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Full Length Research Paper

Development of protocol for *in vitro* culture and rapid propagation of *Wedelia chinensis* (Osbeek) Merr.

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Three different explants of *Wedelia chinensis* (Osbeek) Merr. namely nodal segment (NS), shoot apex (SA) and leaf segment (LS) were grown on MS basal medium supplemented with different combinations and concentrations of cytokinin (BAP and Kn) and auxin (IAA and NAA). Nodal segment and shoot apex underwent direct organogenesis giving rise to multiple shoot buds and the response was dependent on both PGR combinations and type of explant. The maximum number of multiple shoot buds/explant (7.5±0.27) was developed from nodal segment culturing on MS +3.0 mg/l BAP+0.5 mg/l IAA. But shoot apex explant produced longest shoot (5.12±0.17 cm) when only 2.0 mg/l Kn was used in MS medium. Elongated multiple shoot buds were grown on rooting media. Half strength MS fortified with 2.0 mg/l IBA was found better for induction and proliferation of roots. The *in vitro* developed complete plantlets were acclimatized in outer environment through successive phases of acclimatization. On an average 80% of the seedlings could be finally established in pots.

Key words: *In vitro*, micropropagation, protocol, *Wedelia chinensis*.

INTRODUCTION

Wedelia chinensis (Osbeek) Merr. commonly known as Mahavringraj, is a widely used medicinal herb of the family Asteraceae. It is extensively used in ayurvedic preparations, modern drug industries and also in various traditional medicinal practices. Leaf extracts of this plant species is mainly used as hair tonic. It is also used in the treatment of cough, cephalalgia, liver and spleen, skin diseases, especially alopecia. This plant is used by the tribal people of Chittagong Hill Tracts for the treatment of dysentery and rheumatic fever (Yusuf et al., 2009). Due to habitat destruction and indiscriminate collection by the local herbalists it is becoming rare and till now no step has been taken in Bangladesh for commercial cultivation of this medicinally important plant species. It is therefore important to take immediate step for mass and rapid propagation of this medicinal plant species and to save as well as to meet up the demand of traditional ayurvedic industries. *In vitro* culture technique has been proved to be very effective for rapid propagation of rare plant species including medicinal plants (Manickam et al., 2000; Hassan and Roy, 2005; Sudha and Seeni, 2006; Biswas et al., 2007; Hussain et al., 2008; Roy, 2008; Bhadra et al., 2009). So far report goes, in case of *W. chinensis* too, *in vitro* protocol was developed for micropropagation (Sultana and Handique, 2005; Islam et al., 2009). But these investigations were of very limited nature where only a few combinations of plant growth regulators (PGRs) were used with the use of either nodal segment or shoot apex as explant. The present study was therefore undertaken with a view to develop an efficient and repeatable protocol for rapid and mass propagation of this medicinally important plant species with detailed evaluation of PGR combinations including those of both auxin and cytokinin group in the background of culture media.

MATERIALS AND METHODS

A few Mahavringraj plants were collected from BCSIR Laboratories, Chittagong and were grown in earthen pots maintained in the departmental medicinal plant nursery (Figure 1A). After one month

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of plantation shoot apex, nodal segments and leaves of the healthy plants were collected and used as explant for *in vitro* experiments. The explants were washed thoroughly under running tap water for 30 min followed by treatment with savlon for 10 min. These were further washed thoroughly in running tap water for 10 min. The explants were finally surface sterilized with 0.1% (w/v) $HgCl_2$ for 5 min followed by a dip in 70% ethanol for 30 s. These were then thoroughly rinsed five times with sterile distilled water. Before inoculation on to the culture media the explants were cut in to small pieces (1.0 cm apprx.) with the help of sterile surgical blade. MS basal medium supplemented with different concentrations and combinations of cytokinin (BAP and Kn) and auxin (IAA and NAA) were used for induction of organogenesis/embryogenesis. After 30 days of inoculation in culture room the response of the explants were thoroughly examined and the data on different aspects were recorded. For each treatment 15 explants were used and finally the data were subjected to statistical analysis for computation of standard error of mean (SE). For rooting, elongated shoot buds were cultured on rooting medium containing half strength MS medium fortified with different concentrations and combinations of IBA and IAA. In all the cases the media were solidified with the use of 0.8% (w/v) agar (Sigma) and pH was adjusted to 5.8 prior to autoclaving for 30 min at 121°C under a pressure of 1.1 kg/cm^2. All the cultures were incubated in a culture room at 25 ± 2°C under a regular cycle of 14 h light and 10 h dark. The complete seedlings thus produced by *in vitro* culture were finally planted in small earthen pots containing a mixture of soil and compost (2:1) through successive phases of acclimatization.

RESULTS AND DISCUSSION

Of the three kinds of explants used in the experiment only nodal segment and shoot apex gave response to all the media combinations used. On the other hand, leaf segment failed to proliferate in any of the media combinations. Within 15 days of culture leaf segment started browning indicating its death. Nodal segments and shoot apices underwent direct organogenesis giving rise to multiple shoot buds (Figure 1B) and the magnitude and kind of response was dependent on both PGR supplements and their concentrations used in the medium. The PGR supplements and their combinations played an important role in the formation of multiple shoot buds in cultured explants. It is evident that the combination of auxin (IAA/NAA) and cytokinin (BAP/Kn) was more effective in production of multiple shoot buds compared the only cytikinin (BAP/Kn). However, the maximum number (7.5/explant) of multiple shoot bud formation was noted in the medium containing 3.0 mg/l BAP+0.5 mg/l IAA and the minimum number (1.7/explant) was recorded in 2.0 mg/l Kn supplemented media when nodal segments were used as explants (Table 1). Multiple shoot buds underwent elongation in the same media combinations (Figure 1C). After a month of culture, data on elongation of shoots were recorded. Maximum elongation (4.52 cm) of the shoot bud took place in the medium supplemented with 2.0 mg/l Kn + 1.0 mg/l NAA. On the contrary, minimum elongation (0.68 cm) was found in the medium fortified with 3.0 mg/l BAP. Thus the PGR combination suitable for induction of maximum multiple shoot buds was not suitable for elongation of induced shoot buds.

When shoot apices were used as explants the media supplemented with 2.0 mg/l BAP+0.5 mg/l IAA produced the highest number (4.5/explant) of multiple shoot buds and the media supplemented with 1.0 mg/1 Kn produced lowest number (2.1/explant) of multiple shoot buds (Table 2). The longest shoot (5.12 cm) was found on the medium fortified with 2.0 mg/l Kn and the shortest shoot (1.71 cm) was found on 4.0 mg/l Kn.

In terms of production of multiple shoot buds, nodal segment was better than shoot apex. The lateral auxiliary bud primordia in the nodal point probably on incision promoted multiple shoot bud formation. It is evident that for micropropagation of this plant species, nodal explant would be more appropriate. In case of *in vitro* culture, a number of external and internal factors of the explant tissue do involve in the process of tissue formation and differentiation. Here the results clearly indicate that the kind of explant used and the PGR combinations and their concentrations are important aspect of tissue differentiation. Only direct organogenesis took place without any intermediate step of tissue formation and proliferation. This observation clearly indicated that the shoot primordia of the nodal point and that in shoot apex underwent division under the influence of PGR supplements and gave rise to multiple shoot buds. As reported earlier (Purohit and Dave, 1996; Hassan and Roy, 2005; Roy, 2008; Manicham et al., 2000; Raja and Arockiasamy, 2008) BAP singly or in combination with IAA/NAA are effective for induction of multiple shoot buds in many medicinal plants. In case of *W. chinensis* it has been also reported that this plant produced multiple shoot buds in MS medium supplemented with BAP (Islam et al., 2009) and Kn + IAA (Sultana and Handique, 2005) which do not agree with our findings.

In order to produce complete plantlets multiple shoot buds were individually transferred to rooting media. Half strength MS medium with IBA (0.5 to 2.0 mg/l) or IAA (0.5 to 2.0 mg/l) or in combination of IBA and IAA (2.0 mg/l IBA+0.5 to 2.0 mg/l IAA) were used for the purpose. Root initiation was noticed after 4 to10 days of inoculation and it became profuse within one month. The IBA or IAA supplemented half strength of MS media gave 100% response for rooting (Table 3). The best response was observed when 2.0 mg/l IBA was added to half strength of MS media. In this combination on an average 15.75 roots were produced which is the highest number of roots/shootbud (Figure 1D). But half strength of MS media containing 0.5 mg/l IAA produced longest root (3.54 cm). The media ½ MS+2.0 IBA+0.5 IAA and ½ MS+2.0 IBA+1.0 IAA did not give any response for rooting. The influence of IBA for induction and proliferation of root has been reported in many medicinal plants (Yadav et al., 1990; Patnaik and Debata, 1996; Sunnichan et al., 1998; Martin, 2002; Chandramu et al., 2003; Biswas et al., 2007; Jawahar et al., 2008). Similar type of observation was also made in *W. chinensis* where IBA proved to be better compared to IAA (Sultana and

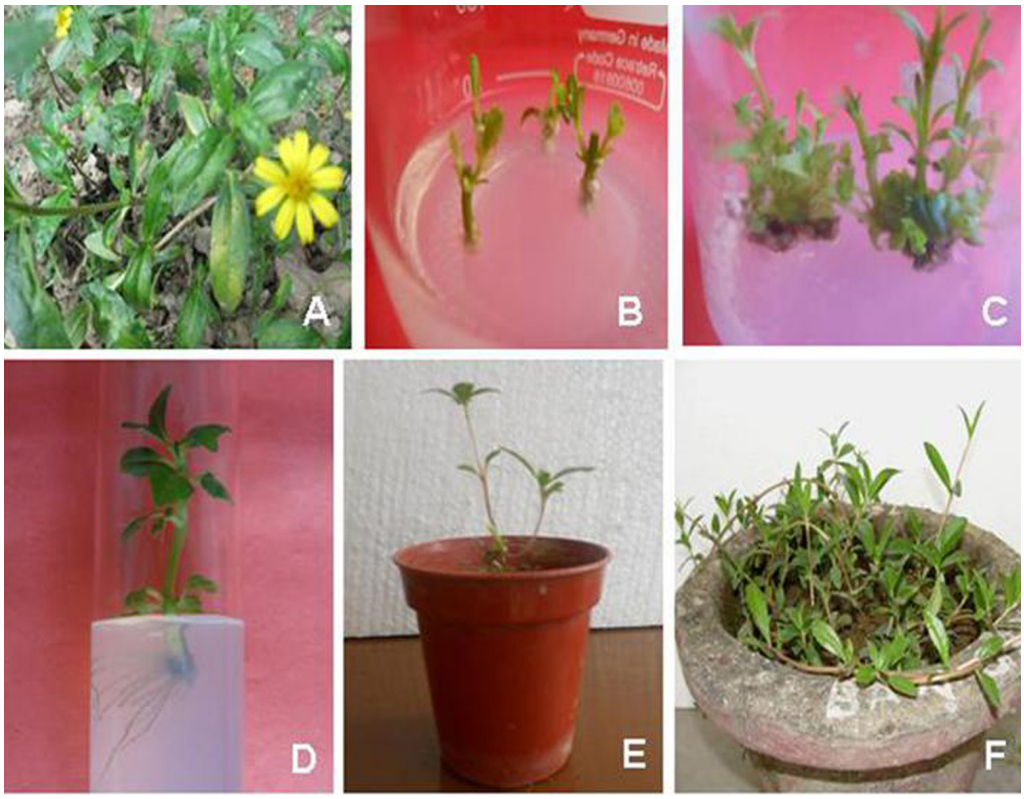

Figure 1. Different steps of micropropagation and establishment of *W. chinensis* through *in vitro* culture technique. A. A *W. chinensis* plant growing in the departmental nursery; B. Nodal segments undergoing direct organogenesis to produce multiple shoot buds; C. Proliferation and elongation of multiple shoot buds; D. Elongated individual multiple shoot bud producing roots in rooting medium; E. *In vitro* grown seedlings established in outside pot F. A four months old *in vitro* grown seedling established in earthen pot.

Table 1. Response of nodal segments (NS) of *W. chinensis* to different kinds of PGR supplemented MS medium.

PGR supplements in the media (mg/l)		Days to response	% of explants giving response	Mean* No. of shoot buds /explant ($\bar{x}$ ±SE)	Mean* length of shoot buds (cm) ($\bar{x}$ ±SE)	Mean No. of roots/ explant
BAP	1.0	7-9	78	2.2±0.17	2.10±0.15	---
	2.0	7-9	85	3.1±0.28	2.00±0.13	---
	3.0	7-9	85	7.0±0.29	**0.68±0.10**	---
	4.0	8-10	85	5.5±0.33	1.25±0.19	---
Kn	1.0	7-9	75	2.2±0.16	1.56±0.15	---
	2.0	7-9	82	**1.7±0.18**	4.06±0.33	2.0
	3.0	7-9	85	2.3±0.28	1.43±0.22	---
	4.0	8-10	85	2.5±0.26	2.55±0.23	3.0
BAP + IAA	2.0+0.1	7-9	78	3.5±0.32	1.75±0.12	---
	2.0+0.5	7-9	85	3.8±0.42	1.62±0.16	---
	2.0+1.0	7-9	85	2.5±0.28	1.37±0.18	---
	3.0+0.1	8-10	85	4.5±0.38	2.19±0.21	
	3.0+0.5	7-9	85	**7.5±0.27**	2.29±0.11	---
	3.0+1.0	8-10	85	3.5±0.29	1.83±0.08	---

Table 1. Contd.

	2.0+0.1	7 - 8	80	2.2±0.24	1.82±0.15	---
	2.0+0.5	7 - 8	85	2.3±0.25	1.71±0.14	---
BAP	2.0+1.0	7 - 8	87	6.8±0.48	3.10±0.32	---
+	3.0+0.1	7 - 8	85	4.2±0.32	2.26±0.18	---
NAA	3.0+0.5	7 - 8	85	5.3±0.48	2.75±0.32	---
	3.0+1.0	7 - 8	85	5.8±0.25	3.00±0.26	---
	2.0+0.1	7-9	75	3.1±0.17	1.62±0.11	---
	2.0+0.5	7-9	82	3.3±0.26	1.32±0.17	---
Kn	2.0+1.0	7-9	85	2.8±0.31	1.25±0.14	---
+	3.0+0.1	8-10	85	4.2±0.32	2.20±0.23	---
IAA	3.0+0.5	8-10	87	6.3±0.28	2.80±0.31	---
	3.0+1.0	8-10	85	3.1±0.24	2.42±0.28	---
	2.0+0.1	7-8	75	2.1±0.18	3.12±0.28	---
	2.0+0.5	7-8	85	2.6±0.40	3.50±0.18	---
Kn	2.0+1.0	7-8	85	2.4±0.24	4.52±0.49	---
+	3.0+0.1	7-8	80	2.3±0.15	2.24±0.16	---
NAA	3.0+0.5	7-8	85	2.4±0.25	2.21±0.13	---
	3.0+1.0	7-8	85	5.0±0.55	2.03±0.19	---

*Based on 15 observations, -- = Indicates no response.

Table 2. Response of shoot apices (SA) of *W. chinensis* to different kinds of PGR supplemented MS medium.

PGR supplements in the media (mg/l)		Days to response	% of explants giving response	Mean* No. of shoot buds/ explant ($\bar{x}$ ±SE)	Mean* length of shoot buds (cm) ($\bar{x}$ ±SE)	Mean No. of roots/ explant
BAP	1.0	7-10	70	2.3±0.18	2.00±0.11	---
	2.0	7-10	80	3.5±0.33	2.20±0.13	---
	3.0	7-10	80	2.8±0.31	2.00±0.13	---
	4.0	8-10	85	2.5±0.31	2.56±0.24	---
	1.0	7-10	72	2.1±0.23	2.10±0.15	---
	2.0	8-10	80	2.5±0.26	5.12±0.17	3.5
Kn	3.0	8-10	85	2.2±0.18	2.38±0.13	4.5
	4.0	8-10	80	2.2±0.20	1.71±0.18	---
	2.0+0.1	7-10	70	3.8±0.26	2.38±0.23	---
	2.0+0.5	7-10	80	4.5±0.33	3.04±0.34	---
BAP	2.0+1.0	7-10	80	2.8±0.23	2.05±0.22	---
+	3.0+0.1	8-10	80	4.2±0.24	2.80±0.23	---
IAA	3.0+0.5	7-10	85	3.5±0.26	2.25±0.24	---
	3.0+1.0	8-10	88	3.3±0.22	2.17±0.17	---
BAP	2.0+0.1	7 - 8	75	2.8±0.25	2.16±0.18	---
+	2.0+0.5	7 - 8	85	3.0±0.41	2.15±0.26	---
NAA	2.0+1.0	7 - 8	85	3.8±0.25	2.94±0.13	---
	3.0+0.1	7 - 8	80	3.1±0.26	2.68±0.22	---
	3.0+0.5	7 - 8	85	3.3±0.25	2.83±0.12	---
	3.0+1.0	7 - 8	85	3.5±0.29	2.70±0.11	---

Table 2. Contd

	2.0+0.1	7-10	72	3.2±0.24	2.18±0.18	---
	2.0+0.5	8-10	80	3.8±0.31	2.80±0.22	---
Kn	2.0+1.0	8-10	85	2.5±0.18	2.02±0.17	---
+	3.0+0.1	8-10	80	3.7±0.26	2.70±0.24	---
IAA	3.0+0.5	7-9	85	3.3±0.24	3.01±0.28	---
	3.0+1.0	7-9	85	3.2±0.21	2.31±0.22	---
	2.0+0.1	7 – 8	70	2.3±0.24	2.53±0.15	---
	2.0+0.5	7 – 8	80	2.3±0.25	2.52±0.09	---
Kn	2.0+1.0	7 – 8	80	2.5±0.29	3.29±0.19	---
+	3.0+0.1	7 – 8	75	2.4±0.20	2.45±0.18	---
NAA	3.0+0.5	7 – 8	85	2.5±0.29	2.48±0.10	---
	3.0+1.0	7 – 8	80	3.5±0.29	2.80±0.07	---

*Based on 15 observations, -- = Indicates no response.

Table 3. Effect of auxins on rooting of *in vitro* grown shoots in half strength MS medium in one month of culture.

PGRs in media (mg/l)		Days to response	% of Response	Mean* No. of root/culture ($\bar{x}$ ±SE)	Mean* length of root(cm) ($\bar{x}$ ±SE)
No PGR	--	4-5	90	4.20±0.20	3.42±0.12
IAA	0.5	4-5	100	7.4±0.24	3.54±0.23
	1.0	4-5	100	8.6±0.24	2.27±0.o6
	2.0	4-6	100	7.8±0.58	3.12±0.27
IBA	0.5	6-7	100	12.75±1.10	2.74±0.27
	1.0	6-7	100	14.25±0.85	2.98±0.06
	2.0	6-8	100	15.75±0.48	2.19±0.10
IBA+ IAA	2.0+0.5	--	--	--	--
	2.0+1.0	--	--	--	--
	2.0+2.0	10-12	70	14.40±0.40	1.93±0.11

*Based on 15 observations, -- = Indicates no response.

Handique, 2005). After rooting the complete plantlets were transferred to outside earthen pots through successive phases of hardening. On an average 80% of the plantlets transferred finally survived in the earthen pots which are now four months old and healthy (Figure 1E and F). It proves the possibility of using this protocol for rapid and mass propagation of *W. chinensis*. Although theprocess of direct organogenesis appears to be less effective in the use of genetic modification experiment but the emergence of direct gene transfer technology like electroporation, electrofusion, microinjection and microprojectile bombardment can be adopted for use of this process in genetic improvement of this medicinal plant through *in vitro* technique.

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