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RESEARCH ARTICLE

Optimization of phytohormone combinations for *in vitro* callogenesis in *Lavatera cashmeriana* : An endemic medicinal plant of Kashmir

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SUMMARY

The state of Jammu and Kashmir is bestowed with diverse variety of plant species especially medicinally important plants due to wide variations in its topography and microclimatic conditions. *Lavatera cashmeriana Camb*. (Malvaceae), which is endemic and endangered to Kashmir valley, has great medicinal importance. Its parts are being used to treat sore throat and common cold. In the present study various phytohormones auxins and cyotkinins either alone or in combination were used for the *in vitro* callogenesis of medicinally important herb of Kashmir. The excellent results from the seeds of *Lavatera cashmeriana* were observed on the Murashaige and Skoog medium supplemented with 2,4-D and BAP. In future a fine tune and refinement of phytohormones are required in terms of the concentration for the organogenesis of medicinally important plant.

Key Words : *Lavatera cashmeriana,* MS, 2, 4-D, NAA, BAP

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Many higher plants are major sources of natural products used as pharmaceuticals, agro-chemicals, flavour and fragrance ingredients, food additives, and pesticides. 90 per cent of plants are herbaceous in nature which possesses the medicinally important compounds.In the past few decades, there has been an ever-increasing global inclination towards herbal medicine, followed by a belated growth in international awareness about the dwindling supply of the world's medicinal plants (Balandrin and Klocke, 1988). Plants are the real treasure

for the discovery of new medicinal products and drug development. Medicinal plants are the most important source of life saving drugs.Many of the recommended drugs sold in market today are either the simple modifications or copies of the naturally obtained substances. About 70-80 per cent of people worldwide rely chiefly on traditional (herbal) medicines for healthcare needs. Worldwide, between 50,000 and 80,000 flowering plants are used medicinally (Marinelli, 2005).Medicinal plants contain a wide range of metabolites that can be used to treat chronic as well as infectious diseases. In spite of tremendous development in the field of synthetic drugs and antibiotics during the 20th century, plants still continue to be a major source of drugs in modern as well as traditional systems of medicine throughout the world (Sonowal, 2013). Many plant extracts are well established in clinical practice and are likely to remain so for some time until better, cheaper, less toxic or more efficacious alternatives become available. Of the pharmacologically active principles found in plant kingdom, higher plants are arguably the most important group. According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994). It may sound an exaggeration of facts, but there may hardly be any plant, which may not be having medicinal or neutraceutical value (Chatervedi *et al.,* 2007). The genetic diversity of medicinal plants in the world is getting endangered at alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines, with little or no regard to the future. Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization etc are other factors, thus, challenging their existence (Gupta *et al.,* 1998). More than 4000 species of medicinal plants are globally threatened. To cope up with alarming situation, biotechnological tools have been increasingly applied for mass propagation, conservation of germplasm, study and production of bioactive compounds and for genetic improvement of the medicinal plants. Tissue culture is useful for multiplying and conserving the species, which are difficult to regenerate by conventional methods and save them from extinction (Kuldeep *et al.,* 2012). *In-vitro* propagation of plants holds tremendous potential for the production of high-quality plant-based medicines (Murch *et al*., 2000). *Lavatera* L. (Malvaceae) is a genus of 21-23 mostly well marked species of herbs, shrubs and tree-like shrubs, occurring in both the old and new worlds (Ray, 1995). Several species of the *Lavatera* genus have been used in traditional medicine. The leaves of *L. arborea* have been used for the treatment of vaginitis and in wound healing (Rojas *et al.,* 2003). The roots and leaves of *Lavatera cashmeriana* are used as mild laxative and for throat problems, flowers are used for treatment of skin irritation in pregnanat women (Aijaz *et al*., 2013). Keeping all this in view, the present study is based on the mass propagation of endangered Kashmiri medicinal plant through *in vitro* culture.

MATERIAL AND METHODS

Plant material :

The plant used in the present study is *Lavatera cashmeriana*. Seeds were used as explant and were collected from the Jammu and Kashmir medicinal plant introduction centre and Kashmir Tibia College.

Sterilization :

Seeds of the plant were soaked overnight in water and after that were washed under running tap water for 30 minutes and then followed by pre treatment with the solution of antifungal agents bavistin (0.1%) and indofill (0.1%) for 5 minutes followed by washing with tween-20 detergent and then the seeds were washed with double distilled water so that no residue of the detergent remain on the explants. Explants were disinfected inside the Laminar air flow with 70 per cent ethyl alcohol followed by varying concentrations of (commercially available) sodium hypochlorite (NaOCl) (10,20 and 30%) and mercuric chloride ($HgCl_2$) (0.1% and 0.2%) for different durations (3,5,8 minutes) to screen the best sterilization treatment. Finally washed with autoclaved distilled water for 6 times to remove the microbial load and dust particles from the surface of the explants to ensure contamination free inoculation.

Medium :

The media used in the study was MS basal media (Murashaige and Skoog, 1962) supplemented with plant growth regulators, vitamins and solidifying agent agar as per requirement of experiments. The medium was supplemented with different combinations of growth regulators like 2,4-D, BAP, NAA, IBA to see their effect on callogenesis of cultured explants. The pH of 5.8 was maintained before autoclaving. The medium was then

autoclaved at 15 psi at 121°C for 20 minutes for proper sterilization. Seeds of *Lavatera cashmeriana* were inoculated carefully on medium supplemented with different combinations of aauxin and cytokinin in culture bottles and bottles were properly plugged and labeled.

RESULTS AND DISCUSSION

The findings obtained from the present investigation as well as relevant discussion have been presented under following heads:

Standardization of sterilization protocol for *Lavatera cashmeriana* :

Explants were treated with different surface sterilizers inside the laminar air flow for the assurance of contamination free inoculation. The surface sterilizers used were mercuric chloride, sodium hypochloride and ethyl alcohol, which were used at different concentrations and various time intervals. Even after three decades of research and development in plant tissue culture microbial contamination by bacteria, fungi, viruses etc. are still the major problems that hampered the establishment of truly aseptic conditions. Table 1 showed that T_2 (0.1%$HgCl_2$ for 3 minutes and 20% NaOCl for 5 minutes) was better surface disinfection for *in vitro* establishment of *Lavatera cashmeriana* seeds than T_{12} (0.2% $HgCl_2$ for 5 minutes and 30% NaOCl for 8 minutes). Further all experiments were carried out with the same T_2 treatment. Ahn *et al*. (2007) and Alam *et al*. (2010) also reported $HgCl_2$ and commercial bleach solution as an effective sterilant to reduce the risk of contamination in cultures.

Callogenesis of *Lavatera cashmeriana* :

Seeds of the herb *Lavatera cashmeriana* were used for the callogenesis at various phytohormone combinations. The explants treated with different phytohormones with time showed response in certain combinations of phytohormones. As per the result in Table 2 and Fig. 1, *Lavatera cashmeriana* seeds showed best response in the phytohormone combination 2,4-D+BAP, moderate response in 2,4-D+IAA+BAP, out of the three plant growth regulators used, BAP was the most suitable for the callus induction in *Lavatera cashmeriana*. Jitendra *et al*. (2013) also reported highest callus induction in *Allium sativum* when cultured on MS medium supplemented with 2,4-D and BAP. According to Toaima *et al*. (2003) the callus induction in *Allium ampeloprasum* was observed in the medium containing

Table 1: Effect of varying concentration and duration of sterilants on sterilization of explants

Sterilizers	Duration (minutes)	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
$HgCl_2$(%)	3	0.1	0.1	0.1	0.2	0.2	0.2	-	-	-	-	-	-
	5	-	-	-	-	-	-	0.1	0.1	0.1	0.2	0.2	0.2
NaOCl (%)	5	10	20	30	10	20	30	-	-	-	-	-	-
	8	-	-	-	-	-	-	10	20	30	10	20	30
Ethyl alcohol	2	70	70	70	70	70	70	70	70	70	70	70	70
Contamination %		60	20	75	80	55	40	60	80	50	40	45	90

Table 2: Effect of different phytohormone combinations for *in vitro* callus induction in *Lavatera cashmeriana* using seed as explant

Phytohormone combinations	*Lavatera cashmeriana*	Type of callus	Colour of callus	Callus diameter(cm)	Callus height (cm)
2,4-D	+	Friable	Brownish	2	1
2,4-D+K	+		Green	2.3	0.8
2,4-D+NAA+BAP	+++	Friable	Whitish green	4.5	1.6
BAP+IAA	+	Friable	Greenish	2	1
2,4-D+BAP+K	+	Friable	Whitish green	1.6	1.1
2,4-D+BAP	++	Compact	Whitish	7	3
2,4-D+NAA	+	Friable	Brownish	3	1.2
2,4-D+IAA+BAP	+	Compact	Greenish	2.8	1
Kn	-	-	-	-	-

- = Noresponse, + = Poor response, ++ = Moderate response, +++ = Excellent

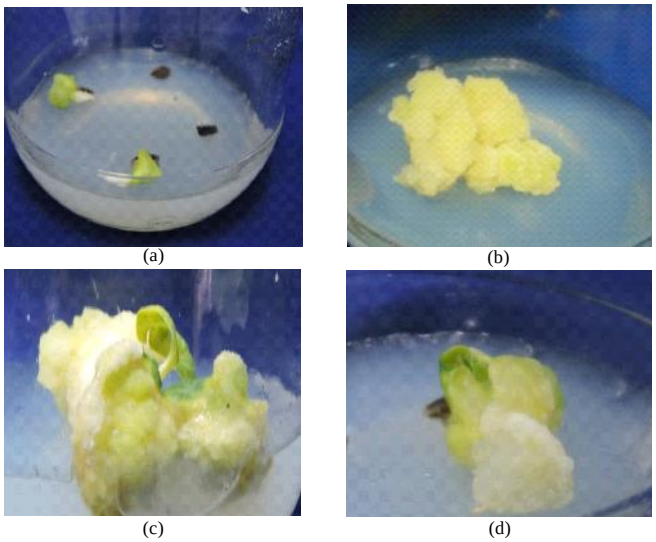

Fig. 1 : Effect of phytohormone combination on callus induction in *Lavatera cashmeriana* when inoculated on MS medium supplemented with (a): 2, 4-D, NAA and BAP after one week; (b): 2, 4-D, NAA and BAP after two weeks; (c): 2,4-D, NAA and BAP after four weeks; (d) 2,4-D and BAP after four weeks

phytohormones BAP and 2, 4-D.

The seeds of *Lavatera cashmeriana* showed response after one week of inoculation and visible callus started forming within 4 weeks. The callus obtained was friable and whitish green in colour.

Callus maintenance :

The most suitable medium for maintenance of callus was selected on the basis of high frequency callogenesis on different auxin and cytokinin combinations. After recording the most suitable medium (2, 4-D and BAP) for the callus induction, the calli were sub- cultured on the same medium after 30 days of interval. Thus, the rapidly growing calli from the seeds of *Lavatera cashmeriana* were carefully maintained after every 30th day on the same media without any changes in morphology.

Conclusion :

The present study concluded that the minimum contamination of the explants of medicinally important herb of Kashmir valley were obtained by the treatment of 0.1 per cent $HgCl_2$ for 3 minutes and 20 per cent NaOCl for 5 minutes. This is the first report for an efficient protocol for *in vitro* callogenesis of the seeds of *Lavatera cahmeriana* by using MS medium supplemented with 2,4-D and BAP. This partial protocol can be used for the further organogenesis and mass propagation of the plant.

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