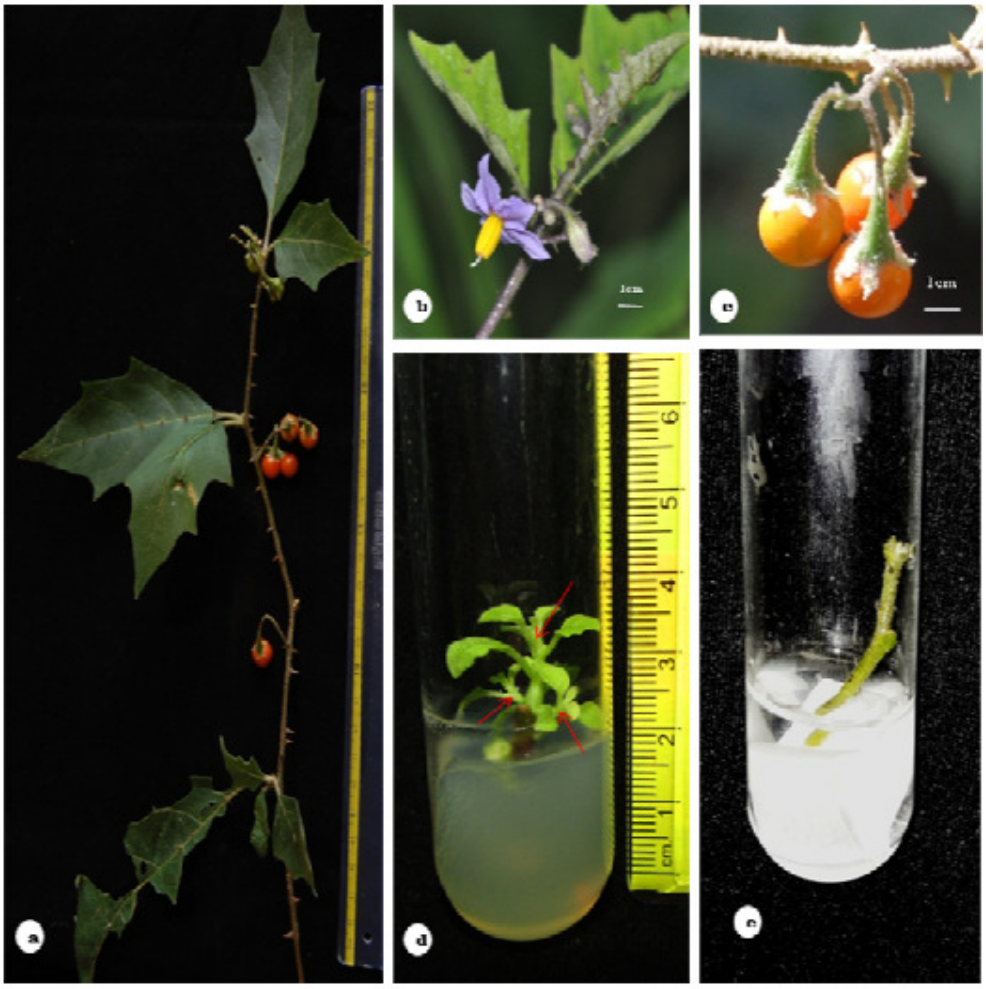

Figure 1 a) Habit of Solanum anguivi b) Flower c) Mature fruit d) Shoot multiplication e) Starting of rooting initiation * Note: Red arrows indication multiple shoots

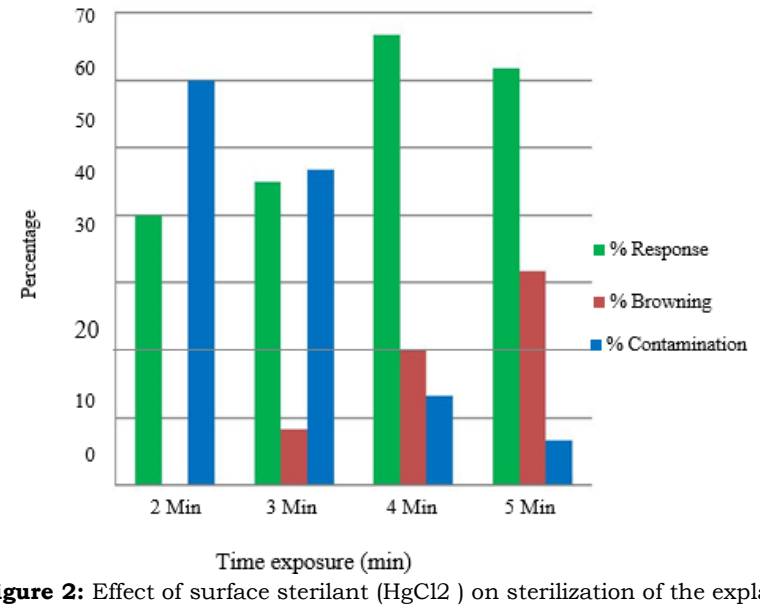

Figure 2: Effect of surface sterilant (HgCl2) on sterilization of the explant

Table 2 : Response of *Solanum anguivi* for shoot induction on MS medium supplemented with growth hormones.

Sr.No.	Medium combination	% Response	Shoot no. ± S. E.*#	Shoot length. ± S. E. (cm)*#
1.	MS	00	00^{n}	00^{q}
2.	MS+0.75BA	60	2.2 ± 0.3178^{bcd}	0.58 ± 0.0876^{cdef}
3.	MS+1BA	60	0.6 ± 0.0637^{lm}	$0.36\pm0.0627^{fghijklmn}$
4.	MS+1.5BA	80	1.2 ± 0.1084^{ghijkl}	$0.3266\pm0.0627^{ijklmnop}$
5.	MS+2BA	33.33	1.0333 ± 0.1343^{ijklm}	$0.5183\pm0.0652^{cdefghi}$
6.	MS+2.5BA	45	0.75 ± 0.099^{klm}	0.47 ± 0.0619^{efghijkl}
7.	MS+0.75Kn	100	3.00 ± 0.100^{a}	1.08 ± 0.0847^{a}
8.	MS+1Kn	50	1.3 ± 0.2182^{ghijk}	$0.39\pm0.0748^{fghijklmno}$
9.	MS+1.5Kn	75	2.166 ± 0.2114^{bcde}	0.566 ± 0.0767^{cdefgh}
10.	MS+2Kn	70	1.00 ± 0.1426^{ijklm}	0.33 ± 0.0558^{ijklmnop}
11.	MS+0.25TDZ	60	2.5 ± 0.320^{ab}	0.99 ± 0.1179^{a}
12	MS+0.5TDZ	70	1.6 ± 0.1948^{efghi}	0.72 ± 0.07382^{bc}
13	MS+0.75TDZ	91.66	1.9166 ± 0.1634^{cdef}	0.666 ± 0.5079^{cde}
14	MS+1TDZ	70	1.2 ± 0.1275^{ghijkl}	0.9 ± 0.1038^{a}
15.	MS+1.5TDZ	90	2.2 ± 0.1822^{bcd}	0.6 ± 0.06106^{cdef}
16.	MS+2TDZ	80	1.8 ± 0.1727^{cdefg}	$0.32\pm0.03673^{ijklmnop}$
17.	MS+2.5TDZ	60	0.7 ± 0.08333^{klm}	0.15 ± 0.02037^{pq}
18.	MS+1.5BA+0.25TDZ	50	1.4 ± 0.2743^{fghij}	0.555 ± 0.0920^{cdefgh}
19.	MS+1.5BA+0.50TDZ	55	1.1 ± 0.1534^{hijklm}	0.35 ± 0.0450^{jklmnop}
20.	MS+1.5BA+0.75TDZ	70	2.25 ± 0.3837^{bc}	$0.415\pm0.0612^{fghijklm}$
21.	MS+1.5BA+1TDZ	70	1.3 ± 0.1432^{ghijk}	0.57 ± 0.08357^{cdefg}
22.	MS+1.5BA+1.5TDZ	58.33	1.5 ± 0.1802^{fghij}	$0.325\pm0.0405^{ijklmnop}$
23.	MS+1.5BA+2TDZ	55	1.65 ± 0.2800^{defgh}	0.25 ± 0.03828^{lmnop}
24.	MS+1.5BA+2.5TDZ	40	1.6 ± 0.3354^{efghi}	0.175 ± 0.0329^{opq}
25.	MS+0.75TDZ+0.5BA	50	0.5833 ± 0.0833^{lm}	0.2166 ± 0.02998^{nop}
26.	MS+0.75TDZ+0.75BA	58.33	0.9166 ± 0.1241^{jklm}	0.275 ± 0.0332^{klmnop}
27.	MS+0.75TDZ+1BA	66.66	1 ± 0.1188^{ijklm}	0.9833 ± 0.1287^{a}
28.	MS+0.75TDZ+1.25BA	58.33	1.0833 ± 0.1451^{hijklm}	$0.325\pm0.0589^{ijklmnop}$
29.	MS+0.75Kn+1.0BA	70	1.1 ± 0.1359^{ghijklm}	0.49 ± 0.0550^{defghijk}
30.	MS+0.75Kn+1.5BA	60	0.7 ± 0.0833^{klm}	0.54 ± 0.579^{cdefghi}
31.	MS+0.75Kn+2.0BA	60	0.7 ± 0.033^{klm}	0.51 ± 0.0568^{cd}
32.	MS+0.75Kn+0.5TDZ	80	1.5 ± 0.1567^{fghij}	0.49 ± 0.0365^{defghijk}
33.	MS+0.75Kn+0.75TDZ	60	0.6 ± 0.0637^{lm}	0.3 ± 0.0386^{jklmnop}
34.	MS+0.75Kn+1.0TDZ	70	1 ± 0.1426^{ijklm}	$0.45\pm0.0444^{efghijklm}$
35.	MS+0.75Kn+1.5TDZ	50	0.5 ± 0.0650^{mn}	0.23 ± 0.0344^{mnop}

Note : * Value represents mean ± standard error of twenty replicates per treatment and all the experiments were repeated at least thrice.

\# Results are presented as means ± S. E. means followed by the different letters (a-q) are significantly different ($p<0.05$) using Duncan**'**s multiple range test.

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