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Induction of Callus from Leaf and Internodal Segments of *Boerhaavia diffusa*, An Important Medicinal Herb.

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ABSTRACT:

In the present studies attempts were made to induce callus on leaf and internodal explants taken from healthy and young branch of *Boerhaavia diffusa*. These explants were cultured in MS medium, supplemented with 3.0% sucrose, gelled with 0.8% agar, and different concentrations and combinations of plant growth regulator. It was noted that leaf explants when culture in MS+1.0 mg/l 2,4-D, the response of explants for induction callus was 80.6% which was the highest. Here increase in concentration of 2,4-D had no impact on the percentage response of the explants for callusing. It was further noted that at 2.0 mg/l and 3.0 mg/l of 2,4-D concentration, the percentage of response was 66.7 and 61.2 respectively. When 1.0 mg/l 2,4-D + 0.5 mg/l BAP was added in the medium the percentage of response for callusing was 88.5, period of response was 8 days, growth rate was best and the texture of callus was white friable. Increase in concentration of BAP had also no promising effect on callusing. Similarly, when NAA at 1.0 mg/l and 1.5 mg/L with BAP at 0.5 mg/l and 1.0 mg/l had no better response and the percentage of response was the lowest that was 44.4 and 45.6 respectively. However, when 1.0 mg/l 2,4-D + 0.5 mg/l BAP + 10 ml of coconut water was added in MS medium the percentage of response was the highest that was 92.65, days of initiation was reduced that was 8.0, growth rate was excellent and the texture was white friable. In the similar culture conditions, internodal explants were also inoculated. But it was observed that the percentage of response for callusing, the time taken for callusing, growth rate of the calli, all were very different from leaf explants. Here the maximum percentage of response for callusing was 56.0, when the internodal explants were inoculated in MS + 1 mg/l 2,4-D + 0.5 mg/l BAP + 10 ml coconut water. The time taken for callusing was 12 days, growth was better and callus texture and colour was white friable respectively.

KEY WORDS:
Boerhaavia diffusa, Callus Induction, Explants, Inoculation, Texture of Callus, Adjuvant.

INTRODUCTION:

Boerhaavia diffusa L known as **Punarnawa** *(Nyctagenaceae),* is a wild medicinal herbs, which is perennial and creeping and has diffused branches in the natural habitat. The plant can be recognized from a distance due to the colour of its flower. Distinct nodes and internodes, nodes swollen, with opposite leaves. Inflorescence panicle. The pink purple colour of flowers is due to its sepals and there are no petals, so flower is incomplete. This plant is being used in traditional medicines. Even local people use this plant that is green leaves and tender leaves when they suffer from jaundice. There are reports regarding the secondary metabolites that have been isolated and identified in this medicinal herb. Mahesh *et al*; (2012); Muthulingam and Chaithanya, (2018), described in detail for the quality and quantity of different phytochemicals present in it. They reported total phenolic contents (TPC). Similarly, flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, proteins, saponnins, tannins, etc. have been isolated and identified. Boeravinone has been reported by Lami *et al*; (1990).

Different Ayruvedic medicines have been prepared from *Boerhaavia diffusa* such as Punarnavastaka, Punarnavakshar and Punarnava taila, which are being used for the treatment of different disease. It is being used to treat renal ailments as diuretic (Anand, 1995) and to treat Seminal weakness and blood pressure (Gaitonde *et al*; 1974). It is being also used to treat stomach ache, anemia, cough and cold, and as a diaphoretic, laxative, expectorant. It is also used as a potent antidote for snake and rat bites. (Chopra *et al*; 1956). It is also used in the treatment of hepatitis, gall bladder abnormalities and urinary disorders. (Mudgal, 1975, Cruz 1995). The flowers and seeds are used as contraceptive (Chopra *et al*; 1956).

MATERIALS & METHODS:

Preparation of Culture Medium:

MS (Murashige and Skoog, 1962) culture medium was prepared in which 3.0% sucrose was added. The medium was jelled with 0.8% agar (Hi-media). Before autoclaving the pH of the medium was adjusted to 5.8 with the help of 1 N NaOH and 1N HCl as was needed. Now different concentration of plant growth regulators such as 2,4-D (2,4-dichlorophenoxy acetic acid, BAP N6-Benzyl amino purine, and Naphthalene acetic acid were added either alone or in combinations).

Above culture medium was dispensed in Borosil make culture tube 150 mm X 25 mm and plugged with non-absorbent cotton plugs wrapped with muslin cloth. 10 such culture tubes were placed in plastic container and the plugs were covered with Aluminum foil to avoid moistening of the plugs during autoclaving. These tubes were autoclaved for 15 min at 15 lb pressure. At this pressure the temperature of steam becomes 121^0C. Then the pressure was released and with the help of cotton gloves, the container with culture tubes was taken out. Some tubes were placed in the slanting position. Next the medium was solidified. Culture tubes with medium were stored at low temperature for two days.

On third day, when inoculation was to be done, then explants were prepared. Healthy and young branches of *Boerhaavia* were collected from its wild habitat. It was collected in a beaker containing water. Just after collection the material was brought in the laboratory. The leaves were separated from the stem. Stem was also cut into small pieces 2 cm internodal segment. These materials were placed in 1L conical flask. The mouth of the flask was covered with muslin cloth with the help of rubber band. The flask was placed under running tap water. Water was going in the

flask and rotating out. Along with this the materials was also circulating but not coming out. This was done for 45 minutes. The explants were taken out and were treated with freshly prepared 0.1% (w/v) mercuric chloride solution for 3-4 minutes. During these flasks containing the explants was shaken vigorously, manually, so that there was uniform contact of the explants with the solution. Then the solution was discarded and the material was rinsed with glass distilled water for 3-4 times where each duration was 4-5 minutes. Each time used water was discarded and fresh water was used. Above explants were stored in pre-sterilized and moist cloth at low temperature. Inoculation was done under aseptic condition of Laminar Air Flow Chamber. The inoculated culture tubes, with leaf segment and internodal segment were incubated in culture room at 26±1^{0}C temperature, 64-66% relative humidity, and 16/8 light and dark photoperiod. Light was maintained at 3000 lux with cool white fluorescent light tubes. Cultures were observed on an alternate day. Tubes showing contamination were discarded after incubation.

RESULTS AND DISCUSSION:

In the present works, experiments were done to induce callus on leaf and internodal explants, cultured in MS medium supplemented with different concentrations and combinations of plant growth regulators. All experiments were done in triplicate and 20 cultures were taken in each cycle. The means of the data obtained for leaf explants were tabulated in table-1, and for internodal explants in table-2.

From the table-1, it may be noted that leaf explants when cultured in MS + 0.5 mg/l 2,4-D, there was 78% response for callusing and initiation of callusing was noted on 10th day of incubation. The growth rate was better and callus was white friable. This response percentage increased when the explants was cultured in MS + 1.0 mg/l 2,4-D, which was 80%, initiation time was 9th day growth was better and callus was white friable. It was further noted that increase in concentration of 2,4-D concentrations there was decrease in percentage of response and increase in callus initiation periods. At 3.0 mg/l 2,4-D, the percentage of response was 61.2% only and time of initiation of callus was 15th day, growth average. However, when the leaf explants were cultured in MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP there was an increase in percentage of response for callusing which was 88.5, decrease in days of initiation which was 8 days and the growth rate was the best. The callus was white friable. Increase in the concentration of 2,4-D + BAP had no promising effect on callus induction. Similarly, MS + 1.0 or 1.5 mg/l NAA + 0.5 or 1.0 mg/l BAP had no good response with respect to callus induction and all parameter were lower than above except the days of initiation. Surprisingly, it was noted that when leaf explants were inoculated in MS + 1.0 mg/l, 2,4-D + 0.5 mg/l BAP + 10% coconut water the percentage of callus induction was the maximum which was 92.65, the initiation days was only 8, growth rate was excellent and callus was white friable.

Internodal explants of *Boerhaavia diffusa* were also inoculated in the same compositions as was for the leaf explants. But here the percentage of response was very low in comparison to the leaf explants. For example in MS + 0.5 mg/l 2,4-D, the percentage of response was only 42.0, time taken for callus induction was 16 days, growth was average and the callus was white loose. Similarly, MS + 1.0 mg/l 2,4-D the response percentage was 48, time taken 15 days and growth was slightly better. Here also there was decrease in response percentage along with the increase in the

concentrations of 2,4-D. At MS + 3.0 mg/l 2,4-D, the response percentage was only 37 and time taken for initiation of callusing was 18 days, growth was average. However, when MS + 1.0 mg/l 2,4-D + 1.0 mg/l BAP was used for inoculation even there was no increase in percentage of response. It was also true for MS + 1.0 or 1.5 mg/l NAA + 0.5 or 1.0 mg/l BAP, there was no increase in percentage of response in callusing or decrease in time taken for its. However, when the internodal explants were inoculated in MS + 1.0 mg/l , 2,4-D + 0.5 mg/l BAP + 10% coconut water the percentage response for callusing increased 56.0, time was 12 days and growth was better.

DISCUSSION:

In the present study it was noted that leaf explants, initiate callusing in MS medium supplemented with 1.0 mg/l 2,4-D and the response was 80.6%. However, when the concentration of 2,4-D was increased the percentage of response for callusing decreased. Similarly, when in MS medium 1.0 mg/l 2,4-D + 0.5 mg/l BAP was added the percentage for response of callusing increased to 88.5. Leaf explants when inoculated in MS + NAA 1.0 or 1.5 mg/l + BAP 0.5 or 1.0 mg/L had no promising impact. But when the leaf explants were cultured in MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP + 10% coconut water the response for callusing was the maximum that was 92.65. Present findings are in agreement with the findings of Harsha Kanfade *et al*; (2011); Souza *et al*; (2014) and Pandey *et al*; (2019).

Internodal explants gave poor response with respect to callus induction in similar culture conditions in which the leaf explants were cultured. Internodal explants responded 54.0% for callus induction in MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP. Here addition of 10% coconut water had no much impact on callus induction.

Tissue culture studies for micropropagation and induction of callus in medicinal plants have been done by different workers. Some of them are being mentioned here such as Rani *et al*; (2003) in *Withania somnifera* L.; Faisal and Anis (2006); in *Mucuna pruriens* L., Sivanesan and Jeong (2007) in *Sida cordifolia*; Ray and Bhattacharya (2008) in *Boerhaavia diffusa* L.., Sudersana *et al*; (2008) in *Boerhaavia diffusa* L.; Biswas *et al*; (2009) in *Boerhaavia diffusa*; Siddiqui and Anis (2009) in *Balanites aegyptica* L., Jeifer *et al*; (2012) in *Boerhaavia diffusa* L.; Ragi and Sibu (2014) in *Boerhaavia diffusa*; Cheruvathur *et al*; (2015); Srivastava *et al*; (2015); Deepak *et al*; (2016) in *Salacia oblonga*, Pandey *et al*; (2019) in *Boerhaavia diffusa*. In *Boerhaavia diffusa*, they have worked for micropropagation in general. In the present work, attempt has been made to induce callus, which may be sued for the extraction of important secondary metabolites without destructing the plants in its natural population. Here it may be concluded that leaf is the best explants had MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP + 10% coconut water is the best culture medium for maximum callusing in *in vitro*.

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CONFLICTS:

There are no conflicts regarding publication of this paper.

TABLES:

Table-1

Induction of callus on leaf explants of *Boerhaavia diffusa* cultured on MS basal medium with different growth regulators and coconut water as adjuvant.

Plant Growth Regulators (mg/l)		% Response	Day after	Growth rate	Texture of the callus
2,4-D	BAP				
0.5	--	78.0	10.0	+ + +	White Friable
1.0	--	80.6	9.0	+ + + +	White Friable
1.5	--	72.5	12.0	+ + +	White Compact
2.0	--	66.7	13.0	+ + +	Yellow Compact
2.5	--	63.4	13.0	+ + +	Yellow Compact
3.0	--	61.2	15.0	+ +	Yellow Compact
1.0	0.5	88.5	8.0	+ + + +	White Friable
2.0	1.0	67.3	12.0	+ +	White Compact
NAA	BAP				
1.0	0.5	45.6	13.0	+	Yellow Compact
1.5	1.0	44.4	14.0	+	Yellow Compact
2,4-D	BAP				
1.0	0.5 +10 ml C.W.	92.64	8.0	+ + + + +	White Friable
+ Poor Growth, + + Good Growth, + + + Better Growth, + + + + Best Growth, + + + + + Excellent					

Table-2

Induction of callus on internodal explants of *Boerhaavia diffusa* cultured on MS basal medium with different growth regulators and coconut water as adjuvant.

Plant Growth Regulators (mg/l)		% Response	Day after	Growth rate	Texture of the callus
2,4-D	BAP				
0.5	--	42	16	+	White Loose
1.0	--	48	15	+ +	White Compact
1.5	--	41	17	+	White Loose
2.0	--	39	17	+	White Loose
2.5	--	36	18	+	White Loose
3.0	--	37	18	+	White Loose
1.0	0.5	54	14	+ + +	White Friable
2.0	1.0	40	16	+ +	White Compact
NAA	BAP				
1.0	0.5	38	17	+	White Loose
1.5	1.0	36	18	+	White Loose
2,4-D	BAP				
1.0	0.5 +10 ml C.W.	56	12	+ + +	White Friable
+ Poor Growth, + + Good Growth, + + + Better Growth, + + + + Best Growth, + + + + + Excellent					

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