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Invitroshoot multiplication response from shoot tips of BergenialigulataEngl. on different nutrient media-A comparative study

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Abstract:

Invitro raised shoot tips of Bergenialigulata were cultured on various media viz. MS (both full and half strength), modified MS, Gamborg's, Nitsch&Nitsch and White's media to score better shoot multiplication response for effective micropropagation. All these media were supplemented with varying concentration of Kinetin (2.5-15 μM) and same concentration of IAA i.e.7.5μM. Although very little shoot regeneration was observed on MS full strength, Gamborg's, and Nitsch&Nitsch media, modified MS medium was found to be best medium for shoot regeneration , supplemented with Kinetin and IAA. MS half strength and White's media did not show any response in terms of shoot formation.

Keywords: *Bergenia, Invitro, MS ,Nitsch&Nitsch ,Nutrient media*

Abbrevations*: MS-Murashige and Skoog; NN-Nitsch&Nitsch, IAA –Indole 3-acetic acid, Kn –Kinetin*

1.Introduction

The relationship between plants and humans has always been close and interdependent. Drugs, used to cure disease and relieve sufferings, are to a great extent plant products. Right from beginning traditional knowledge especially on medicinal uses of plants has provided many drugs of modern day [1].Even today this area holds much more hidden treasure as almost 80% of human population in developing countries is dependent on plant resources for health care [2][3]. Nowadays due to increasing awareness towards the herbal products there is tremendous pressure on Himalayan medicinal plants. There has been a rapid decline in biodiversity of the world, more particularly during the last two decades or so. Biodiversity losses have been alarming in developing countries in tropics and occur due to habitat destruction, over harvesting, pollution, inappropriate and often accidental introduction of exotic plants. Herbal Conservation of Biodiversity is one of the paramount concerns the world over, there is an urgent need to conserve the genetic diversity of medicinal plant resource as the interests in herbal medicine is increasing.

Invitro culture techniques have been employed on a number of instances for example to obtain virus free plant material [4] to regenerate recalcitrant woody species or species which do not set seeds [5] [6] to establish rapid and effective micropropagation protocols [7] [8] .Recently, particular attention has been paid to invitrotechniques as a means to preserve endangered species [9] [10].

Bergenialigulata commonlyknown as Rock foil belongs to family saxifragaceae, is distributed in Temperate Himalayan region (from Kashmir to Nepal) from 2000-2700m, and is very common on moist rocks and under forest shade 1900-2600m in Kashmir. The plant has medicinal importance and is used traditionally to treat a variety of diseases. Plant extracts of B. ligulata have been reported to possess antioxidant activity [11]. It has been listed as official herb in the Indian Herbal Pharmacopoeia [12].The alcoholic extract of the plant has been reported to exhibit significant anti-inflammatory, analgesic and diuretic properties. It is therapeutically categorized as an anti-inflammatory [13] and antiurolithiatic [12]. Plant has considerable antiviral activities against influenza virus (RNA) and HSV (DNA) [14]. In Kashmir B. ligulatahas been reported to be used in fever, diarrhoea and applied to bruises and boils [15]. The plant contains afzelechin , a type of flavin -3-ol [16] and its dried rhizomes constitute the drug that contains an active principle bergenin as well as gallic acid, glucose, mucilage , wax apart from β-sitosterol and four flavonoids. Due to indiscriminate collection and over exploitation of natural resources this

plants is rapidly disappearing and is therefore listed under vulnerable category [17] [18] [19]. No steps have been taken for conservation of this species so far. Realizing the threat of extinction there is need to develop quick propagation protocols for its conservation strategies.

2. Materials and Methods

Invitro grown shoot tips of B. ligulata were used as explants, these shoot tips were inoculated on different media viz. MS [20] both full and half strength, modified MS (with salts of MS and vitamins of Nitsch and Nitsch), Gamborg's [21], Nitsch&Nitsch [22] and White's media [23]. All these media were enriched with Kn (2.5-15μM) and IAA (7.5 μM).The pH of the medium was adjusted to 5.5 before gelling the medium with 0.8% Difco- bactoagar. The cultures were maintained at 25 ± 2^0 with 55-65% relative humidity and exposed to 12 h photoperiod by cool fluorescent tubes (3000lux).

3. Results and Discussions

Effect of different nutrient media supplemented with Kn and IAA on induction of shoot formation, and callus formation from shoot tips is summarised in Table 1. A comparative response of shoot multiplication induction from the shoot tips on these media is depicted in Fig1. Data was scored after a time interval of 12 weeks, with sub culturing after every 6 weeks.

Medium	Auxin conc. (µM)	Cytokinin conc. (µM)	Avg .no. of shoots/explant Mean(S.D)
MS(x1/2) medium	0	0	No response
	IAA 7.5	Kn 2.5	No response
		Kn 5	No response
		Kn 7.5	Callus
		Kn 10	Callus
		Kn 12.5	No response
		Kn 15	No response
MS medium	0	0	No response
	IAA 7.5	Kn 2.5	$2.40(0.52)^{o}$
		Kn 5	$3.00(0.82)^{n}$
		Kn 7.5	$5.70(0.48)^{f}$
		Kn 10	$7.70(0.48)^{c}$
		Kn 12.5	$3.60(0.52)^{k}$
		Kn 15	No response
Gamborg's medium	0	0	No response
	IAA 7.5	Kn 2.5	No response
		Kn 5	No response
		Kn 7.5	$1.40(0.52)^{r}$
		Kn 10	$1.50(0.53)^{p}$
		Kn 12.5	$1.00(0.00)^{q}$
		Kn 15	No response
White's medium	0	0	No response
	IAA 7.5	Kn 2.5	No response
		Kn 5	No response
		Kn 7.5	No response
		Kn 10	No response
		Kn 12.5	No response
		Kn 15	No response

Modified MS	0	0	No response
	IAA 7.5	Kn 2.5	6.60(0.52)^{d}
		Kn 5	10.30(0.82)^{b}
		Kn 7.5	18.20(0.92)^{a}
		Kn 10	5.80(0.42)^{e}
		Kn 12.5	4.00(0.82)^{j}
		Kn 15	3.50(0.53)^{l}
Nitsch&Nitsch	0	0	No response
	IAA 7.5	Kn 2.5	3.00(0.94)^{n}
		Kn 5	4.30(0.82)^{i}
		Kn 7.5	5.00(0.82)^{h}
		Kn 10	5.20(0.79)^{g}
		Kn 12.5	3.40(0.70)^{m}
		Kn 15	No response

Table 1:Effect of MS (half and full strength), modified MS , Gamborg's, Nitsch&Nitschand White's media either alone or in combinations with different concentration of Kn and IAA on shoot tip culture of B. ligulata.

Values given are means (standard deviation). Fisher's LSD was applied when value of analysis of variance (ANOVA) was significant (P < 0.05), and values within a column followed by same alphabet in superscript don't differ significantly. Data scored after 12 weeks of culture period :Ten replicates taken in each treatment

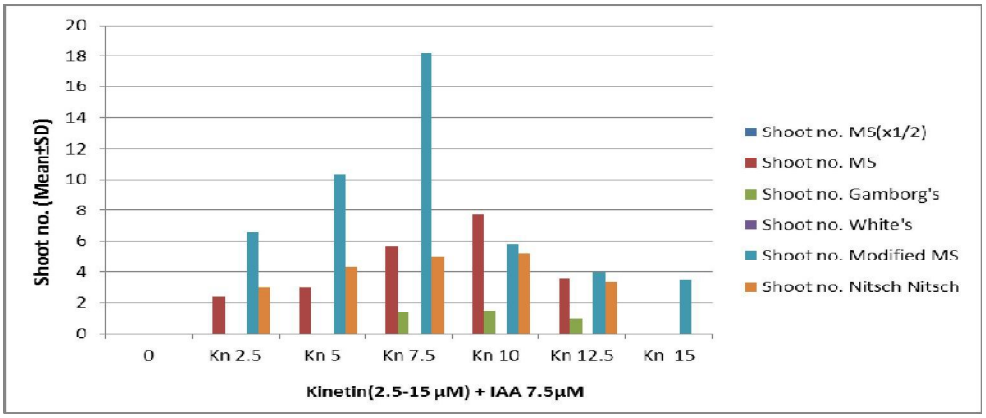

Figure 1:Response of shoot tip segments of B. ligulataon various media combined with different concentrations of phytohormones

From the observations it is clear that shoot tips did not show any response in terms of shoot formation when cultured on MS (half strength), however some callus formation was observed at Kn 7.5-10 µM with 7.5 µM of IAA but on White's media no response was observed. However some response in terms of shoot formation was observed onMS(full strength) medium with average number of shoots reaching only up to7.70 (0.48) at10 µM of Kncombined with 7.5 µM of IAA (Fig 1), but the shoots formed were not vigorous and healthy and did not respond upon sub culturing instead the shoots suffered necrosis which is in agreement with the work of Carmen [24] who reported necrosis of callus of B. crassifolia on MS with different auxin/cytokinins in the ratio of 1:2. Gamborg's medium also resulted in formation of indirect multiple shoots when cultured under the influence of different concentration combinations of Kn and IAA (Fig 2).It was observed that the number of shoots raised to the average of 18.20(0.92) when the shoot tips were cultured on modified MS medium supplemented with 7.5 µM of Kn and 7.5 µM of IAA (Fig 3). However the average number of shoots on Nitsch&Nitsch medium was 5.20(0.79) (Fig 4), which was again less than the number of shoots obtained on modified MS medium, there was also the formation of intense brown callus that hindered further proliferation. The reason for unsatisfactory response on different media may be due to the fact that nutritional requirements for the optimal growth of plants invitro vary from species to species. The concentrations and chemical forms of components vary considerably. According to Arditti [25] considerable variations are reported to exist between media used for species of same genus or different genera.

The best medium for obtaining the maximum number of shoots was recorded on modified MS medium. This might be due to the fact that B. ligulatanot only needs high salt concentration of MS medium but also needs more vitamins like biotin, folic acid and cysteinHCl which are not present in MS medium but are present in Nitsch&Nitsch medium which also has more concentration of thiamine HCl and Nicotinic acid than MS medium. These findings are in conformity with Welander [26] who reported that the requirements of the cells for added vitamins vary according to nature of plant and the type of culture; George [27] reported that folic acid shows tissue proliferation in dark while enhancing it in light as it is hydrolyzed to p-aminobenzoic acid. According to Gamborget al. [28] the basal nutrient medium is one of the most important factors influencing the success of culturing plant material. The difference in response lies in the concentration and constituents in the media. These results are in agreement to some

extent with the reports of Furmanovaet al. [29] who reported multiple shoot formation of B. crassifolia on NN medium when supplemented with Kinetin . Duskovaet al.[30] reported the formation of callus only on MS combined with Kinetin in B. crassifolia.

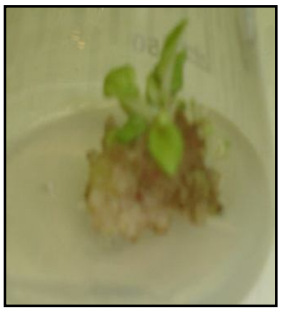

Figure 1: Indirect shoot formation from shoot tips on MS +Kn(10µM)+IAA (7.5 µM) after 12 weeks of culture period

Figure 2: Indirect shoot formation from shoot tips on Gamborg's medium + Kn (10µM) + IAA (7.5 µM) after 12 weeks of culture period

Figure 3: Indirect shoot formation from shoot tips on modified MS medium + Kn(7.5µM) +IAA (7.5 µM) after 12 weeks of culture period

Figure 4: Indirect shoot formation from shoot tips on Nitsch&Nitsch +Kn(10µM)+IAA (7.5 µM) after 12 weeks of culture period

4 .Conclusion

The above findings reveal preliminary information regarding morphogenetic potential of shoot tip explants of B. ligulata on different media, which is a vulnerable plant of medicinal importance to produce large number of plantlets in short period from the single explant without destroying the mother plant and subsequently their restoration in the natural habitat, thus conserving the biodiversity.

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