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Callus induction and shoot regeneration from rhizome explants of *Rheum webbianum* Royle- a threatened medicinal plant growing in Kashmir Himalaya

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Abstract

Rheum webbianum Royle is an important medicinal plant of family Polygonaceae growing in Kashmir Himalaya. It possesses hepatoprotective, spasmolytic, anticholesterolaemic, antitumor, antiseptic, antifungal, anti-microbial, anti-Parkinson's, anti-proliferative, immuno-enhancing, antiviral and antioxidant properties. During the present study an attempt has been made to standardize a protocol for large scale propagation of this threatened Himalayan plant species using rhizome cuttings as explants. Callus was obtained when the rhizome explants were inoculated on MS medium containing different concentrations of auxins either alone or in combination, 2, 4-D at a concentration of 0.5 mg/l was found to be most effective in callus production in a time period of 30 days. Callus derived from rhizome explants differentiated into shoots on MS medium supplemented with different concentrations of auxins (IAA, IBA) and (BAP). Maximum number of shoots (2.8±0.2) was regenerated on MS medium supplemented with BAP (5.0 mg/l) and IAA (2.0 mg/l) after 16 days.

Keywords: Polygonaceae, Explant, Callus, Inoculation, Auxin, Cytokinin.

Introduction

Rheum webbianum is an important medicinal plant of family Polygonaceae. It is commonly known as 'Pambhakh' (leaves) or 'Pambchalan' (rhizome) (Fig.1). *Rheum webbianum* is found in China, India, Pakistan and Nepal at an altitude of 2,400-4,300 m asl. In India it occurs in Jammu & Kashmir, Himachal Pradesh and Uttar Pradesh. The main secondary metabolites present in *Rheum webbianum* to which it owes its medicinal importance are anthraquinones like rhein, emodin, aloe-emodin, physcion and chrysophanol. Due to the presence of these active components it is used to cure various diseases like cancer, renal disorders, and hyperlipedemia and improves the memory of senile patients.[1] Anthraquinones present in *Rheum webbianum* are helpful in managing cancer as anthraquinones have been found to have antitumour and antiangiogenic action. Apoptosis and cell cycle inhibition of many human cancer cell lines has been observed *in vitro*. 'Rhubarb' extract is also suggested as an adjunct to chemotherapy.[2] *Rheum webbianum* is a potential source of dietary fiber with lipid lowering effect. It is suggested that rhubarb exerts its effect on cholesterol by inhibition of enzyme squalene epoxidase.This enzyme is thought to catalyze the rate limiting step of cholesterol biogenesis.[3] The genus is also reported to have anti-microbial, anti-oxidant and anti-diabetic properties.[4, 5]

Owing to the above mentioned potentialities this plant is being exploited at an alarming rate. Beside indiscriminate exploitation, overgrazing also poses a serious threat to this plant species. These threats if not controlled will lead to the extinction of the plant very soon. Hence an efficient conservation measure is necessary in order to save this plant from extinction. In this regard plant tissue culture technique offers an alternative for conservation of endangered

species. The significance of an efficient *in vitro* protocol would be to obtain maximum number of plantlets in minimum period of time. Their restoration in the natural habitat will subsequently help in conserving the biodiversity. The present study was carried out to develop a conservation protocol *Rheum webbianum.*

Figure 1: *Rheum webbianum*-Royle in natural habitat

Materials and Methods

Rheum webbianum was collected from Jawahar Tunnel Jammu and Kashmir. Rhizome explants were kept overnight under running tap water in order to remove dirt and dust followed by washing with detergent laboulene and surfactant tween-20. This was followed by rinsing the explants with double distilled water. Under laminar air flow hood the explants were treated with chemical sterilant 50% sodium hypochlorite for 15-20 min.This was followed by washing with autoclaved double distilled water and finally inoculation on sterilized nutrient medium.

Medium and culture conditions

Murashige and Skoog's (MS, 1962) medium, gelled with 8% agar was supplemented with different concentrations of auxins and cytokinins both individually and in combination.[7] Auxins like 2, 4- D; IAA; IBA and cytokinins like BAP were used in concentration range of 0.1-5 mg/l. The pH of the media was adjusted to 5.8 before autoclaving at 121 °C and 15 lb pressure. The cultures were incubated at 22±4 ^{0}C and exposed to a regular photoperiod of 24 hours.

Results

Callus production from rhizome explants

Callus was produced when the rhizome explants were inoculated on MS medium fortified with 2,4-D (0.5mg/l), BAP (3mg/l), BAP 3mg/L +IAA 2g/L , 2,4-D 1mg/L + and BAP 3mg/l + 2,4-D 0.5 in a time period of 30, 34, 45,44 and 47 days of inoculation respectively (Fig.2, Table:1).

Shoot regeneration

For shoot regeneration callus derived from the rhizome was sub cultured on MS medium supplemented with different concentrations of BAP individually or in combination with auxin. BAP at a concentration of 5mg/l individually was found to be effective in producing 1.4±0.24 shoots with mean shoot length of 0.6±0.06 cm in a time period of 10 days. 2,4-D also induced shoot regeneration at a concentration of 0.5mg/l and 1 mg/l. 2.6±0.24 shoots with mean shoot length of 0.8±0.15 and 1.8±0.2 shoots with mean shoot length of 0.96±0.11 in a time period of 30 and 19 days respectively were produced. BAP at a concentration of 1.6mg/l in combination with IBA 1 mg/l produced 2.6±0.24 shoots with mean shoot length of 1.6±0.22 within 11 days. However BAP at a concentration of 5mg/l in combination with IAA at a concentration of 2mg/l was found to be the best medium in terms of shoot induction whereon 2.8±0.2 shoots with mean shoot length of 3.86±0.37 within 16 days were produced.(Fig. 3, Table:2)

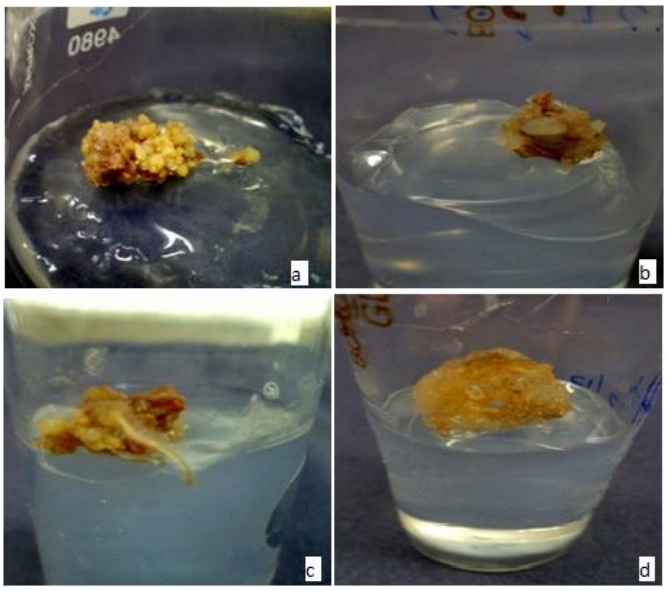

Figure 2: Callus production from rhizome explant [a MS 2,4-D (0.5 mg/l) [b MS +BAP (3 mg/l) [c MS +BAP (3 mg/l) + IAA (2 mg/l)]. [d MS+BAP(3mg/l)+2,4-D(0.5mg/l)]

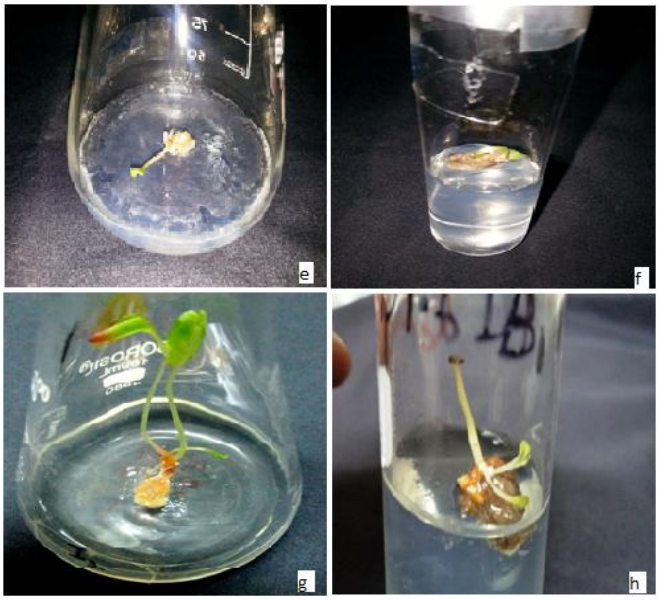

Figure 3: Shoot formation in *Rheum webbianum* on [e MS +2,4-D (0.5mg/l)], [f:MS+2,4-D(1mg/l)],[g:MS+BAP(5mg/l)+IAA(2mg/l)[h:MS+BAP (1.6mg/l)+IBA(1mg/l)]

Table 1: Effect of different hormones on callus production from rhizome explants

Treatments	Mean No. of days taken for callus production	Amount of callus produced	Texture and color of callus	% Culture response
MS +2,4-D	30	High	Friable, Brown colored	100
MS+BAP(3mg/l)	34	Little	Friable ,Brownish green	70
MS+BAP(3mg/l)+ IAA(2mg/l)	45	High	Friable, Brown colored	90
MS+2,4-D(1mg/l)+KN(2mg/l)	44	Moderate	Friable, Brown colored	70
MS+BAP(3mg/l)+2,4-D(0.5mg/l)	47	High	Friable,Brown colored	60

(30 replicates per treatment)

Table 2: Effect of different hormones on shoot regeneration from callus obtained from rhizome explants

Treatments	Mean no. of shoots ± SE	Mean length of shoots (cm) ± SE	Mean No. of days taken for shoot regeneration	% Culture Response
MS+BAP(1.6mg/l)+IBA(1mg/l)	2.6±0.24	1.6±0.22	11	70
MS+BAP(5mg/l)	1.4±0.24	0.6±0.06	10	30
MS+2,4-D(0.5mg/l)	2.6±0.24	0.8±0.15	30	40
MS+2,4-D(1mg/l)	1.8±0.2	0.96±0.11	19	60
MS+BAP(5mg/l)+IAA(2mg/l)	2.8±0.2	3.86±0.37	16	90

(30 replicates per treatment)

Discussion

There are no reports of callus production from rhizome explants in any plant of the family Polygonaceae. However during present study; callus was produced from rhizome explants of *Rheum webbianum* on MS medium supplemented with different growth regulators. 2,4-D at a concentration of 0.5 mg/l was found to be best for callus production. Callus was also produced when MS medium was supplemented with BAP at a concentration of 3 mg/l. Auxin cytokinins combinations like BAP(3mg/l)+IAA(2mg/l), 2,4-D(1mg/l)+KN(2mg/l) and BAP(3mg/l)+2,4-D(0.5mg/l) were also effective in callus production but the percent response at these concentrations was lesser. Callus after subculture produced maximum shoot regeneration on MS medium supplemented with BAP (5 mg/l) in combination with IAA (2 mg/l). Malik *et al*. also used rhizome as explants in case of *Rheum emodi* but they obtained direct shoot organogenesis on MS medium supplemented with 10.0 mM (BAP) and 5.0 mM (IBA).

Conclusion

An efficient *in vitro* protocol was standardized for callus induction and shoot regeneration of *Rheum webbianum* from rhizome explants on MS medium supplemented with different growth regulators. Maximum callus development was achieved on MS medium supplemented with 2,4-D (0.5 mg/l) and maximum number of shoots developed on MS medium supplemented with BAP (5 mg/l) in combination with IAA (2 mg/l)

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