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TISSUE CULTURE STUDY FOR EFFICIENT CALLUS INDUCTION FROM INTERNODAL EXPLANTS OF *Asparagus racemosus* WILLD AN IMPORTANT MEDICINAL HERB

RITIKA KUMARI[a1], ANUPAM KUMARI[b] AND A.K. SINGH[c]

[abc]Department of Botany, B.R. Ambedkar Bihar University, Muzaffarpur, Bihar, India

ABSTRACT

Tissue culture study was performed to standardize the technique and select suitable plant growth regulators and their concentrations for efficient callus induction from the nodal explants of *Asparagus racemosus* willd, an important medicinal herb. Different concentrations of NAA, 2,4-D, BAP and KN were supplemented in MS basal medium in which the nodal explants were inoculated. It was observed that MS + 0.2 mg/l NAA was the best culture condition in which maximum percentage of response 90.36 was obtained. This was followed by the culture conditions that were MS+ 2.0 mg/l where the percentage response was 88.68%. Similarly, next higher percentage 88.62 was observed in MS+1.0 mg/l BAP + 1.0 mg/l NAA. It was further observed that growth rate was excellent where the percentage response was higher. Second it was noted that the calli had both compact and friable texture and their colour varied from green yellow to brown even where the growth was excellent. It was further noted that, NAA or BAP alone at higher concentration and KN alone at lower concentration when used alone had no response for callusing. However, KN at higher concentration 2.0 mg/l gave the highest percentage of response for callusing. NAA + BAP and NAA + KN at the similar concentrations gave different result. In the MS medium having 1.0 mg/l BAP + 1.0 mg/l NAA the percentage of response was 88.62 while MS + 1.0 mg/l KN + 1.0 mg/l NAA the percentage of response was 62.34 only. Growth rate of the calli also differed in different culture conditions.

KEYWORDS: Nodal Explants, Callus, Friable, Compact, *Asparagus racemosus*, Plant Growth Regulator

ABBREVIATIONS: MS = Murashige and Skoog, NAA = Naphthalene acetic acid, 2,4-D = 2,4- Dichlorophenoxy acetic acid, BAP = 6, Benzyl amino purine, KN = Kinetin

Asparagus racemosus willd, of family *Liliaceae* /*Asparagaceae* is a thorny herbaceous climber. It is commonly called Shatavari or Shatavar in Hindi. The stem is woody, much branched and roots are tuberous. These roots bear several medicinal properties. Shatavari has been mentioned in Ayurvedic text like Charak Sanhita and Sushrut Sanhita, (Asha and Pawan, 2012). In Ayurveda the plant is called the queen of herbs and is the primary herb recommended for female health. There are several diseases for which the powdered roots of Shatavar are being given. It is beneficial in the treatment of infertility, impotency, leucorrhoea, menopause syndrome and incase of herpes and syphilis. It is also used to cure epilepsy, kidney disorders, chronic fevers, stomach ulcers, liver cancer, to increase milk secretion. *Asparagus racemosus* is considered as the most potent female health tonic. Its use reduces the sizzling emotions such irritability, anger, jealousy, resentment, and hatred. There are several experimental evidences that support the above action. Some of them may be cited here such as, Mathur *et al*., (1994), Shao *et al*., (1997), Mandal *et al*., (2000), Saxena and Chauraisa (2001), Datta *et al*., (2002), Goel *et al*., (2006), Bopana and Saxena (2007), Singh *et al*., (2009), Pise *et al*., (2011), Sharma *et al*., (2011), Trivedi *et al*., (2011), Acharya *et al*., (2012), Joshi *et al*., (2012), Alok *et al*., (2013), Bansode *et al*., (2014). Medicinal plants do produce secondary metabolites of different groups. These phytochemicals are extracted from different parts. Because these secondary metabolites are produced at low concentration, so large number of plants is required for the extraction of these metabolites. This has posed a threat on the survival of such species. Alternative technique for the production of such secondary metabolites is tissue culture. Here the callus may be induced and such metabolites can be extracted. From these calli the cell suspension cultures can be prepared. Such cultures may exploit for enhanced rate of production of desired secondary metabolites.

Tissue culture studies of *Asparagus racemosa* as well other medicinal plants have been done by different workers. Some of them may be mentioned here, such as Gyulai *et al* (1992), Kohnura *et al*., (1994), Mathur *et al*., (1994), Mustapha *et al*., (1996), Dihan *et al*., (2001), Saxena and Chaurasia (2001), Martin (2004), Goel *et al*., (2006), Mehta and Subramaniyan (2005), Zhang *et al* (2006), Bopana and Saxena (2008), Lin *et al*., (2008), Nishitha and Sanjay (2008), Bakheeet (2010), Bojnauth *et al*., (2010), Ojha *et al*., (2010), Chaudhary and Dantu (2011), Sharma *et al*., (2012), Mahendra and Bai (2012), Renjiana *et al*., (2012), Anandan *et al*., (2012), Sharma and Sharma (2013), Bansode *et al*., (2014), Patel and Patel (2015), Elimira *et al*., (2017), Azad and Momad (2017), Pank and Joshi (2017), Chandra *et al*., (2018),

[1]Corresponding author

Chaudhary and Dantu (2019). Keeping all these ideas in mind, tissue culture study was preformed to induce callus on nodal explants of *Asparagus racemosus* willd.

MATERIALS AND METHODS

Plant Materials

For obtaining sterile plant materials to be used as an explants, healthy seeds were collected from the plants growing in the university campus. Seeds were first washed with running tap water, flowed by washing with liquid detergent for 15 minutes. Above seeds were washed again with glass distilled water 3-4 times to remove the detergent. These seeds were then treated with 0.2% Bavistin solution for 5 minutes. The flasks were shaken time to time for uniform treatment of the seed surface. Above seeds were germinated on MS (Murashige and Skoog, 1962) medium, containing 3% sucrose and 0.7% agar. The pH of the medium was adjusted to 5.8, before autoclaving at 15 lb pressure. There were no growth hormones. 12 ml of above autoclaved medium was dispensed in 25X150 mm Borosil culture tubes under aseptic condition. When the medium solidified, it was stored and next day used for inoculation of seeds. 2-3 seeds were placed on the above medium under Laminar flow air chamber. These tubes were incubated in culture room at low light intensity. After 40 days seedlings were seen. They were sub-cultured in the same medium to get much branched and well grown plants.

In the mean time stock solutions for different constituents of MS basal medium as well different plant growth regulators were prepared separately. These stock solution had 20X and 200X concentrations. Now for the preparation of 1 liter of medium desired amount form different stock solutions were taken in one liter conical flask. With the help of sterilized distilled water the volume was made 500 ml. In another 500 ml, 8 g of agar powder was dissolved carefully. These two solutions were mixed and 30 g sucrose was dissolved in it. The pH was adjusted to 5.8. Above culture medium was supplemented separately, 0.2, 0.5, 1.0, 1.5 and 2.0 mg/l of NAA, 2,4-D, BAP and Kinetin. In separate experiments the medium was supplemented with 0.2, 0.5, 1.0, 2.0 mg/l NAA along with 0.2, 0.5, 1.0, 2.0 mg/l BAP and 0.2, 0.5 and 1.0 mg/l NAA + 0.2, 0.5 and 1.0 mg/l KN separately. 40ml of the molten media were dispensed in culture jar, of 250 ml. The jar was covered properly and was sterilized at 15 lb pressure for 20 min. All the culture jars were taken out and allowed to cool at room temperature. They were stored in freeze and used for inoculation.

Plants of *Asparagus racemosus* grown in the laboratory on hormone free MS medium were used for explants. Nodes were excised and pieces of 1.0 cm were inoculated in above prepared media, inside the laminar air flow chamber. Above culture jars with explants were incubated in culture room at 26 ± 1^0C and at 3000 lux, light produced by the cool white fluorescent tubes. The photoperiods were adjusted at 16 h light and 8 h dark. All the experiments were repeated thrice and each time 15 culture jars were inoculated and incubated. Observation was made on an alternate day. Jars showing contamination were discarded after autoclaving. Observation was also done for the total number of explants, responding for callus induction, the growth rate of calli, their texture and colour etc. Mean of the data was tabulated and used for discussion.

RESULTS

From the table 1, it may be noted that the response of the nodal explants inoculated in MS medium supplemented with different plant growth regulators at different concentrations either alone or in combinations varied considerably. It may be noted from the table 1, that maximum percentage of response for callus induction was in MS + 0.2 mg/l NAA alone that was 90.36. This was followed by the explants inoculated in MS + 0.5 mg/l NAA that was 82.48. The explants, in MS + 0.2 mg/l 2,4-D had lower percentage of response which was 63.54. However, in MS + 0.5 mg/l 2,4-D the percentage of response was 72.18. It may be further noted that the percentage of response in MS + 0.2 mg/l BAP was 62.74 and in MS + 0.5 mg/l BAP 58.45 respectively. There was no callusing when the explants were inoculated in MS + 0.2 mg/l KN or MS + 0.5 mg/l of KN. However, the percentage response for callusing in MS + 2.0 mg/l KN was 88.78. It may be noted from the table that in MS+ 2.0 mg/l NAA medium there was zero response. When 1.0 mg/l NAA + 1.0 m/gl BAP were supplemented in MS medium the percentage of response for callus induction was 88.62. Whereas MS + 0.5 mg/l NAA + 0.5 mg/l KN induced callusing in 78.62% of the explants.

Growth rate, texture and colour of the calli were also observed. From the table it was noted that excellent growth rate was among the cultures where the percentage of response was the maximum. Similarly, the texture of the calli varied form compact to friable. However, here the texture and nature of the calli were not dependent on

the percentage of response for callus induction. The colour of the calli varied form green, to green yellow, to brown or dark brown- or light green to light brown.

Table 1: Impact of different concentrations of cytokinins and auxins, supplemented in MS basal medium individually or in different combinations on callus induction and its growth, induced form nodal explants of *Asparagus racemosus* willd

NAA	2,4-D	BAP	KN	% Response for callusing	Growth rate	Texture	Colour
0.2	--	--	--	90.36	+ + + +	Compact	Greenish yellow
0.5	--	--	--	82.48	+ + +	Compact	Green
1.0	--	--	--	58.24	+ +	Friable	Green
1.5	--	--	--	46.58	+ +	Compact	Brown
2.0	--	--	--	--	- -	--	--
--	0.2	--	--	63.54	+ +	Friable	Greenish
--	0.5	--	--	72.18	+ + +	Compact	Green
--	1.0	--	--	42.64	+ +	Compact	Brown
--	1.5	--	--	61.28	+ +	Compact	Dark Brown
--	2.0	--	--	68.30	+ + +	Compact	Brown
--	--	0.2	--	62.74	+ +	Friable	Brown
--	--	0.5	--	58.45	+ +	Friable	Dark Brown
--	--	1.0	--	51.26	+ +	Friable	Light Green
--	--	1.5	--	--	- -	--	--
--	--	2.0	--	--	- -	--	--
--	--	--	0.2	--	--	--	--
--	--	--	0.5	--	--	--	--
--	--	--	1.0	47.28	+ +	Compact	Brown
--	--	--	1.5	62.34	+ +	Compact	Green
--	--	--	2.0	88.78	+ + + +	Friable	Dark Brown
0.2	--	0.2	--	43.56	+ +	Compact	Light Brown
0.5	--	0.5	--	68.74	+ +	Compact	Green
0.5	--	1.0	--	57.62	+ +	Friable	Brown
0.5	--	2.0	--	73.56	+ + +	Friable	Green
1.5	--	1.0	--	62.38	+ +	Friable	Green
1.0	--	1.0	--	88.62	+ + + +	Friable	Brown
2.0	--	1.0	--	66.54	+ +	Compact	Brown
0.1	--	--	0.1	38.74	+ +	Friable	Green
0.5	--	--	0.5	78.62	+ + + +	Compact	Brown
1.0	--	--	1.0	62.34	+ +	Friable	Light Brown

+ + + + Excellent, + + + Best, + + Better, + Growth

DISCUSSION

In the present study, experiments were done to induce calli on nodal explants of *Asparagus racemosus*. It was observed that nodal explants responded for callusing in MS basal medium, supplemented with different concentrations of NAA and 2,4-D alone as well as with BAP and KN. Similarly, BAP and Kinetin alone were also tested for callusing. Here best response for callus induction and growth was noted on MS + 0.2 mg/l NAA. It was further noted that in this case increasing concentrations of the auxins had no promising impact rather there was no response for callusing in MS + 2.0 mg/l NAA. This was also noted in case of MS + 0.5 mg/l 2,4-D where there was maximum response and at MS + 0.2 mg/l BAP alone the response was better but at higher concentration there was no response at all. Just reverse to the above findings MS + KN at higher concentration maximum percentage of response for callus induction 88.78 was found. It was further noted that MS + 1.0 mg/l NAA + 1.0 mg/l BAP gave better response, but either NAA or BAP at 1.0 mg/l had lower response. This may be due to the fact that for callusing, interaction of

cytokinin & auxins is essential. However, for NAA + KN there was lower percentage of response. Present findings corroborate with the findings of Gyulai *et al*., (1992), Kohmura *et al*., (1994), Mustapha *et al*., (1996), Sharma *et al*., (2011), Dezfuli *et al*., (2013), Patel and Patel (2015), Azad *et al*., (2017), Pank and Joshi (2017), Lamor *et al*., (2018), and Chaudhary and Dantu (2019). These workers induced calli for morphogenetic studies. Trivedi *et al*., (2010), used Thidiazuron to induce callus in *A. racemosus*. They also found that the explants had different response for callusing in different concentrations of Thidiazuron.

CONCLUSION

The calli induced are being further cultured to get embryoids, or for morphogenesis. It may be used for generation of suspension culture and production of secondary metabolites. In suspension culture we can use the precursors for specific secondary metabolite or can enhance the rate of secondary metabolite synthesis by adding elicitors. It has been reported that biomass accumulation was correlated with saponin production over 30 day culture cycle. The saponin concentration in callus raised from the explant taken from *A. racemosus* was 20 fold higher than the plants found in natural habitat. (Pise *et al*., 2011). Therefore, induced calli may be exploited for various purposes.

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