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Effects of Growth Regulators on *In vitro* Callogenesis of *Taxus baccata* L.

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ABSTRACT: Interest in over exploitation, possibly illegal, of the existing resources of *Taxus* has been intensified with the discovery of Taxol which is identified as one of the most promising anti-cancer drugs. In recent years, plant tissue culture techniques have become a powerful tool for the propagation of many plant species. In this research weight of callus was analyzed to determination of appropriate medium culture. This study was performed in complete randomized design with three replicated in 2014. The MS medium culture was contains NAA and Kinetin hormones. ANOVA showed significant difference at 1% probability level. Maximum callus weight belongs to 0.25 mg/l Kinetin and 2 mg/l NAA treatment. Therefore, hormones amount which used in this research can induced callus in *Taxus baccata* L.

Keyword: *Taxus* (*Taxus baccata*), Callus, Kinetin, NAA

INTRUDUCTION

Interest in over exploitation, possibly illegal, of the existing resources of Taxushas been intensified with the discovery of Taxol which is identified as one of the most promising anti-cancer drugs (Heinstein 2006). *Taxus* is propagated by seeds and rooted cuttings though these methods are slow and would not conserve the plant traits people value. In vitro culture of *Taxus* has been reported mainly through embryo culture and somatic embryogenesis. The estimated need of Taxol per year is 250 kg of the purified drug that need 750,000 trees. The ever increase demand of Taxol in the treatment of cancer need a large source of plants for extraction. Therefore, *Taxus* is exposed to the risk of extension (Liao *et al*, 2006). Many studies on in-vitro regeneration of *Taxus* species viz. *Taxus cuspidata*, *Taxus baccata*, *Taxus media*, *Taxus canadensis* (Globa *et al*, 2009), *Taxus brevifolia* (Chee, 1995a), TaxuswallichianaZUCC (Hien *et al*, 2004) have been undertaken earlier. So, alternative biotechnological method such as cell suspension culture for the production of Taxol and tissue culture for the rapid propagation and conservation of *Taxus wallichiana* should be considered as demonstrated by Hussain *et al*, (2011).

Flavonoids are ubiquitous in plants (Krauze-Baranowska, 2004, Hergert, 1998). These so-called secondary products have many functions mainly in protecting against biotic and abiotic aggressors (Treutter, 2006). The flavanols as a subgroup of the flavonoids were selectively detected in nuclei by their blue histochemical stain in fresh tissue. This finding was also confirmed by a highly selective physical method (Mueller-Harvey, 2012). The flavanols appear to be bound to histones in nuclei of several coniferous tree species and their intranuclear distribution is highly influenced by developmental and environmental signaling networks (Feucht, 2012). The extraction of paclitaxel from naturally growing *Taxus* trees is quite limited because a large number of trees need to be harvested to obtain a sufficient amount of paclitaxel (Jaziri *et al*, 1996) developed a high-yield procedure for the in vitro propagation of juvenile material of *Taxus baccata* involving a combination of seed handling and induction of shoot proliferation from nodal explants obtained from 2-month old plantlets raised from seedlings. This procedure allowed the fast screening of individuals for their taxane content (Majada *et al*, 2000).

Demands on the supply of taxol also continue to grow as a result of its expanding use in early intervention therapies and in combination with other chemotherapeutic agents. At present, taxol is successfully obtained in cell cultures of different *Taxus* species (Cragg *et al*, 1993, Srinivasan *et al*, 1995, Cusido *et al*, 1999, Navia-Osorio *et al*, 2002). The estimated need of Taxol per year is 250 kg of the purified drug that need 750,000 trees. The ever increase demand of Taxol in the treatment of cancer need a large source of plants for extraction. Therefore, *Taxus* is exposed to the risk of extension (Liao *et al*, 2006). Tissue culture has helped to develop new strain of food crops, cereals, vegetables flowers, oil seeds and plantation crops such as spices, coffee, tea and rubber. Therefore the present preliminary study was we have chosen callus mediated shoot organogenesis as an alternative method to achieve a higher rate of leaf multiplication for Hortical crops improvement, during spring, 2014, University of Zabol, Iran.

MATERIAL AND METHODS

A. Preparation of explants

Explants were taken from *Taxus baccata* tree growing in the Herb garden of province Golestan, Iran. Leaf was used as explants. The explants was washed with tape water up to 15 min to remove any mud or dust particle and reduce the microbial load. Then washed with distilled water and sterilized with 0.1% mercuric chloride for 1min. after sterilization with mercuric chloride, the explants were washed 3 times with autoclaved distilled water to reduce the toxic effect of mercuric chloride.

B. Propagation media

WPM basal media (pH 5.2) containing wpm mineral and vitamins supplemented with 25g/L sucrose as energy source were used. Different concentration of Kin, NAA and a photoperiod of 16 hr light/8 hr dark condition at 25±5°C, relative humidity 15-30% were used throughout the experiment. Activated charcoal was also used in some experiment to reduce the browning effects of exudates. Media was solidified with 6 g/l agar, added before autoclaving.

C. Inoculation of explants

The sterilized explants (Leaf) was cut into small pieces and aseptically placed in the test tubes/flasks containing WPM media under laminar air flow hood. Culturing was carried out in 50 ml test tubes or 250 ml Erlenmeyer flasks. All the cultures were sub-cultured using same media supplemented with aforementioned growth regulators and carbohydrates to regenerate the species.

Organogenic Callus Induction

leaf segments from in vitro grown 20 days old seedlings were used as explants and placed on callus initiation medium which contained WPM, B5 vitamins (Gamborg *et al.*, 1968) supplemented with diverse concentration of Kin (0- 0.25- 0.5- 0.75- 1 mg/l) and NAA (0- 0.5- 1- 1.5- 2 mg/l) alone or in combination Kin and NAA for callus induction.

RESULTS AND DISCUSSION

A. Callus culture induction

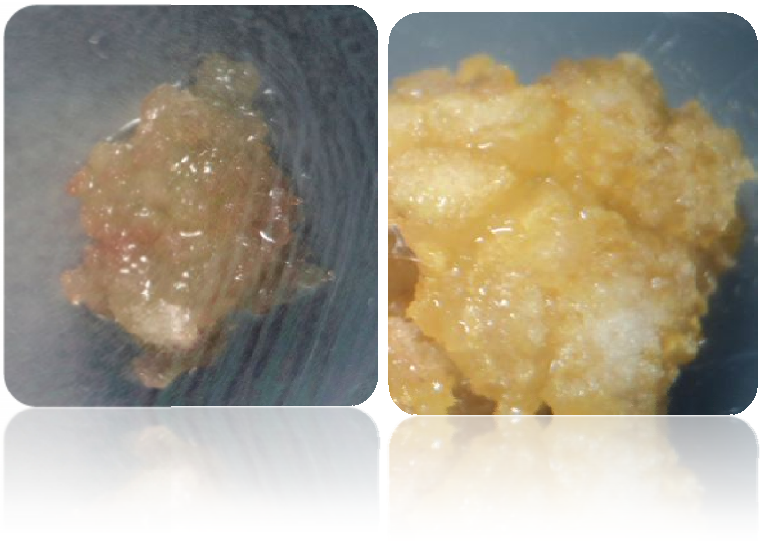
The leafs were cut into small segments and used as explants. They were cultured on callus induction medium (WPM) consisting of auxin and cytokinin. Among the two auxin and cytokinin investigated Kin with NAA were more effective than the other auxin and cytokinin with the highest percentage (78 %) of callus initiation (Table 1). The auxin and cytokinin in different concentration produced different types of callus. However, used Kin and NAA at mid-level concentrations gave best percentage of organic callus induction (78 %, Table 1) in the present study. Result of ANOVA statistical analysis according by Complete Random Design (CRD) with three replicates showed that the leaf in MS medium with Kin (0.25 mg/l) and NAA (2 mg/l) has produced high quality callus (Table 2). The results were obtained has many differences with Omer *et al* (2011) in amount of using Kin and NAA hormones. Their result in compare with our research has significant difference in weight of callus. Therefore, we can introduce these hormone concentrations' for *Taxus* callus induction.

Table 1: Data on effects of Kin and NAA on callus induction and callus growth of leaf explants.

Plant Growth Regulators (mg/l)	Percentage of organogenic callus induction	Type and nature of callus
Kin		
0	0	—
0.25	0.16	Brown
0.5	0.17	Brown and brittle
0.75	0.25	Brown and brittle
1	0.29	Brown and brittle
NAA		
0	0	—
0.5	0.37	Brown
1	0.39	Brown
1.5	0.44	Brown and brittle
2	0.46	Brown
NAA+KIN		Brown
0.5+0.25	0.49	Dark Brown
0.5+0.5	0.45	Brown
0.5+75	0.57	Brown and brittle
0.5+1	0.59	Brown
1+0.25	0.38	Brown
1+0.5	0.57	Light Brown
1+75	0.53	Brown
1+1	0.45	Dark Brown
1.5+0.25	0.67	Light Brown
1.5+0.5	0.52	Brown
1.5+75	0.44	Brown
1.5+1	0.55	Brown
2+0.25	0.78	Brown
2+0.5	0.64	Dark Brown
2+75	0.59	Brown
2+1	0.66	Dark Brown

Table 2: *Taxus* ANOVA statistical analysis.

source	df	weight
NAA	4	44.56^{**}
KIN	4	3.56^{**}
NAA×KIN	16	0.40^{ns}
Error	50	0.34



KIN*NAA(0.25+0.5) KIN*NAA(0.75+2)

Fig. 1. Callus induction of *Taxus* by KIN and NAA hormones.

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