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TISSUE CULTURE STUDY IN *Solanum xanthocarpum* FOR EFFICIENT MICROPROPAGATION

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ABSTRACT

Tissue culture study was carried out by culturing nodal explants of *Solanum xanthocarpum* in MS medium supplemented with 6 different (0.5-3.0 mg/l) concentrations of 6-Benzyl amino purine and kinetin separately and two different concentrations of IBA and IAA (0.5-1.0 mg/l). Highest percentage of response (92.5), higher mean number of shoots (9.6) and highest growth (2.8 cm) were observed in MS +2.0 mg/l BAP + 1.0mg/l 1BA .The percent response at similar concentration of IAA was 80.6 ,mean number of shoots 6.4 and mean length of shoots 2.4cm respectively .It was further noted that MS +2.0 mg/l KN + 1.0 mg/l IBA induced multiple shoots among the explants that was 72.2% mean number 5.2 and mean length 2.0 cm respectively .Multiplication of shoot was found in the same medium having similar concentration.60-70 days plants were used for rooting in half strength MS basal medium supplemented with (0.5-5.0mg/l) concentration of IBA . 1/2 MS + 3.5 mg/l IBA gave better response for rooting in which the percent response was 78% and the mean number of roots 12.4.Such rooted plantlets were use for hardening in the poly chamber and then transferred in poly bag containing sterile soil, Sand and compost in the ratio of 1:1:1.The survival rate was above 60%.

KEYWORDS : Tissue Culture, Micro Propagation, Multiple Shoot, Nodal Explants, *Solanum xanthocarpum*

Solanum xanthocarpus (Kantkari) of the family Solanaceae is an important medicinal herb . Plants are found growing wild in the bare land, on the side of railway track or in the rail yard during June- October. Common people use this plant to cure asthma, vomiting of blood, phlegmatic rheumatism, leprosy etc.

It is also used to reduce enlargement of liver and spleen, muscular pain and stone in the urinary bladder. It is also used in case of migraine and headache. The fruit juice is used to cure throat sore. It is also used in piles .Roots and seeds are used as an expectorant in asthma, Cough and pain in chest(Roshy, 2012).The paste prepared from the root with lemon juice is used to cure snake bite and scorpion sting .The use of fruits promote ejaculation of semen and cures fevers, itching and reduce fat. The seeds are used to cure irregular menstruation and dysmenorrheal. It is also used in heart diseases (Ramaswamy et al., 2005) have described different properties in the plant .Due to this, the traders and local vaidaya exploit the plant brutally and the species is becoming rare. Tissue culture techniques may help in the production of large number of plantlets and thereby in conservation.

We get limited works with respect to its micropropogation .Arulmozhi and Ramanujam, (1997) in S.trilobattem; Powar et al., (2002) in *S. surattense* and Jawahar et al., (2004) in *S. trilobatum* have reported micropropagtion and organogenesis. Rahman et al., (2011), (Sunder and Jawaher, 2011; Rita and Animesh, 2011).

In the present work it has been reported that nodal explants are the best material for the multiple shoots initiation in vitro and MS basal medium and BAP is the suitable growth hormones.

MATERIALS AND METHODS

New healthy stems were collected from the plants growing in wild condition. In the laboratory leaves were removed and the stems were cut into pieces containing single node. All these explants were washed in running tap water in a flask whose mouth was closed with thin clothes for half hour. Above explants were surface sterilized in 70% ethanol for 30-40 seconds ,followed by 0.1% (w/v) mercuric chloride for 1-2 minutes , and were thoroughly washed three four times in sterile distilled water , each having 3-4 minutes duration.

MS (Murashige and Skoog, 1962) basal medium was supplemented with 3% sucrose and solidified with 0.8% agar (Hi media Mumbai). Before this the culture medium was supplemented with (0.5 -5.0 mg/l) BAP and KN separately 0.5 -1.0 mg/l IBA and 1AA were also added in the above for shoot bud initiation. The pH was adjusted at 5.8 and the dispensed medium was autoclaved at 15 lb pressure for 20 minutes. Culture tubes containing the explants were incubated in culture room at 26 $\pm 1^0$C and white fluorescent light with 16h photoperiod. The

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Table 1: Response of Nodal Explants of *Solanum xanthocarpum* (White flowers) on MS Basal Medium Supplemented With Different Concentrations of BAP , KN and NAA in Various Composition

Growth Regulators Mg/l			% response	Mean number of shoots	Mean length (cm^{-1})
BAP	IBA	IAA			
0.5	0.5	-	62.4± 1.22	4.5±0.58	1.5
1.0	0.5	-	68.6± 1.26	4.8± 0.58	1.8
1.5	0.5	-	78.2± 1.22	6.4± 0.56	2.0
2.0	0.5	-	82.5± 1.26	8.7± 0.58	2.6
2.5	0.5	-	76.3± 1.24	6.2± 0.56	1.8
3.0	0.5		56.8 ±1.26	4.5 ±0.58	1.4
0.5	-	0.5	58.6± 1.24	3.8±0.57	1.2
1.0	-	0.5	66.4 ±1.32	4.2±0.57	1.5
1.5	-	0.5	73.5 ±1.36	4.8±0.58	1.8
2.0	-	0.5	78.8 ±1.38	5.2±0.58	2.2
2.5	-	0.5	68.7 ±1.26	4.7±0.57	1.6
3.0			52.3 ±1.22	4.2±0.56	1.0
0.5	1.0	-	68.4 ±1.26	5.2±0.58	1.8
1.0	1.0	-	72.6 ±1.24	5.8±0.58	2.0
1.5	1.0	-	80.5 ±1.28	6.8±0.64	2.4
2.0	1.0	-	92.5 ±1.32	9.6±0.72	2.8
2.5	1.0	-	70.5 ±1.26	6.7±0.64	2.0
3.0	1.0	-	62.4 ±1.23	5.6±0.54	1.6
0.5	-	1.0	62.6 ±1.22	4.2±0.57	1.4
1.0	-	1.0	68.8 ±1.24	4.6±0.57	1.8
1.5	-	1.0	76.4 ±1.32	5.2±0.58	2.2
2.0	-	1.0	82.5 ±1.36	5.8±0.59	2.6
2.5	-	1.0	72.3 ±1.33	5.2±0.58	2.2
3.0	-	1.0	64.6 ±1.26	4.6±0.57	1.6

multiplication of shoot buds was obtained in the same medium after subculture. The plantlets (60 days old) were rooted in half strength MS + sucrose + .8% agar and various concentrations of 1BA (0.5-5mg/l) .Well grown plants with vigorous roots were taken out .Roots were washed to remove culture medium and were transferred in pot containing sterile soil, sand and compost in 1:1:1 ratio. The hardening was done in artificial moist chamber made with the help of poly bag .Experiments were done in triplicates and each experimental set had 20 explants.

RESULTS

Internodal segments of *Solanum xanthocarpum* were cultured on MS basal medium supplemented with six different concentrations of BAP, KN along with 0.5mg/l and 1.0mg/l IBA and IAA separately. Mean of the data were tabulated and have been presented in the table, 1, 2 and 3.

From the table it is seen that at 0.5mg/l BAP +0.5mg/l IBA induced auxiliary buds among 62.4% explants only. However, at 2.0mg/l BAP +0.5mg/l IBA the per cent response was 82.5. When the concentration was increased the percent response decreased. It may be noted that BAP 0.5mg/l +1.0mg/l IBA induced budding among 68.4% of the explants. Similarly 2.0mg/l BAP +1.0 mg/l IBA induced auxiliary buds among 92.8% of the explants. At the similar above concentrations of BAP+IAA induction of auxiliary budding was noted among 58.6 and 78.8, and 62.6 and 82.5% of the explants respectively Fig(B)

From the table 2 Kinetin at 0.5 mg/l +IBA 0.5 mg/l induced auxiliary budding among 48.2% and at 2.0 mg/l +0.5 mg/l of IBA it was 75.5% respectively. Kinetin at 0.5 mg/l+1.0 mg/l IBA promoted budding among the 52.4% of the explants while at 2.0mg/l KN +1.0 mg/l IBA, the percent response was 52.4 and 78.2% respectively.At the similar above concentration of KN, 0.5and 1.0 mg/l IAA induced vegetative buds among 46.8 and 70.6 and 48.5 and 72.7% of the explants. Here also increasing concentrations

Table 2: Impact of Kinetin, IBA and IAA on Shoot Regeneration From The Nodal Explants of *S.xanthocarpum*

S.N.	Growth regulator KN	IBA	IAA	% response	Mean number of shoots	Mean length
	0.5	0.5	-	48.2 ±1.20	4.4±0.58	1.2
	1.0	0.5	-	58.6 ±1.24	4.8±0.58	1.4
	1.5	0.5	-	69.8 ±1.26	5.6±0.62	1.8
	2.0	0.5	-	75.5 ±1.38	6.0±0.68	2.2
	2.5	0.5	-	64.2 ±1.26	5.4±0.62	2.0
	3.0	0.5	-	58.4 ±1.24	4.8±0.58	1.8
	0.5	-	0.5	46.8±1.18	3.2±0.56	1.0
	1.0	-	0.5	54.4±1.24	3.8±0.56	1.2
	1.5	-	0.5	64.5±1.28	4.2±0.58	1.4
	2.0	-	0.5	70.6±1.30	4.8±0.56	1.8
	2.5	-	0.5	56.6±1.26	3.2±0.48	1.6
	3.0	-	0.5	50.4±1.24	3.0±0.46	1.4
	0.5	1.0		52.4± 1.32	4.8±0.58	1.2
	1.0	1.0		62.6 ±1.34	5.2±0.62	1.4
	1.5	1.0		73.8 ±1.38	5.6±0.64	1.8
	2.0	1.0		78.2 ±1.42	6.2±0.68	2.4
	2.5	1.0		66.4± 1.30	5.4±0.58	1.6
	3.0	1.0		60.2±1.26	5.2±0.50	1.2
				56.8±1.32	4.6±0.60	1.2
	0.5	-	1.0	46.5 ±1.20	3.8 ±0.57	1.0
	1.0	-	1.0	55.2 ±1.24	4.2 ±0.57	1.2
	1.5	-	1.0	67.6 ±1.25	4.6 ±0.58	1.6
	2.0	-	1.0	70.4 ±1.28	5.2 ±0.60	2.0
	2.5	-	1.0	62.3 ±1.24	4.6 ±0.58	1.6
	3.0	-	1.0	55.4 ±1.24	4.0±0.57	1.4

Table 3 :Showing Initiation of Roots From The Tissue Culture Raised Plantlets of *S.xanthocarpum*

Growth Regulators IBA Mg/l	% response for root induction	Mean number of roots +SD
0.5	18.5	3.6±1.26
1.0	30.5	3.8±1.26
1.5	42.6	6.2±1.24
2.0	48.4	8.4±1.24
2.5	51.7	8.6±1.24
3.0	58.2	9.2±1.23
3.5	78.8	12.4±1.26
4.0	56.3	6.2±1.22
5.0	26.5	4.0±1.24

of KN had reduced promoting impact.

From the table 1, the mean number of shoots and their length can be noted. It was observed that at 0.5 mg/l BAP + 0.5mg/l IBA the mean number of axillary branches were 4.5 and at 2.0 mg/l BAP + 0.5 mg/l IBA it was 8.7 respectively. At BAP 0.5mg/l + 1.0 mg/l IBA and 2.0 mg/l BAP +1.0 mg/l IBA the number was 5.2 and 9.6 respectively. At the similar above concentration of IAA the number of branches was 3.8 and 5.2, and 4.2 and 5.8 respectively.

Kinetin at 0.5 mg/l + IBA 0.5 mg/l induced somatic buds which were 4.4, while at 2.0 mg/l Kinetin +0.5 mg/l IBA it was 6.0. At 0.5 mg/l Kinetin + 1.0mg/l IBA the number was 4.8 and at 2.0 mg/l Kinetin + 1.0 mg/l IBA , it was 6.2 . At the similar above concentration of Kinetin with IAA induced budding which were 3.2 and 4.8 and3.8 and 5.2

Figure A: Multiple Shoot Induction From Callus

Figure B: Multiplication of Shoots Raised Through Tissue Culture

respectively Figure (A).

The mean length of the branch was also calculated. At 0.5mg/l BAP + 0.5 mg/l IBA it was 1.5 cm while at 2.0 mg/l BAP +0.5 mg/l IBA the length was 2.6 cm. At 0.5 mg/l BAP +1.0 mg/l IBA it was 1.8 and at 2.0 mg/l BAP +1.0 mg/l IBA 2.8 cm. At the similar conditions of BAP and IAA the length was 1.2, 2.2 and 1.4 and 2.6 cm respectively.

At the same concentration of kinetin the length was 1.2, 2.21.2 and 2.4 cm respectively. While kinetin with 0.5mg/l and 1.0 mg/l IAA had 1.0 and 1.8,1.0 and 2.0 cm respectively.

DISCUSSION AND CONCLUSION

Multiple shoot proliferation was obtained from nodal explants .The multiple shoot initiation from the explants was observed after 15 days of inoculation. Highest no. of multiple was 9.6 in the MS medium supplemented with 2.0mg/l BAP +1.0mg/l IBA, followed by similar combination of BAP + 0.5 mg/l IBA that was 8.7. Kinetin at the same concentration had lesser no. of shoots.

The multiple shoots induction and elongation of shoots was obtained at the same medium. It is clear from the above finding that the relative effectiveness of BAP and KN varied for in vitro multiple shoot regeneration from the nodal explants. 2.0 mg/l BAP with 1.0mg/l IBA was found to be the best concentration for generation of maximum number of shoot buds (9.6). At the same concentration the elongation was also higher than other combination of growth regulators. In this way the ability of shoot proliferation from the nodal explants of *S. xanthocarpum* (white flower) depended on hormonal variations. Bud initiation took place only in the presence of Cytokinin and no bud initiation was found in the basal medium alone .Tulika and Shukla ,2009 in *Withenia somnifera* also reported similar findings. Pawar et al., (2000) reported that BAP and KN in combination induced higher frequency of adventitious shoots from the nodal explants of

S.xanthocarpum. The present report is thus also being supported by the above finding.

Experiments for rooting Table, 3 in the above plantlets,raised through tissue culture. IBA at 3.5 mg/l concentration, the average number of roots was 12.4.It was further noted that both lower concentration that is 1.0 mg/l and higher concentrations i.e. 5.0 mg/l IBA had less promising.

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