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Investigation of the Effects of Growth Regulators on Callus Induction in *Taxus baccata* L.

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ABSTRACT: *Taxus baccata* is classified as a conifer in the order Pinales, family Taxaceae and genus *Taxus*. The leaves are thin and long, with spiral arrangement, upper surface of the lamina is dark green and glossy but the lower surface is light green. In recent years, plant tissue culture techniques has 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 randomized design with three replicated in 2014. The MS medium culture was containing NAA and Kin hormones. ANOVA showed a significant difference at 1% probability level. Maximum callus weight belongs to 0.25 mg/l Kinetin and 2 mg/l NAA. Therefore, hormones amount used in this research can induce callus in *Taxus baccata*.

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

INTRUDUCTION

Taxus baccata is classified as a conifer in the order Pinales, family Taxaceae and genus *Taxus*. Three species of genus *Taxus* are found in Iran of which only *T. baccatais* native (Yazdani *et al,* 2005). *T. baccata* is an evergreen species with high longevity, slow growth, straight stem and Sinusoidal profile and may reach the height of 30m and the diameter of 2.5m (in Siyahkal) (Ahmadi *et al,* 2011, Poorbabaei *et al,* 1998). Its crown is extended and semi-spherical. The bark is scaly, laminated and reddish brown. The leaves are thin and long, with spiral arrangement, upper surface of the lamina is dark green and glossy but the lower surface is light green. Flowers are without petal and sepal and appear as female and male on separate female and male trees. Its fruit is called aril and is berry-like, red, sweet and edible. Its annual height growth has been estimated about 10cm and annual diameter growth is about 0.5mm (0.64mm of average annual diameter growth) (Lessani 1999, Danehkar and Mahmoodi 2008, Dargahi 2000). Axillary bud induction from juvenile shoot tips or segments derived from rooted cuttings (Ewald, 2007, Jun, 2007), in vitro-germinated seedlings (Majada, 2000) or even from up-to 5-year-old plants (Nhut, 2007) has also been studied. Meanwhile, using mature plant materials which could be up to 1000-year-old is limited (Chang, 2001, Abbasin, 2010), attributable to the fact that micropropagation of mature trees is generally more difficult comparing to their juvenile counterparts. Nevertheless, mature trees are often preferred for cloning because one can select trees that have been in the field long enough to have demonstrated their superior value. Using shoot tips as initial explants results in a problem of elongated single shoot without axillary bud induction (Nhut, 2007), which could be overcome by shoot apical decapitation (Majada 2000). The objective of our study was, therefore, to refine a procedure for initiation, establishment and proliferation of an in vitro shoot culture from mature explants of *Taxus*. The leaves and bark of *Taxus* has been exploited for the extraction of Taxol. It has unique property of preventing the growth of cancerous cells, and being used in the treatment of breast and ovarian cancer (Kovacs *et al,* 2007).

It inhibits cell proliferation through inhibiting microtubule dissociation, due to its tubulin binding affinity (Ashrafi *et al.*, 2010). It is obtained from all the *Taxus* species and for the first time it was obtained from the bark of *Taxus*. Its anticancer activity was discovered in 1971 (Wani *et al*, 1971).and even in the controlled conditions the pericarp of the seed acts as the barrier for seed germination. Based on the current bark extraction procedures, nearly 7, 000-10,000 kg of bark is needed to produce 1 kg of Taxol (Cragg *et al,* 1993; Wann and Goldner, 1994). 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 were 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

MS basal media (pH 5.8) containing MS mineral and vitamins (Murashige & Skoog, 1962) supplemented with 30g/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 8 g/l agar, added before autoclaving.

C. Inoculation of explant

The sterilized explant (Leaf) was cut into small pieces and aseptically placed in the test tubes/flasks containing MS 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.

D. 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 MS salts (Murashige and Skoog, 1962), 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

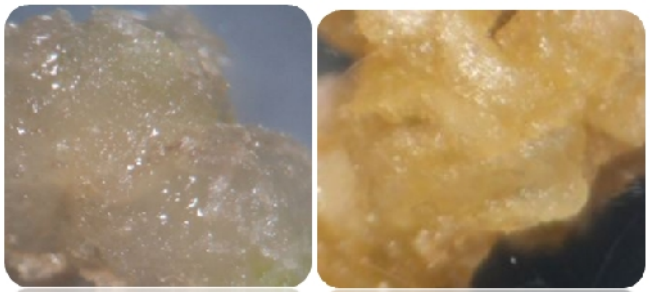
The leaves were cut into small segments and used as explants. They were cultured on callus induction medium (MS) consisting of auxin and cytokinin. Among the two auxin and cytokinin investigated Kin with NAA were more effective than the other auxin and cytocinin with the highest percentage (73 %) of 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 (73 %, 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 Hussian *et al* (2013) study in amount of using Kin and NAA hormones. Their results comparing with our research have significant difference 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	0.12	Brown
0.5	0.14	Brown and brittle
0.75	0.22	Brown
1	0.20	Brown
NAA		
0	0	—
0.5	0.34	Brown and brittle
1	0.37	Brown
1.5	0.40	Brown and brittle
2	0.38	Brown
NAA+KIN		Light Brown
0.5+0.25	0.47	Dark Brown
0.5+0.5	0.43	Light Brown
0.5+75	0.54	Brown and brittle
0.5+1	0.56	Brown
1+0.25	0.36	Brown
1+0.5	0.54	Light Brown
1+75	0.52	Brown
1+1	0.43	Dark Brown
1.5+0.25	0.62	Light Brown
1.5+0.5	0.51	Brown and brittle
1.5+75	0.41	Brown
1.5+1	0.54	Dark Brown
2+0.25	0.73	Brown
2+0.5	0.63	Dark Brown
2+75	0.58	Light Brown
2+1	0.65	Dark Brown

Table 2: *Taxus* ANOVA statistical analysis.

Source	df	Weight
NAA	4	45.95**
KIN	4	2.76**
NAA×KIN	16	0.54**
Error	50	0.09
CV	-	11.94



1. KIN*NAA(1+1.5) 2. KIN*NAA(0.25+2)

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

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