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Multiple shoot proliferation in *Tridax procumbens* L. through *in vitro* method

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Abstract

The present study was performed to find out an efficient in vitro multiple shoot proliferation method for Tridax procumbens L. using shoot tip and nodal segment cultured in Murashige and Skoog's (MS) medium. Dipping in 0.1% HgCl2 was most effective for surface sterilization of explants. Among the different growth regulators, maximum number of shoot proliferation per culture was observed in MS medium supplemented with 6-benzylaminopirine (BAP) and Nodal segments are more responsive for micropropagation than shoot tip explants. Micro cuttings were rooted most effectively in half MS medium supplemented with 0.5 mg/l Indole-3-butaric acid (IBA). Propagated plantlets were successfully acclimatized in soil and more than 90% of the transplanted clones were survived.

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Introduction

Tridax procumbens L, known as Mexican daisy (Coatbuttons) is hardy, perennial, procumbent herb (Asteraceae). The plant is valued for its divergent pharmaceutical properties. The leaf juice has antiseptic, insecticidal and parasiticidal properties. It is used to check hemorrhage from cuts and wounds, bruises and wounds, dysentery, diarrhea and also for preventing premature hair fall. The species is widely used in traditional medicine in India and is in great demand in the Indian pharmaceutical industry (Malik and Chitra, 2009). An aqueous extract of this plant also has marked depressant action on respiration. Earlier workers have already reported the presence of dexamethasone luteolin, glucoluteolin, b-sitosterol and quercetin in this plant (Reddy *et al.*, 2001). The plant harbours immense medicinal potential. It is used to cure hepatitis. Its extract is used to increase immune inflammatory reactions, such as increase in phagocytic index, leucocyte count and antibody secret- ing cells. It has been shown to exhibit dexamethasone effects on wound contraction and granulation. *T. procumbens* develops granulation tissue in rats. It also affects lysyl oxidase activity. The extracts of *T. procumbens* have been reported to have various pharmacological effects, anti-microbial activity against both gram positive and gram negative bacteria and stimulate wound healing. Flavones, glycosides, polysaccharides and monosaccharides have been isolated from the leaves of the plant. The species is widely exploited and its distribution has been declining over the years. It is not possible to micropropagate it through vegetative means, for example cuttings. Propagation through seeds is likely to cause variations. Recently methods have been developed for its *ex situ* conservation through micropropagation (Saini *et al.*, 2008). Advanced biotechnological methods of culturing plant cells and tissues should provide new means of conserving and rapidly propagating valuable, rare and endangered medicinal plants (Nalawade *et al.,* 2003). Successful micro propagation protocols for various medicinal plants have been developed and their conservation has also become feasible through synthetic seeds and cryopreservation technologies. However, no efforts have been made so far for the micropropagation of *Tridax procumbens*. The present studies were undertaken to investigate an efficient method for *in vitro* multiple shoot proliferation of *Tridax procumbens* L.

Materials and methods

Plant material and explant collection

Plant material was collected from Rajshahi University campus. Shoot tip and nodal segment were both used for establishing maximum number of multiple shoots. The explants (1-2 cm.) were washed thoroughly under running tap water for 10 min and then treated with few drops of tween-20 (Polyoxyethylene sorbitan monolaurate) for one min with constant shaking by hand. The shaking followed three successive washings again with distilled water. The surface sterilization was carried out with 0.1% $HgCl_2$ followed by gentle shaking. The segmented parts were thoroughly washed for several times with sterile distilled water and explants were transferred in 25x 150mm.The culture tubes were incubated at 25±4 °C under the warm fluorescent light with intensity varying from 2000-3000 lux. The pH of the media was adjusted to 5.8 prior to all autoclaving. The cultures were incubated for 8 hours photoperiods.

Shoot induction and multiplication

The shot tip and nodal segment explants were cultured on MS medium supplemented with different concentrations (0.5, 1.0, 2.0, 3.0 & 4.0) of BAP and KIN. Data for percentage shoot regeneration, shoot number per explants and shoot length was recorded after 45days of culture.

In vitro rooting

Regenerated microshoots (4-5 cm) were excised and transferred to rooting medium comprising half strength MS without any growth regulator or with auxins – IBA (0.05, 0.10 mg/l) or IAA (0.50 mg/l). The rooting percentage, number of roots per shoot and root length was recorded after 45 days of culturing.

Hardening and acclimatization

Plantlets having well developed root system were removed from the rooting medium, washed properly under running tap water to remove any adherent gel and transferred to thermocol cups containing sterilized soilrite. Thermocol cups were covered with transparent polythene bags to ensure high humidity and irrigated with ¼ strength MS salt solution (without vitamins) for initial 2 weeks followed by tap water. Hardening and acclimatization was performed under diffuse light conditions (16:8 h photoperiod). Polythene bags were removed gradually in order to acclimatize plantlets. After 4 weeks, they were successfully transferred to soil pots and maintained in green house under normal day length conditions.

Data collection and statistical analysis

All the experiments were conducted with 10 replicates per treatment and repeated three times. The data were analyzed statistically using MS excel.

Results

Surface sterilization

After one week of inoculation 0.1% $HgCl_2$ (for 5 min.) treated explants 90% were found contamination free and healthy. $HgCl_2$ used for short duration (1, 2, 3 min) failed to kill the microorganisms and long time (8 minutes), causes no contamination but partial or complete tissue killing was observed.

Multiple shoot development from shoot tip and nodal segment explants:

Shoot tip explants

The highest culture response (90%) was observed in 1.0 mg/l BAP and lowest (35%) was in 4.0 mg/l KIN. Highest mean number of shoots per culture was 10.5±0.27 in 1.0 mg/l BAP and lowest mean number of shoots per culture was 1.0±0.32 in combination of 1.0 mg/l KIN +0.1 mg/l IBA. Highest mean length of shoots was 6.2±0.54 cm in combination of 2. 0 mg/l BAP+0.1 mg/l GA_3 and lowest mean length of shoots was 2.0±0.62 cm in combination of 2.0 mg/l BAP+1.0 mg/l KIN. The highest (10%) and lowest (1%) root formation was in combination of 2.0 mg/l KIN+ 1.0 mg/l NAA and 3.0 mg/l KIN, respectively. To ensure the necessity of growth regulators for rapid micropropagation from shoot tip, a control medium (MS_0) was also used in this experiment. Only 35% shoot tips responded with a lowest mean number 1.3±0.21 of shoots per culture. (Table 1)

Nodal segment explants

The highest culture response (95%) was observed in 1.0 mg/l BAP and lowest (30%) was in combination of 2.0 mg/l BAP+1.0 mg/l KIN. Highest mean number of shoots per culture was 10.6±0.18 in 2.0 mg/l BAP and lowest mean number of shoots per culture was 1.9±0.24 in combination of 1.0 mg/l KIN +0.1 mg/l IBA. Highest mean length of shoots was 6.1±0.12 cm in combination of 2.0 mg/l BAP+0.1 mg/l GA_3 and lowest mean length of shoots was 2.0±0.12 cm in 4.0 mg/l KIN. The highest (9%) root formation was in combination of 2.0 mg/l KIN+0.1 NAA and 2.0 mg/l KIN+0.1 mg/l IBA. Lowest (1%) root formation was in 4.0 mg/l KIN and in combination of 2.0 mg/l BAP+1.0 mg/l KIN. To ensure necessity of growth regulator for rapid micropropagation form nodal segment, a medium (MS_0) was also used in this experiment only 40% nodal segments responded with a lowest mean number (2.1±0.31) of shoots per culture in this medium. (Table 2)

Root induction

Root induction of the shoot cutting Spontaneous rooting was observed in the micro shoots developed in medium supplemented with different concentrations of KIN, KIN+ NAA, and KIN+ IBA. But shoots which were developed in different concentrations BAP, BAP+GA3, BAP+NAA, BAP+IBA produced a very few or no roots. So these micro cutting are further taken for high frequency root formation in different concentrations of auxin (NAA, IBA and IAA) in full and half strength of MS medium (Table-3). In this investigation, the highest response of explant, mean no. of shoot/culture and mean length of shoot was 92%, 12.0±0.24, and 4.3±0.47 cm, respectively in 0.5 mg/l IBA+ half MS. On the other hand the lowest response of explant, mean no. of shoot/culture and mean length of shoot was 50%, 3.5±0.25, and 2.0±0.45cm, respectively in 2.0 mg/l IAA+ full strength MS. Half strength of MS medium

with IBA was best for rooting. The plantlets developed from different *in vitro* culture were successfully established in soil (Fig. 1 & 2). The survival rate was 90%.

Table 1. Effect of different concentrations of Cytokinins and Auxins on *in vitro* propagation of *T. procumbens* using shoot tip explants. Data were recorded after 45 days of inoculation.

Treatment (mg/l)	Days to response	Response of explants (%)	Mean no. of shoot/ culture ($\overline{X}$ ±SE)	Mean length of shoot (cm) ($\overline{X}$ ±SE)	Basal callus formation	Root formation (%)
MS_0	13-14	35	1.3±0.21	2.0±0.42	-	-
BAP						
0.5	8-9	50	5.6±0.44	2.6±0.34	-	-
1.0	10-11	90	10.5±0.27	3.0±0.12	-	-
2.0	9-10	80	8.5±0.31	2.7±0.23	-	-
3.0	11-12	75	8.1±0.43	2.7±0.24	-	-
4.0	12-13	65	7.2±0.14	2.8±0.12	-	-
KIN						
0.5	11-12	55	2.9±0.10	2.6±0.10	-	2
1.0	10-11	60	3.0±0.23	2.2±0.31	-	2
2.0	10-11	65	3.1±0.08	3.0±0.14	-	3
3.0	11-12	50	2.7±0.12	2.4±0.24	+	1
4.0	12-13	35	2.2±0.11	2.1±0.09	+	1
BAP+KIN						
1.0+0.5	10-11	60	2.1±0.22	2.8±0.51	-	2
1.5+0.5	12-13	70	2.5±0.50	2.6±0.60	-	3
2.0+0.5	13-14	72	3.0±0.23	2.2±0.64	+	4
1.0+1.0	9-10	55	2.2±0.30	2.1±0.32	-	2
2.0+1.0	11-12	45	2.0±0.25	2.0±0.62	+	1
BAP+NAA						
1.0+0.1	12-13	50	4.5±0.93	2.1±0.15	+	1
1.0+0.5	11-12	60	4.8±0.31	2.8±0.63	+	3
2.0+0.1	10-11	75	5.3±0.25	3.0±0.31	+	4
2.0+0.5	10-12	70	4.8±0.52	2.8±0.16	++	2
BAP+IBA						
1.0+0.1	11-12	55	3.9±0.90	2.7±0.91	-	1
1.0+0.5	11-12	65	4.7±0.85	2.9±0.09	-	3
2.0+0.1	10-11	70	5.1±0.09	3.4±0.18	+	5
2.0+0.5	11-12	60	4.8±0.25	3.2±0.35	+	2
BAP+GA3						
1.0+0.1	11-12	55	3.3±0.24	4.4±0.36	-	-
1.0+0.5	10-11	60	4.1±0.37	5.2±0.66	-	-
2.0+0.1	10-11	80	4.3±0.32	6.2±0.54	-	-
2.0+0.5	11-12	65	4.1±0.51	6.0±0.16	-	-
KIN+NAA						
1.0+0.1	12-13	40	2.1±0.32	2.5±0.27	-	5
1.0+0.5	10-11	52	2.6±0.21	3.8±0.14	-	7
2.0+0.1	9-10	60	3.0±0.52	4.2±0.54	-	10
2.0+0.5	10-11	48	2.7±0.17	3.5±0.20	-	8
KIN+IBA						
1.0+0.1	11-12	35	1.0±0.32	3.3±0.48	-	4
1.0+0.5	11-12	46	1.3±0.32	3.4±0.53	-	7
2.0+0.1	10-11	55	2.6±0.10	4.0±0.28	-	9
2.0+0.5	11-13	40	1.9±0.16	3.2±0.48	-	6

[Note: -: No callus/root formation; +: Few callus formation and ++: Moderate callus formation]

Table 2. Effect of different concentrations of Cytokinins and Auxins on *in vitro* propagation of *T. procumbens* using nodal segment isolated from field grown plant. Data were recorded after 45 days of inoculation.

Treatment (mg/l)	Days to response	Response of explant (%)	Mean no. of shoot/ culture ($\bar{X}$ ±SE)	Mean length of shoot (cm) ($\bar{X}$ ±SE)	Basal callus formation	Root formation (%)
MS_0	14-15	40	2.1±0.31	1.5±0.11	-	-
BAP						
0.5	9-10	65	5.5±0.32	2.5±0.41	-	-
1.0	10-11	95	12.2±0.32	3.5±0.43	-	-
2.0	9-10	85	10.6±0.18	3.1±0.59	-	-
3.0	9-10	80	10.2±0.21	2.9±0.26	-	-
4.0	10-11	65	9.0±0.32	2.2±0.26	-	-
KIN						
0.5	10-11	55	3.0±0.25	2.2±0.21	-	2
1.0	8-9	56	3.2±0.40	2.9±0.19	-	2
2.0	10-11	65	3.9±-0.44	3.8±0.53	-	5
3.0	11-12	45	2.9±0.23	3.1±0.65	-	2
4.0	12-13	40	2.6±0.15	2.0±0.12	-	1
BAP+KIN						
1.0+0.5	9-10	65	2.3±0.35	3.2±.42	-	2
1.5+0.5	11-12	70	2.5±0.34	2.9±0.61	-	3
2.0+0.5	10-11	75	3.0±0.41	2.6±0.65	-	4
1.0+1.0	12-13	50	2.6±0.30	2.5±0.32	-	2
2.0+1.0	10-11	30	2.2±0.21	2.1±0.35	-	1
BAP+NAA						
1.0+0.1	11-12	60	5.0±0.53	2.3±0.15	-	3
1.0+0.5	10-11	75	5.3±0.31	2.7±0.63	-	4
2.0+0.1	10-11	80	6.4±0.20	3.6±0.35	+	6
2.0+0.5	10-11	70	5.1±0.52	2.9±0.16	++	7
BAP+IBA						
1.0+0.1	10-11	55	5.1±0.03	2.5±0.12	-	2
1.0+0.5	9-10	60	5.8±0.25	2.5 ±0.31	-	3
2.0+0.1	9-10	70	6.0±0.85	2.8±0.71	+	5
2.0+0.5	8-9	60	5.7±0.25	3.2±0.18	+	6
BAP+GA_3						
1.0+0.1	9-10	65	4.6±0.42	4.8±0.22	-	-
1.0+0.5	8-9	70	5.1±0.37	5.9±0.66	-	-
2.0+0.1	10-11	85	5.5±0.42	6.1±0.12	-	-
2.0+0.5	11-12	75	4.8±0.51	6.0±0.41	-	-
KIN+NAA						
1.0+0.1	10-11	45	2.5±0.09	2.5±0.13	-	4
1.0+0.5	9-10	60	3.0±0.31	3.8±0.16	-	6
2.0+0.1	8-9	80	4.0±0.10	4.2±0.11	-	9
2.0+0.5	7-8	55	3.2±0.34	3.4±0.23	-	7
KIN+IBA						
1.0+0.1	9-10	35	1.9±0.24	2.9±0.73	-	5
1.0+0.5	9-10	50	2.5±0.63	3.2±0.90	-	7
2.0+0.1	9-10	70	3.0±0.37	4.0±0.54	-	9
2.0+0.5	10-11	65	2.5±0.17	3.8±0.27	-	7

[Note: -: No callus/root formation, +: Few callus formation and ++: Moderate callus formation.]

Discussion

In the present investigation, important medicinal plant *Tridax procumbens* of Bangladesh was used for micropropagation. *In vitro* propagation of plants holds tremendous potential for the production of high-quality plant based medicines (Murch *et al.*, 2000). It is reported that achievements and advances in the application of tissue culture for the *in vitro* regeneration of medicinal plants from various explants and enhanced production of secondary metabolites (Tripathi and Tripathi, 2003). Plants regeneration from shoot and stem meristems has yielded encouraging results in medicinal plants like *Catharanthus roseus*, *Cinchona ledgeriana* and

Digitalis spp, *Rehmannia gluthinosa*, *Rauvolfia serpentina*, *Isoplexis canariensis*.i (Perez *et al*., 2002; Roy *et al*., 1994).

Table 3. Effect of different concentrations of Auxin (NAA, IBA and IAA) in half strength and full strength of MS medium for root induction of *T. procumbens* micro cutting.1/2 MS.

Treatment (mg L^{-1})	Days to response	Response of explant (%)	Mean no. of shoot/culture ($\overline{X}$ ±SE)	Mean length of shoot (cm) ($\overline{X}$ ±SE)
NAA+$\frac{1}{2}$ MS				
0.1	8-9	60	5.2±0.41	2.2±0.28
0.5	10-11	90	10.2±0.30	3.5±0.26
1.0	9-10	72	7.4±0.25	3.1±0.71
2.0	10-11	60	6.4±0.30	2.2±0.52
IBA+$\frac{1}{2}$ MS				
0.1	16-18	80	8.2±0.44	3.1±0.21
0.5	12-14	92	12.0±0.24	4.3±0.47
1.0	10-12	90	9.3±0.41	3.9±0.37
2.0	14-15	55	7.5±0.25	3.3±0.28
IAA+$\frac{1}{2}$ MS				
	12-13	60	5.1±0.71	2.2±0.25
0.1	14-15	75	8.4±0.56	3.0±0.71
0.5	16-18	65	7.3±0.46	2.4±0.60
1.0	15-16	55	5.0±0.25	2.1±0.31
2.0				
NAA+MS				
0.1	10-12	51	4.0±0.23	2.8±0.21
0.5	11-12	65	7.5±0.27	3.5±0.32
1.0	12-14	60	6.4±0.60	3.0±0.24
2.0	12-13	52	4.2±0.40	2.2±0.32
IBA+MS				
0.1	17-18	80	5.1±0.44	2.9±0.62
0.5	15-16	75	9.0±0.38	3.5±0.19
1.0	14-16	85	11.4±0.27	4.0±0.26
2.0	14-15	60	5.0±0.32	3.0±0.28
IAA+MS				
0.1	15-16	55	3.6±0.54	2.6±0.34
0.5	14-15	60	4.8±0.40	2.8±0.60
1.0	16-17	70	9.6±0.60	3.0±0.39
2.0	18-19	50	3.5±0.25	2.0±0.45

In surface sterilization of explants, many workers used different type of sterilizing agents in different concentrations. In the present study 50% (v/v) of 5.2% sodium hypochlortie and 0.1% mercuric chloride ($HgCl_2$) solution were used as surface sterilizing agents for different duration of time. There are many reports of using $HgCl_2$ (Alam *et al*., 2004; Ahsan *et al*., 2003; Das *et al*., 2001 and Borthakur *et al*., 2000). It is observed about 90% explants of *T. procumbens* was pathogen free with no tissue damage when treated with 0.1% HgCl2 solution for 5 minutes, respectively which similar with other workers (Alam *et al*., 2004; Anand and Jeyachandran, 2004; Nagib *et al*., 2003 and Venkateshwarulu *et al*., 2001). Shoot tip and nodal segment explants from field grown plant of *T. procumbens* were used as explant source. There have been several reports of micropropagation with nodal and shoot tips of tropical and subtropical medicinal plants in the juvenile phase of development (Jaiswal *et al*., 1989). Sahoo and Chand (1998) reported that Shoot tips and nodal explant of *T. procumbens* the highest percentage of shooting response of the inoculated explants was 95% when the explants were cultured in MS medium with 1.0 mg

L^{-1} BA. Both the explants responded in the same medium but the highest number of micro shoots were found to be induced in nodal explants. In the present study, different concentrations and combinations of cytokinin or in combination with NAA, IBA and GA_3 were used to test multiple shoots proliferation potentiality from shoot tip and nodal segments. Among all BAP showed the best performance for multiple shoot induction. Highest mean number of shoots was obtained in in combination of 2.0 mg/l BAP+0.1 mg/l GA_3 from shoot tip and nodal segments. More or less similar observation were noted by Ramesh *et al.* (2005) in *T. bellirica*, Arya et al. (2003) in *Leptadenia*, Ahmad, *et al.* (2002) in *Rauvolfia*. This explant had initiated growth of axillary shoots from the first week of culture. Multiple shoot formation was observed from almost all the treatment. Shoot multiplication rate and growth varied according to the media composition. Different growth regulators played an important role in shoot multiplication and elongation, root induction and basal callus formation. In contrary, Zaman *et al.* (1992) and Yadav *et al.* (1990) also reported the better effect of BAP (2.0 mg/l) in shoot multiplication and elongation in mulberry plant. In pear plants 2.2 mg/l BAP proved to be most effective concentration for shoot tip culture (Lane, 1979) and in strawberry through meristem tip culture 2.5 mg/l BAP was most suitable for shoot regeneration (Khanam *et al.*, 1998). Lakshmidevi and Rajeevan, (1991) and Hommode (1992) have also observed the positive effect of BAP for shoot multiplication in *Dendrobium* sp. Choudhary, (1991) reported the effect of BAP on shoot proliferation of Indian rose. He noted that 2.5 mg/l BAP was optimum for shoot proliferation. In the present inspection, nodal explants are better than shoot tips explants for shoot multiplication. It was found in case of *Vitis vinfiera* (Fanizza and Hadiuzzaman, 1996), *Gladioulus* sp. (Arora *et al.*, 1996) *Prosopie juliflora* (Nandwani and Ramawat, 1991) and *Asclepias curassivica* (Pramanik and Datta, 1986) so many species. Single use of KIN in different concentrations was found not efficient for mass in vitro propagation. Only (3.1 ±0.08) and (3.9 ±0.44) shoots were produced in this medium from shoot tip and nodal explants, respectively in 2.0 mg/l concentration. KIN promoted shoot elongation but results decline in shoot number, as observed in *Balanites aegyptica* by Ndoye *et al.* (2003). This result is contrary to Borthakur *et al.* (2000), who observed high frequency of shoot multiplication along with, maximum shoot length using low concentration of KIN as noted earlier by Franca *et al.* (1995). Although Franca *et al.* (1995) did not obtain spontaneous rooting, but in the present investigation spontaneous rooting was obtained as supported by Borthakur *et al.* (2000). BAP in combination with NAA or IBA produced relatively less number of shoots than alone but a number of roots were formed in these combinations. BAP in combinations with NAA was found effective in many species (Ndoye *et al.*, 2003; Ahmad *et al.*, 2002) but in the present investigation single use of BAP was considered effective.

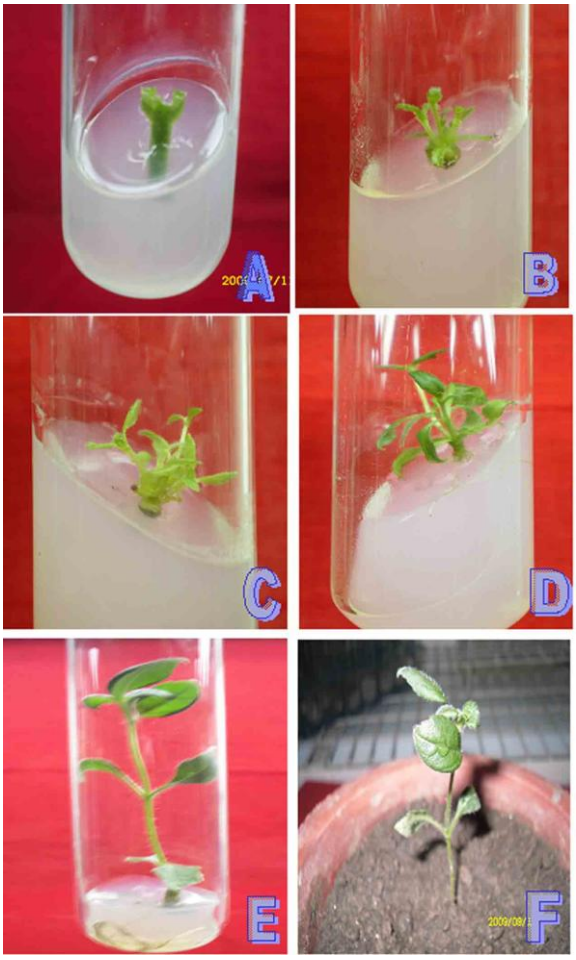

Fig. 1. Clonal propagation of *T. procumbens* from shoot tip: A: Inoculation of shoot tip on MS semi-solid medium, B: Initiation of multiple shoots from

shoot tip, C & D: Development of multiple shoot, E: Development of root, and F: Acclimatization of plantlet.

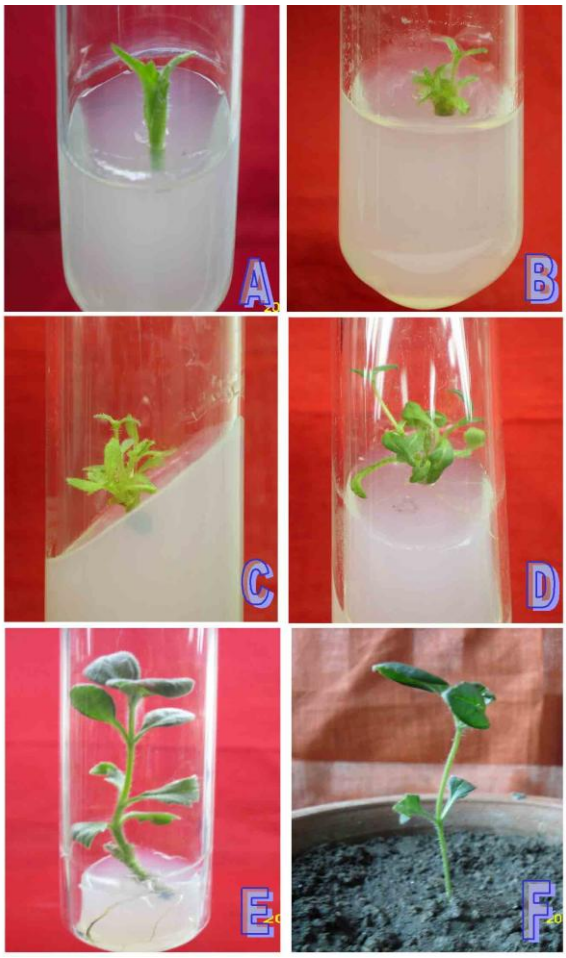

Fig. 2. Clonal propagation of *T. procumbens* from nodal segment: A: Inoculation of nodal segment on MS semi-solid medium, B: Initiation of multiple shoots from nodal segment, C & D: Development of multiple shoot, E: Development of root and F: Acclimatization of plantlet.

KIN in combination with auxin simply increased shoot number but reduce shoot length in the same time. Although, the difference was not seem significant. Barna and Wakhlu (1988) also indicated that production of multiple shoot is highest in *Plantago ovata* in KIN+NAA combination. Under the combined effect of cytokinin with auxin and gibberellic acid in different combinations, BAP+NAA showed better performance than KIN+NAA for shoot multiplication both in shoot tip and nodal explants. As noted by Nodye (2003) and Ahmad *et al.* (2002). Beside these different concentrations of cytokinin (KIN and BAP) in combination with IBA were also tested to form shoot multiplication from shoot tips as well as nodal explants, but these were found less effective than NAA. In the present study, nodal explant was found better than shoot for high frequency shoots multiplication probably due to their more herbaceous nature. It was found in the case of *Holorrhena antlidysenterica* (Datta and Datta, 1984), *Asclepias curassivica* (Pramanik and Datta, 1986), *Prosopis Juliflora* (Nandwani and Ramawat, 1991), *Eucalyptus citriodora* (Gupta *et al.*, 1981), *Carica papaya* (Hossain *et al.*, 1992) and *Gladiolus* sp., (Arora *et al.*, 1996). GA_3 enhance shoot length in a wide range of species (Alam *et al.*, 2004; Ahsan, 2003). In the present investigation BAP in combination with GA_3 produces relatively long shoots than BAP alone but mean shoot number reduced drastically, that was also not the primary objectives in this phase of multiplication. BAP+ GA_3 combination failed to produce root spontaneously. NAA, IBA and IAA were used in different concentration in full and half strength of MS basal medium. Half MS medium having 0.5 mg/l IBA was found best for high frequency root formation from the microcuttings. Similar results were also observed by Jha and Jha (1989) in *Caphaelis ipecacuanha*, Amin *et al.* (2003) in *Centella asiatica*, Barna and Wakhlu (1988) in *Plantago ovata*. NAA also produced satisfactory number of root (Sikdar *et al.*, 2003; Hoque and Fatema, 1995). MS of full strength response also produced satisfactory number of roots but these roots were proved less effective during acclimatization probably due to less mineral supply. The multiplication rate of shoots for mass *in vitro* propagation was found to be dependent on the number of the subsequent subculture. Gautheret (1959) suggested first the importance of auxin in root induction. Bhojwani and Razdan (1983) reported the common range of NAA or IBA is 0.1 to 1.0 mg/l and NAA is the most suitable for root induction for *in vitro* grown plantlets. In the present experiment, IBA was superior to other auxin (NAA), when used singly for rooting. Among the different concentrations tested in both half and full strength of MS medium supplemented with 0.5 mg/l IBA and 1.0 mg/l IBA

was found to be the best level for root induction and growth of roots. From the overall experiment, it can be concluded that BAP was most effective for high frequency shoots multiplication and half strength of MS medium with IBA was best for rooting. During initial culture establishment it was observed that BAP+GA_3 combination produced relatively lower number of shoot with increased shoot length. So GA_3 can be added during sub culture. So, for high efficiency micorpropagation, it is recommended that nodal explant can be cultured for 6-7 weeks in 1.0 mg/l BAP. As the single use of BAP result reduced shoot length. So, during subculture in addition to BAP, 2.0 mg/l of GA_3 should be added. Micro shoots should be cultured in half MS (0.5 mg/l IBA) for root induction. The plantlets developed from different *in vitro* culture were successfully established in soil. The method used for acclimatization in this study was also used by Dhir *et al.* (1998). Thus the utilization of rapid clonal propagation, germplasm conservation and distribution of *T. procumbens* this important medicinal plant of Bangladesh is possible. In conclusion, the protocol described in this investigation can be used for the efficient production of *Tridax procumbens*.

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