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STUDY ON EFFECT OF DIFFERENT PLANT GROWTH REGULATORS AND ADJUVANT ON CALLUS INDUCTION AND GROWTH OF THE CALLI, RAISED FROM DIFFERENT EXPLANTS OF *Bacopa monnieri* (LINN) PENNELL AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

Tissue culture experiments were done to observe different concentrations of 2,4-D and KN on callogenic potential of three different explants viz., young leaf, mature leaf and internodal segment of *Bacopa monnieri*, and impact of adjuvant on the growth rate of the above calli. In the present work it was noted that all the three explants responded for callusing in different concentrations of 2,4-D and Kinetin, but the highest percentage of response 93.78 and 91.56 for swelling and callus induction was in MS + 1.5 mg/l 2,4-D + 0.4 mg/l Kinetin, respectively. This was followed by the internodal explants in the same medium that was 87.48 for swelling and 76.38 for callusing. Here lowest percentage of response for swelling in young leaf explants was 74.38 and 70.62 for callus induction. It was further observed that all the three explants when cultured in MS + 1.0 mg/l 2,4-D + 0.3 mg/l Kinetin revealed the similar trends as noted above. In the next experiment callus raised from the mature leaf explants in MS + 1.5 mg/l 2,4-D + 0.4 mg/l Kinetin were sub cultured in the same medium but with coconut milk as an adjuvant in the ratio of 0.5%, 1.0% and 1.5%. After four weeks of incubation the growth rate and biomass of the calli were calculated. Here better growth of the calli as evident from the diameter and fresh weight, was in MS + 1.5 mg/l 2,4-D + 0.4 mg/l KN + 1.5% coconut milk. The growth rate, and fresh weight of the callus was lowest when cultured in MS + 1.5 mg/l 2,4-D + 0.4 mg/l KN + 0.5% coconut milk added as an adjuvant.

KEYWORDS: Explant, Adjuvant, Coconut Milk, Swelling, Subculture, Fresh Weight, *Bacopa monnieri* (L)

Bacopa monnieri (Linn) Pennell, belongs to family *Serophulariaceae*. This plant is commonly called as “Brahmni” by the local people. Plants are much branched, annual, and grows in moist soil. Its plants can be observed in wild habitat from June to October. There is natural senescence after this. Plants may survive in submerged state for several days and among the green leaves, white flowers may attract the plant collectors. On the prostrate branches, roots are produced from the node that touches the ground.

Based on the medicinal values, its demands at national and international market, potential for research, for the presence of most important secondary metabolites –Bacosides, this plant has been placed second in priority list of the most important medicinal plants (Srivastava and Rajani, 1999). According to Tripathy *et al*; (1996) due to its antioxidant property it stimulates brain circulation and enhances level of serotonin, which reduces stress and other brain disorders. It has been used as a brain tonic in Ayurvedic system of medicine to improve memory, concentration, learning as well as to cure mental illness. (Srivastva and Rajani,1999). Mehta (2017) reported that the plant is being used for the treatment of Asthma, insanity, epilepsy, hoarseness, enlargement of spleen, against snake bites to cure, rheumatism, leprosy, eczema, and other skin diseases. Presence of most important saponins such as, Bacoside A, B, C, and D in *Bacopa monnieri* has been reported by Majumdar *et al*; (2013). Bacoside-A is being used for the production of memory enhancing capsule. Ali *et al*; (2000), reported that *Bacopa monnieri* is being used in phytoremediation of heavy metal such as Cadmium, mercury and zinc.

Tissue culture studies in medicinal plants for micropropagation and callus induction, cell suspension cultures and production of desired secondary metabolites have been reported by different workers, in India and abroad. Some of them are being mentioned here such as Arumugam and Bhojwani (1990); Martin (2004); Wood Ward and Bartel (2005); Sakakibara (2006); Chaturvedi *et al*; (2007); Wang *et al*; (2008); Siva *et al*; (2009); Das and Mandal (2010); Joshi *et al*; (2010); Juan *et al*; (2010); Madhukar *et al*; (2011); Rao *et al*; (2011); Subashri and Kolipalli (2012); Jay Kumar and Lingam (2013); Kalsaitkar *et al*; (2014); Kumari Ritika *et al*; (2014); Thangavel *et al*; (2014); Anita (2017); Abiri *et al*; (2017); Khattak *et al*; (2017); Rajeev *et al*; (2018). In the present work, impacts of plant growth regulators and adjuvant were observed on three different explants of *Bacopa monnieri* Linn.

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MATERIALS AND METHODS

The Plant Material

Plants of *Bacopa monnieri* (L.) growing in wild habitat were located in the campus of University Department of Botany, on the basis of its morphological characters. Different weeds growing near it were uprooted. In this the plant was free to grow there. Few healthy braches were carefully excised with pre-sterilized blade. These branches were collected in a poly bag and within 6 minutes were brought to the laboratory. The basal and apical portion of the branches were trimmed and washed under running tap water. Because they were placed in a 2 L, conical flask whose mouth was covered with muslin cloths. Therefore, they were circulating in water but not going out. This was done for 45 min. All the branches were taken out and kept in a pre-sterilized Petri Plate. In the aseptic condition of laminar airflow cabinet, above materials were treated with 0.1% mercuric chloride aqueous solution for two minutes. For uniform contact with the solution of the flaks in which treatment was done. It was shaken manually. All the materials were transferred in another conical flask and were rinsed thrice carefully to remove even a trace of the chemical from the surface of the plant material.

From the above branches, mature leaves, immature leaves and the internodal explants were prepared. These explants were stored in pre-sterilized and moistened muslin cloth at low temperature.

Preparation of Culture Medium

All the chemicals and plant growth regulators were of Hi-Media products. Stock solutions of higher concentrations were prepared for different ingredients of Murashige and Skoog (1962) medium. Similarly, stock solutions of plant growth regulators were prepared. Required volumes of different stock solutions were taken for one liter of medium. The volume was made 500 ml by adding distilled water. 30 g (0.3%) of sucrose was added. By adding N HCl the pH was adjusted to 5.8. To this 8 g of molten agar was added. Now desired amounts of plant growth regulators were added. 25 ml of the above medium was taken in 125 ml culture flask. In this way all the medium was dispensed into culture flasks. The mouth was plugged with non-absorbent cotton plugs, covered with muslin cloth. Finally the plugs were wrapped with Aluminum foil to avoid wetting of the plugs during autoclaving. All the culture flasks with medium were autoclaved at 15 lb pressure for 20 min. Flasks were taken out and allowed to cool at room temperature. All the flasks were stored in freeze and were used for inoculation after three days.

All the tools such as forceps, scalpel, blade, scissors, beakers and Petri plates were sterilized in hot air oven before use. Inoculation was done in the aseptic cabinet of laminar airflow. All precautions were taken to avoid contamination. Inoculated culture flasks were incubated in culture rooms where temperature 26±1^{0}C, moisture 66% and light 3000 lux were maintained artificially. Observations for contamination, response for callus induction time taken for callusing, the texture and growth rate of calli were done on an alternate day. All the experiments were done in triplicate and each time 20 cultures were used. Mean of the data were present in tables 1-3.

Impact of three different concentrations of coconut milk, 0.5%, 1.0% and 1.5% were observed on callus biomass in subculture. Fresh coconut milk was taken in a pre-sterilized beaker. It was boiled to deproteinate. This was filtered through double layered muslin cloth. Calli of known biomass were subcultured in the same medium. Here the deproteinated coconut milk was added to get 0.5%, 1.0%, and 1.5%. The mean of the data was represented in table-4. Observations were made on alternate day. Final reading was taken after 4 weeks of inoculation. 200 mg callus mass was sued for inoculation. MS medium with growth regulator but without coconut milk was used as control. Data were placed in table-4.

RESULTS AND DISCUSSION

From the table 1-3, it was noted that all the three explants such as young leaf, mature leaf and the internodal explants responded in all the cultures used here but with different percentage of response.

When inoculated in MS + 0.5 mg/l 2,4-D + 0.2 mg/l KN, the percentage response for callusing was 64.65 for mature leaf explants followed by 59.82 for internodal explants and 49.52 for the immature leaf explants, which was the lowest. When the explants were inoculated in MS + 1.0 mg/l 2,4-D + 0.3 mg/l KN, the percentage of response for callusing was again highest 69.56 in the case of mature leaf explants, followed by 64.72 for internodal and 61.72 for the immature leaf explants which was the lowest. From the table-3, it was noted that percentage response for callusing in mature leaf explants was 91.56, for internodal explants 76.38 and for immature leaf explants 70.62 in MS + 1.5 mg/l 2,4-D + 0.4 mg/l Kinetin.

Table 1: Showing effect of concentration of auxin and kinetin on callus induction in three explants of *Bacopa monnieri* (L.)

Concentration	MS + 0.5 mg/l 2,4-D + 0.2 mg/l Kinetin			
Explant	% of swelling	% response for callusing	Time taken for callusing	Colour of the callus
Young leaf	62.35	49.52	15 Days	Green White
Mature leaf	71.54	64.76	13 Days	Greenish
Internodal segment	68.28	59.82	14 Days	Yellow Green

Table 2: Showing effect of concentration of auxin and kinetin on callus induction in three explants of *Bacopa monnieri* (L.)

Concentration	MS + 1.0 mg/l 2,4-D + 0.3 mg/l Kinetin			
Explant	% of swelling	% response for callusing	Time taken for callusing	Colour of the callus
Young leaf	66.45	61.72	14 Days	Dull White
Mature leaf	77.35	69.56	11 Days	Green White
Internodal segment	68.25	63.70	12 Days	Yellow White

Table 3: Showing effect of concentration of auxin and kinetin on callus induction in three explants of *Bacopa monnieri* (L.)

Concentration	MS + 1.5 mg/l 2,4-D + 0.4 mg/l Kinetin			
Explant	% of swelling	% response for callusing	Time taken for callusing	Colour of the callus
Young leaf	74.38	70.62	12 Days	White
Mature leaf	93.68	91.56	10 Days	Green White
Internodal segment	86.42	76.38	11 Days	Yellow White

Table 4: Showing effect of concentration of auxin and kinetin on callus diameter, weight and % response, colour of callus after 6 weeks of incubation period in three different concentrations of coconut milk in *Bacopa monnieri* (L.)

Concentration	MS + 1.5 mg/l 2,4-D + 0.4 mg/l Kinetin			
Coconut milk	Colour after Period of incubation (6 weeks)	Diameter of calli in (mm)	Weight of the calli (mg)	% Response
+0.5%	Greenish	33.18	960.82	86.26
+1.0%	Dark Green	36.75	974.56	90.38
+1.5%	Dark Green	38.24	989.24	92.72
Control	White Green	22.16	675.38	76.42

Time taken for callus induction was 10 days for mature leaf explants, 11 days for internodal explants and 12 days for the immature leaf explants. Here maximum periods for callusing in mature leaf explants was 12 days, for internodal, 14 days and for immature leaf explants 15 days in MS + 0.5 mg/l 2,4-D + 0.2 mg/l KN. Here percentage of explants showing was maximum for all the explants in MS + 1.5 mg/l 2,4-D + 0.4 mg/l KN and minimum in MS + 0.5 mg/l 2,4-D + 0.2 mg/l KN.

Impact of three different concentrations 0.5%, 1.0%, and 1.5% of coconut milk on callus biomass was also observed. From the table-4, it was noted that maximum biomass of calli was in the medium MS + 1.5 mg/l 2,4-D + 0.4 mg/l KN + 1.5% coconut milk. Here the fresh weight was 989.42 mg and diameter was 38.24 mm and the response was 92.64% respectively after 6 week of culture. It was followed by the calli cultured in MS + 1.5 mg/l 2,4-D + 0.4 mg/l KN + 1.0% coconut milk, where

the fresh weight was 974.54 mg, diameter 36.75 mm and percentage of response was 90.38. The fresh weight of calli 960.32 mg, diameter 33.18 mm and percentage of response 86.26 were obtained in MS + 1.5 mg/l 2,4-D + 0.2 mg/l KN + 0.5% coconut milk.

DISCUSSION

Different explants such as immature, mature leaves, internodal segment of *Bacopa monnieri* were inoculated in MS basal medium along with 3% sucrose and different concentrations of 2,4-D and KN alone or with NAA. Here mature leaf explants were the best for callus induction than that of the immature leaf and internodal explants. Further in comparison to the auxins alone, auxins and cytokinin when used together was the best medium because here the percentage response for callusing was the maximum, while time taken for callus induction was the minimum. That mature leaves are the best explants for callus induction has also been reported by Patra *et al*; (1998) in *Centella asiatica*, Reddy *et al*; (2001) in Coleus, Matkowaski (2004) in *Nigella sativa, Azadirachta indica* and *Pueraria lobaba*. Sharath *et al*; (2007) in *Bacopa monnieri*, Osman *et al*; (2013) in *Lycium barbatum* L., and Sheikh *et al*; (2015). Thus findings of the preset work corroborate with the findings of above workers as here also mature leaf explants were more promising with respect to callogenic potential.

Addition of coconut milk in the form of adjuvant enhances the biomass production of calli in comparison of control. This finding is in agreement with the findings of Ardkani *et al*; (2017) who also reported impact f coconut milk and Casein hydrolysate on callus induction and somatic embryogenesis in *Stevia* spp.

CONCLUSION

Use of *Bacopa monnieri* L. for extraction of different Secondary metabolites in general and the Bacoside-A in particular have increased like anything. The heavy pressure due to unplanned harvesting of this plant from its natural population has forced the plant to be on the verge of extinction. *In vitro* micropropagation and enhanced callus biomass way rescue the species form the above threat. Here, callus biomass and suspension cultures may be done in presence of suitable elicitors. This will enhance the production of desired secondary metabolites in general and the Bacosides in particular. Thus finding of the present work may be exploited at commercial scale.

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