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Impact of BAP and different auxins on *Invitro* shoot proliferation of *Rheum emodi* Wall.

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Abstract*:Invitro raised shoots of R. emodi were cultured on MS nutrient medium to assess the influence of different concentrations of BAP (2.5-15μM) separately, and also its combination with different auxins i.e. NAA/2,4-D/IAA/IBA. Out of all the trials tested the best response for shoot proliferation was observed on 7.5μM of BAP supplemented with 5μM of IBA. Each isolated shoot was then sub cultured for rooting under the influence of different concentrations of IAA(2.5-15 μM) .The best rooting response was observed at 12.5 μM of IAA. The plantlets were transferred to pots containing autoclaved potting mixture of sand: peat: soil: vermiculite in the ratio of 1:1:1:1 and were finally acclimatized and hardened to green house where about 80% of plantlets survived.*

Key words**:** *Rheum, Invitro, BAP , MS, IAA*

Abbrevations *:Rheum,Invitro,MS –Murashige and Skoog;BAP- 6-benzyl amino purine,IAA- Indole - 3 acetic acid, IBA- Indole 3-butyric acid*

I. Introduction

India is one of the twelve mega diversity countries of the world with a rich diversity of biotic resources [1]. A total of 560plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, out of which 247 species are in the threatened category. On a global basis, the IUCN has estimated that about 12.5% of the world's vascular plants, totalling about 34, 000 species are under varying degrees of threat [2].IUCN[3] recognises the following categories: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient and not evaluated. Critically endangered, endangered and vulnerable together form the threatened category.

*Rheum emodi*Wall. exMeissn. (Polygonaceae) is a perennial stout herb belonging to a large genus '*Rheum'* and is distributed in the temperate and subtropical regions of Himalaya from Kashmir to Sikkim, between an elevation of 2800 and 3800 m. In Garhwal Himalaya it is generally found between 2800and 3600 m in an alpine zone on rocky soil, between boulders and near streams. It has been pointed out that nearly 60,000 plant species may be in danger of extinction leading to gene erosion during the next 30–40 years[4], and *Rheum emodi*is among the top of that list, particularly for Garhwal Himalaya.It *has* been categorised as vulnerable[5][6] and recently it has been categorised as critically endangered[7] and is therefore identified as a top-priority species for conservation and cultivation .

1.1 Active compounds and Ethnomedicinal importance

The most important constituents from rhubarb roots are the anthraquinone derivatives such as Chrysophanol, Aloe-emodin, Emodin, Physcion, Rhein and its glycoside, Glucorhein etc. Tannins are also present in rhubarb which includes hydrolysable tannins, containing ester or glycosidic bonds composed of gallic acid, glucose and other monosaccharaides and condensed tannins, derived primarily from the flavone derivatives catechinand leucocyanidin[8] [9] [10]. The history of rhubarb dates back to ancient China and the Mediterranean region as a highly popular laxative drug and a general tonic [11]. Indian rhubarb is used as purgative and astringent tonic; its stimulating effect combined with apparent properties renders it especially useful in atonic dyspepsia. Powdered roots are sprinkled over ulcer for healing and is also used for cleaning teeth. Leaf stalks are eaten either raw or boiled, sprinkled with salt and pepper.

1.2 Need for Conservation

Due to these properties, the species has excessive demand, which leads to its illegal over-exploitation from natural habitat, resulting in habitat destruction and now this species is on the verge of becoming a rarity. The development of reliable *in vitro* protocol is of great importance for conservation of rare and threatened plant speciesby virtue of producing uniform planting materialfor offsetting the pressure on the natural populations especially for medicinal andornamental plants. Indiscriminate over exploitation of this natural

resource to meet the ever increasing demand and insufficient attempts for replenishment and cultivation have resulted in severe depletion of wild stock resulting in critically low population levels of this species[12]. Keeping in view the importance of*Rheum emodi*, and the threat to it, this species needs to be conserved, for this reason present investigation was undertaken to conserve the wild stocks of this species.

II. Materials And Methods

Invitro grown shoot tips of *R. emodi*were used as explants which were inoculated on MS [13] medium augmented with BAP (2.5-15µM) either separately or in combination with different concentrations of NAA/2,4-D/IAA/IBA. 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).The cultures were sub cultured after every 3-4 weeks of culture period.

III. Results And Discussion

Shoot tips cultured under the influence of different concentrations of BAP separately or in combination with NAA/2,4-D/IAA/IBA showed different degrees of response (Table 01). Most of the combinations tested resulted in shoot proliferation but the best response in terms of shoot multiplication and proliferation was observed when the medium was supplemented with 7.5µM of BAP and 5 µM of IBA with 25.07 (1.20) as the average number of shoots (Fig 1), however the shoots of longer size were observed at lower concentration combination of both BAP and IBA i.e. 2.5 µM. Similar results were reported by Lal and Ahuja[14] who reported the propagation of Indian rhubarb (*R.emodi*) on MS medium supplemented with BAP and IBA.Roggemans and Claes[15],Roggemans and Boxus [16] also reported the similar observations in *R.rhaponticum;*Farzami*et al. [*17*]*in *R.ribes*; Thomas *et al*. *[*18] in *R. rhubarbarum, R. rhaponticum, R. undulatum*; Malik *et al*.[19*]* in *R. emodi* also reported formation of multiple shoots on MS supplemented with BAP and IBA and Verma*et al.[*20*]*who reported formation of multiple shoots in *R. emodi* on MS with BAP and IAA .These reports does not agree with the results of Walkey[21]who reported plantlet formation of *R. rhaponticum* on LS medium.

These micro shoots were separated carefully and were cultured on different concentrations of IAA for rooting, although rooting was observed at all the concentrations of IAA 2.5-15 µM but the best rooting response was observed at 12.5 µM of IAA with the size of shoots varying insignificantly (Fig 2.)(Table 02).Similar results were observed by Thomas *et al* .[18] on MS +IAAin *R. rhubarbarum, R. rhaponticum, R. undulatum.*Plantlets with at least 2-4 rootswere transferred to pots containing autoclaved potting mixture of sand: peat: soil: vermiculite in the ratio of 1:1:1:1 and were finally acclimatized in mist house (Fig 3) .After one month the plants were repotted in pots containing mixture of soil, sand and compost in the ratio of 2:1:1and were kept in net house for acclimatization . It was noticed that about 80% of plants showed new leaf formation , became photosynthetically active and showed normal growth patterns till the season ended.Lal and Ahuja[14][22]also reported hardening of *invitro* raised plantlets of *R. emodi* with 89%-90% survival rate.

IV. Figures And Tables

Table 01.Impact of different concentrations of BAP separately or in combination with NAA/2,4- D/IAA/IBA on shoot regeneration from shoot tips of *R. emodi*

Auxin conc. (µM)	**Cytokinin conc. (µM)**	**Response**	**Average no. of shoots/explant (n=10)** **Mean(S.D.)**	**Average length of shoots in cm. (n=10)** **Mean(S.D.)**
	BAP 2.5	Callus	–	–
	BAP 5.0	Direct shoots	2.99 (1.23)^{d}	3.37 (0.52)^{e}
	BAP 7.5	Direct shoots	3.73 (0.79)^{c}	3.67 (0.48)^{c}
	BAP 10	Direct shoots	18.08 (0.88)^{a}	4.64 (1.23)^{a}
	BAP 12.5	Direct shoots	3.90 (0.94)^{b}	4.10 (0.92)^{b}
	BAP 15	Direct shoots	2.55 (0.52)^{e}	3.52 (0.84)^{d}

BAP with NAA

NAA 2.5	BAP 2.5	No response		
	BAP 5.	Callus	–	–
	BAP 7.5	Direct shoots	2.55 (0.52)^{h}	4.08 (1.03)^{e}
	BAP 10	Direct shoots	2.83 (1.15)^{g}	3.50 (0.97)^{g}
	BAP12.5	Direct shoots	1.71 (0.88)^{i}	2.69 (0.63)^{j}
	BAP 15	Callus	–	–
NAA 5.0	BAP 2.5	Callus	–	–
	BAP 5.	Callus	–	–
	BAP 7.5	Direct shoots	4.13 (0.79)^{d}	3.32 (0.84)^{h}
	BAP 10	Direct shoots	10.08 (0.74)^{a}	5.79 (0.42)^{a}
	BAP12.5	Direct shoots	4.83 (0.88)^{c}	5.48 (0.53)^{b}
	BAP 15	Direct shoots	1.64 (0.79)^{j}	4.28 (0.48)^{d}
NAA 7.5	BAP 2.5	Callus	–	–
	BAP 5	Callus	–	–
	BAP 7.5	Indirect shoots	3.17 (1.26)^{f}	4.57 (0.52)^{c}
	BAP 10	Indirect shoots	5.40 (1.08)^{b}	3.81 (0.88)^{f}
	BAP12.5	Indirect shoots	3.37 (0.52)^{e}	3.46 (0.53)^{g}
	BAP 15	Indirect shoots	1.32 (0.52)^{k}	3.09 (0.32)^{i}

BAP With 2,4-D

2,4-D (2.5)	BAP 2.5	Callus	–	–
	BAP 5	Indirect shoots	1.32 (0.52)^{e}	3.18 (0.42)^{f}
	BAP 7.5	Indirect shoots	1.47 (0.70)^{c}	3.46 (0.53)^{d}
	BAP 10	Indirect shoots	2.96 (0.99)^{a}	4.16 (0.63)^{a}
	BAP12.5	Indirect shoots	1.52 (0.52)^{c}	3.27 (0.48)^{e}
	BAP 15	No response	–	–
2,4-D (5)	BAP 2.5	Callus	–	–
	BAP 5	Callus	–	–
	BAP 7.5	Indirect shoots	1.37 (0.71)^{d}	4.08 (1.03)^{b}

	BAP 10	Indirect shoots	2.70 (0.79)[b]	3.57 (0.52)[c]
	BAP12.5	Indirect shoots	1.00 (0.00)[f]	3.18 (0.42)[f]
	BAP 15	No response	–	–

BAP with IAA

IAA 2.5	BAP 2.5	No response	–	–
	BAP 5	No response	–	–
	BAP 7.5	Indirect shoots	1.32 (0.52)[b]	2.35 (0.52)[d]
	BAP 10	Indirect shoots	2.70 (0.79)[a]	2.66 (0.48)[b]
	BAP12.5	Indirect shoots	2.66 (0.48)[a]	2.17 (0.42)[e]
	BAP 15	No response	–	–
IAA 5.0	BAP 2.5	No response	–	–
	BAP 5.0.0	No response	–	–
	BAP 7.5	Indirect shoots	1.00 (0.00)[c]	2.45 (0.53)[c]
	BAP 10	Indirect shoots	2.66 (0.48)[a]	2.77 (0.42)[a]
	BAP12.5	Indirect shoots	1.32 (0.52)[b]	2.17 (0.42)[e]
	BAP 15	No response	–	–

BAP with IBA

IBA 2.5	BAP 2.5	Direct shoots	2.26 (1.08)[k]	5.79 (0.42)[a]
	BAP 5.0	Direct shoots	2.76 (0.99)[i]	5.48 (0.53)[b]
	BAP 7.5	Direct shoots	8.56 (0.97)[e]	4.47 (0.53)[c]
	BAP 10	Indirect shoots	4.72 (0.92)[g]	4.28 (0.48)[d]
	BAP 12.5	Indirect shoots	2.55 (0.52)[j]	4.09 (0.32)[e]
	BAP 15	Indirect shoots	1.00 (0.00)[o]	3.73 (0.79)[g]
IBA 5.0	BAP 2.5	Direct shoots	1.64 0.79 ()[m]	3.89 (0.32)[f]
	BAP 5.0	Direct shoots	10.07 (0.88)[d]	3.46 (0.53)[i]
	BAP7.5	Direct shoots	25.07 (1.20)[a]	3.25 (0.67)[k]
	BAP 10	Indirect shoots	13.77 (1.03)[b]	3.25 (0.67)[k]
	BAP 12.5	Indirect shoots	11.43 (1.35)[c]	3.46 (0.53)[i]
	BAP 15	Indirect shoots	1.76 (1.05)[l]	3.18 (0.42)[l]
IBA 7.5	BAP 2.5	Indirect shoots	5.03 (0.88)[f]	3.68 (1.03)[h]

	BAP 5.0	Indirect shoots	3.57 (0.52)^{h}	3.25 (0.67)^{k}
	BAP 7.5	Indirect shoots	2.55 (0.52)^{j}	3.37 (0.52)^{j}
	BAP 10	Indirect shoots	1.32 (0.52)^{n}	3.09 (0.32)^{m}
	BAP 12.5	Indirect shoots	1.00 (0.00)^{o}	2.23 (0.67)^{n}
	BAP 15	No response	–	–

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

Table 02.Response of *invitro*raised microshoots of *R. emodi* for rooting on different concentrations of IAA

Medium	**Auxin conc. μ M**	**Response**	**Average no. of roots/shoot (n=10) Mean(S.D.)**	**Average length of roots /shoot in cm.(n=10) Mean(S.D.)**
MS	IAA 2.5	Long ,Thin adventitious roots	3.00(0.940)^{f}	7.20(1.22)^{ns}
	IAA 5	Long ,thin adventitious roots	11.20(0.919)^{e}	7.50(1.269)^{ns}
	IAA7. 5	Long ,thin adventitious roots	16.80(1.229)^{d}	7.80(1.229)^{ns}
	IAA 10	Long ,thin adventitious roots	21.00(0.943)^{c}	8.40(1.075)^{ns}
	IAA 12.5	Long ,thin adventitious roots	29.60(1.174)^{a}	7.10(1.37)^{ns}
	IAA 15	Long ,thin adventitious roots	23.00(0.943)^{b}	6.60(1.776)^{ns}

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 8 weeks of culture period; Ten replicates takenin each treatment

Fig.1.Directmultiple shoot formation of shoot tips on MS +BAP 7.5µM +IBA 5 µM

Fig.2.Roots formed on MS+IAA (12.5µM) after 8 weeks of culture period

Fig3.*Invitro*raised Plantlets for hardening

V. Conclusion

The concentration and ratio of hormones varies from plant to plant and needs to be standardized for a particular plant tissue. In the present study a number of concentrations and combinations were tried for exploiting maximum potential for organogenesis /regeneration of this species. The present findings summarises the protocol for invitro propagation and conservation of this important threatened plant species and the feasibility of its large-scale propagation.

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