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Research Article

THIDIAZURON MEDIATED CALLUS AND MULTIPLE SHOOT INDUCTION IN *NOTHAPODYTES FOETIDA* (WIGHT) SLEUMER- AN IMPORTANT MEDICINAL PLANT

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A B S T R A C T

Mature embryo and hypocotyl explants of *Nothapodytes foetida* were cultured on Murashige and Skoog's medium (MS) supplemented with different concentrations of Thidiazuron. Both multiple shoots and nodular callus from the base of shoots were induced. TDZ at 0.1 mg/l was found most effective in induction of maximum number of (14.60±0.32) shoots per explant. With an increase in the concentration of TDZ (0.2-0.4 mg/l) decrease in tendency of multiple shoot induction was noticed from both the explants. The nodular callus when subcultured again on TDZ (0.1 mg/l) supplemented media produced multiple shoots on an average of 9.25±0.29 shoots per callus. Both the explants when cultured on MS medium supplemented with 2, 4-D (2.0-4.0 mg/l) resulted in callus formation. When this callus was cultured in MS medium supplemented with TDZ (0.1-0.4 mg/l) did not responded for organogenesis.

INTRODUCTION

Nothapodytes foetida (Wight) Sleumer belongs to the family Icacinaceae, distributed in few parts of Western Ghats in India. The plant contains a quinolone alkaloid a potent anticancer drug Camptothecin. Camptothecin was first isolated from *Camptotheca accuminata* (Wall *et al*. 1966). It is the third most promising alkaloid of the twenty first century and is also isolated from the *N. foetida.* It is used in the treatment of different types of cancer viz., lung, breast, ovarian and cervical (Priel *et al*. 1991; Takeuchi *et al*. 1991; Potmesil, 1994; Taguchi, 1995). The accumulation of camptothecin and its derivative 9-methoxy camptothecin in *N. foetida* was first reported by Govindachari and Vishwanathan (1972) from different parts of the plant viz. bark, stem, root and leaves. Hence, toextract and isolate the CPT from in vitro cultures, many authors reported callus induction from different explants viz., leaves (Ciddi and Shuler 2000; Sundravelan *et al*. 2004), cotyledon (Thengene *et al*. 2003; Karadi *et al*. 2008), whole seed (Singh *et al*. 2009), nodal explant (Dandin and Murthy 2012), zygotic embryo (Fulzele and Satdive 2003) and shoot induction from hypocotyl (Rai, 2002), Leaf (Thengane *et al*. 2001), cotyledon (Thengane *et al*. 2001), nodal explant (Dandin and Murthy, 2012) and very few reports regarding the induction of multiple shoots through callus (Tejavathi *et al*. 2012; Dandin and Murthy, 2012).

Many reports suggest that TDZ is a potent cytokinin in inducing multiple shoots and have multiple role during in vitro/ micropropagation studies (Tejavathi *et al*. 2012) and it act as an effective bio-regulant in cell and tissue cultures (Li *et al*., 2000; Hosseini-Nasr and Rashid, 2000; Svetla *et al*., 2003; Matand and Prakash, 2007). In the present investigation too TDZ played dual role in induction of callus and multiple shoots.

MATERIALS AND METHODS

Preparation of explants and culture conditions

Seeds of *N. foetida* were collected from the forest area of Mahabaleshwar and seed coat was removed manually and seeds were washed with running tap water. These seeds were soaked in distilled water for 24 h in dark and disinfected with 70% ethyl alcohol and treated with 4% Sodium hypochlorite for 10min and finally surface sterilized with 0.1% (w/v) aqueous Mercuric chloride ($HgCl_2$) for 2-3 min in a laminar airflow and washed with deionized water 3-4 times to remove the traces of $HgCl_2$ (Kaveri and Rao, 2015). Then seeds were vertically dissected in such way that mature embryos (explants) can be easily taken off from the decoated seeds. MS medium used throughout the study was gelled with 0.8% Agar (Himedia) and pH of the medium is adjusted to 5.7 and autoclaved at 1.2 kg/cm^2 for 20min. Explants were aseptically inoculated on MS medium containing different concentrations of TDZ, 2, 4-D or NAA. After inoculation all the cultures were incubated in light and dark (16x8 h) conditions, providing a quantum flux density of 30mol$S^{-1}m^{-2}$ provided by cool white fluorescent bulbs at 25±2^{0}C.

Statistical analysis

For all experiments 30 explants and three replications per study were maintained and the data obtained was subjected to statistical analysis one way ANOVA to know the standard error (SE) and level of significance with Duncan multiple range test.

RESULT AND DISCUSSION

Callus induction and multiple shoot formation

The morphogenetic response of mature embryo explants varied in frequency and percentage of callus and multiples shoot induction. On all the concentrations of TDZ explants turned green after 10 days of inoculation (Fig: 1.a), developed like seedling, multiple shoots were appeared after 3 repeated sub-culture for every 15 days on same medium. Shoot with primary root and also callus formation from the transition zone/proximal end of embryo was observed after 28-30 days (after 2nd sub-culture) on TDZ supplemented medium (Fig: 1b) MS medium containing 0.1 mg/l TDZ and produced maximum number of multiple shoots (14.60±0.32) and callus from the base of shoots with a frequency of 37%. With an increase in concentration of TDZ both frequency and number of shoots formation decreased (Table: 1) (Fig: 1c).

Hypocotyl explants when inoculated on TDZ supplemented medium failed to produce direct shoots but resulted only in formation of nodular callus (Fig: 1d). Hypocotyl explants started to swell within 10-12 days and continued incubation for 40-42 days formation of nodular callus was observed from the cut ends. This callus when sub-cultured on 0.1 mg/l TDZ supplemented medium produced average of 7.25±0.87 multiple shoots (Fig: 1e) (Table: 2). However with an increase in the concentration of TDZ number of shoot formation decreased (Table: 3). Explants inoculated on 2, 4-D supplemented media responded only for callus formation, which was friable and yellow in colour. With an increase in the concentration of 2, 4-D frequency and fresh and dry weight of callus decreased (Table: 1).

Table 1 Effect of different growth regulator and their response on mature embryo explants

PGR (mg/l)	Response	No. of multiple shoots	Callus		Frequency
			Fresh weight (mg)	Dry weight(mg)	
TDZ					
0.1	Callus and multiple shoots	14.60±0.32[a]	755.96±1.29[a]	74.04±0.32[a]	37
0.2	Callus and multiple shoots	10.80±0.48[b]	642.13±0.47[b]	64.69±0.27[b]	31
0.3	Callus and multiple shoots	07.00±0.58[c]	421.72±0.22[f]	40.93±0.26[d]	25
0.4	Multiple shoots	03.00±0.41[d]	-	-	11
2,4-D					
2.0	Callus induction	-	631.83±0.50[c]	63.32±0.34[b]	30
3.0	Callus induction	-	572.26±0.19[d]	46.07±0.34[c]	30
4.0	Callus induction	-	432.05±0.41[e]	45.92±0.39[c]	25
NAA					
4.0	Shoot induction	1	-	-	14
5.0	Shoot induction	1	-	-	11
6.0	Shoot induction	1	-	-	8

The data represent the average of three replicates, each replicate consist of 30 cultures. Values (Mean ± SE) in a column followed by the same letter are not significantly different P< 0.001. n=30.

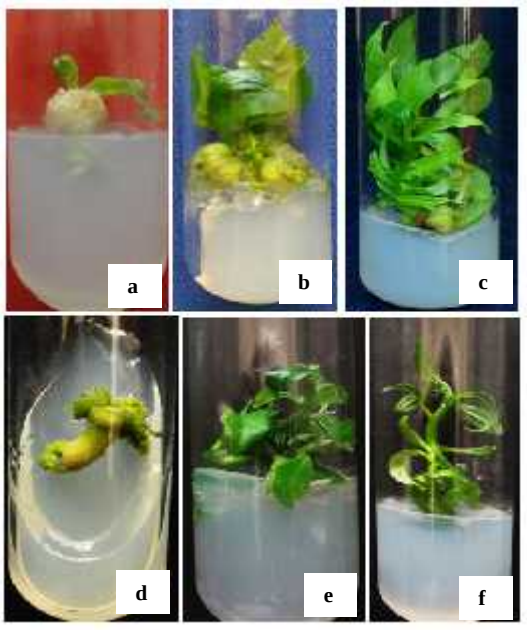

Fig: 1, a. Initiation of callus and shoot from embryo explant on 0.1 mg/l TDZ, **b.** 45 day old cultures of embryo explant on 0.1 mg/l TDZ, **c.** formation of multiple shoots from embryo derived callus cultures, **d.** Initiation of callus from hypocotyl explant on 0.1 mg/l TDZ, **e.** formation of multiple shoots from hypocotyl derived callus and **f.** Single shoot let on NAA supplemented medium.

Where mature embryo explants inoculated on medium containing NAA failed to form callus, however only single shoot formation was observed (Fig:1. f). Callus obtained from both hypocotyl and mature embryo explants on medium supplemented with 2, 4-D failed to produce multiple shoots even after repeated sub culturing on TDZ supplemented media. Dual role of TDZ in formation of multiple shoots and callus from the base of the shoots is reported in this species (Tejavathi *et al*., 2012). Singh *et al*., (2003) also reported the dual nature of TDZ in initiating somatic embryos in the intact seedlings of Pigeon pea. TDZ can have beneficial effect in inducing multiple shoots directly from hypocotyl explants (Rai, 2002) and from leaf explants (Thengane *et al*., 2001) rather inducing callus. Guo *et al* (2011) reported that TDZ is a plant growth hormone can bring both auxin and cytokinin like effects during in vitro culturing of tree species, during the present investigation too TDZ exhibited a cytokinin like activity by promoting shoot differentiation and an auxin like activity by inducing callus in *N. foetida*. The role of TDZ is not clear, doesn't have purine ring which is common to adenine but it stimulates the synthesis of endogenous cytokinins and also inhibits their degradation (Thomas and Katterman, 1986) during the in vitro culturing.

Table 2 Effect of different concentrations of TDZ/ 2, 4-D on Hypocotyl explants

PGR (mg/l)	Response	Number of multiple shoots	Callus		Frequency
			Fresh weight (mg)	Dry weight (mg)	
TDZ					
0.1	Callus and multiple shoots	13	553.24±0.68[a]	59.19±0.30[a]	30
0.2	Callus and multiple shoots	08	440.30±0.82[c]	46.15±0.27[c]	25
0.3	Callus and multiple shoots	07	413.34±2.02[d]	44.28±0.52[d]	10
0.4	Callus and multiple shoots	05	310.77±0.74[g]	32.69±0.77[g]	10
2. 4-D					
2.0	Callus induction	-	481.35±0.39[b]	48.61±0.28[b]	25
3.0	Callus induction	-	351.58±0.52[e]	39.58±0.33[e]	10
4.0	Callus induction	-	328.13±0.39[f]	35.22±0.37[f]	10

The data represent the average of three replicates, each replicate consist of 30 cultures. Values (Mean ± SE) in a column followed by the same letter are not significantly different P< 0.001. n=30.

Fig: 2, a *In vitro* rooting on 1/4 MS + 2.0mg/l IBA. **b**. potted plant.

Table 3 Effect of TDZ on induction of multiple shoots from callus

TDZ (mg/l)	No. of multiple shoots	
	Embryo derived callus	Hypocotyl derived callus
0.1	9.25±0.29[a]	7.25±0.87[ab]
0.2	5.50±0.58[b]	5.00±1.41[b]
0.3	2.75±0.55[bc]	1.00±0.47[c]
0.4	1.25±0.73[c]	-

The data represent the average of three replicates, each replicate consist of 20 cultures. Values (Mean ± SE) in a column followed by the same letter are not significantly different P< 0.01. n=20.

Rooting of in vitro raised shoots

The in vitro formed shoots from the cultures were excised individually and inoculated onto medium containing (1/4 strength MS medium nutrients) ¼ MS+ 2.0mg/l IBA for rooting (Fig 2. a). Root initiation was observed after 20-22 days of culture. Many authors reported rooting from ½[nd] (half strength MS medium nutrients) and ¼[th] strength MS medium (Thengane *et al*. 2001). Induction of roots at different concentration of IBA either alone (Tejavathi *et al*., 2012) or in combination with NAA (Dandin and Murthy, 2012) is reported earlier. Well rooted plants were then transferred to small polycups containing soil:sand:vermicompost in 1:1:1 ratio. The plantlets were covered with polythene bags made with small pores for proper aeriation and left for hardening (Fig 2. b).

After successful hardening of plants in laboratory conditions they were then transferred to pots and maintaining in the green house of Department of Botany, Gulbarga University, 41% survival rate of the plants was recorded.

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