



Effect of Growth Regulators and Explant Types on Callus Induction from *Oroxylum indicum* (L.) Vent.

KEYWORDS

Oroxylum indicum, callus, plant growth regulators.

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ABSTRACT

The present work is based on developing a protocol for callus induction in *Oroxylum indicum* (L.) Vent. Hypocotyl, apical bud and axillary bud excised from *in vitro* raised seedling were used as explant. Callus induction depended on the different concentration of plant growth regulators and types of explants in the callus inducing medium. Among the three explants used, hypocotyl was best for callus formation as compared to other explant. Highest efficiency of callus formation was observed in the medium containing different concentration of auxin and cytokinin. *In vitro* generated callus can be used as a source for phytochemical screening.

Introduction

Oroxylum indicum (L.) Vent. commonly known as Sonapatha, Syonaka and Midnight horror which is widely found in India, Sri Lanka, China, Philippines and Malaysia (Bennet et al., 1992). This plant possesses antioxidant, antifungal, antimicrobial, anti-inflammatory, antibacterial, antiarthritic, anticancer property (Warrier et al., 1995). Root extract of this plant has been used in Ayurvedic preparations like Dashmularisht and Chyawanprash (Yasodha et al., 2004). It is also used in many important ayurvedic formulation such as Amartarista, Dantadyarista, Narayana taila, Dhanawantara ghrita, Brahma rasayana, Awalwha (Anonymous, 1998). This plant are reported to contain flavonoids namely, Chrysin, oroxylin- A, scutellarin, baicalein (Sankara and Nair, 1972). Because of its indiscriminate collection and over exploitation for medicinal purpose has pushed this plant to the list of endangered plant species of India (Darshan and Ved 2003).

The development of rapid callus formation protocol for this important medicinal plant has become a necessity in order to multiply the plant which will help in conservation and meet the pharmaceutical needs and also to prevent the plant from becoming endangered.

MATERIALS AND METHODS

Collection of the plant material

The seeds from mature fruits were collected from the selected plant during December- January, 2012 – 2013 at Hemchandracharya North Gujarat University, Patan, Gujarat.

Selection of explants

Different explants like hypocotyl, apical bud and axillary bud were excised from 6-7 weeks old *in vitro* raised seedlings for callus induction.

Culture media and Culture conditions

The explants were surface sterilized in 0.1% (w/v) HgCl₂ for 2 minutes. Each treatment was followed by repeated washing minimum of 3 times in autoclaved distilled water, and then inoculated in glass tubes containing MS Media. Explants were cultured on MS basal medium supplemented with different concentrations and combinations of 2, 4-dichlorophenoxyacetic acid, BAP and Kinetin for callus formation. in growth room in controlled conditions at a

temperature of 25 ± 2°C, 16 h dark/8 h light photoperiod, 0-70% humidity and continuous illumination was provided by cool white fluorescent tubes at 2000 lux. Each treatment consisted of at least 8 explants and the experiment was repeated four times. Morphological changes were recorded on the basis of visual observations at 2-week intervals.

RESULTS AND DISCUSSION

There was no sign of callus formation when explants were cultured in media without auxin or cytokinin. The nature of callus was measured by the callus graded into two categories: friable, spongy, also graded according to their color in a symbol of pale white, whitish green, whitish brown.

Effect of 2,4-D

Maximum induction of callus occurred at 2.0 mg/l 2, 4-D (90.62 %) from Axillary bud. Whereas minimum callus (56.25 %) induced at 0.5 mg/l 2,4 -D from hypocotyls. Increase the concentration of 2,4-D from 0.5 mg/l to 2.5 mg/l, increased the frequency of callus. 2,4-D at lower concentration produced lower response in all the explants. While callus from hypocotyls was spongy in nature and pale white in color. In apical bud callus induction at 2.5 mg/l 2, 4-D (87.5 %) was friable in nature and green in color while highest frequency of callus induction observed in hypocotyls at 2.0 mg/l 2, 4-D, (87.5%). The callus from axillary bud and apical bud explants was friable in nature and white in color.

Effect of 2, 4-D with BAP

Various explants cultured on MS Media containing 2, 4-D (2.0mg/l) + BAP (0.5- 2.5mg/l) exhibited different frequency as well as nature of callus. In hypocotyl explants, 2,4- D (2mg/l) + BAP (2mg/l) produced spongy, pale white callus with 100 % frequency followed by 90.62 % from apical bud (2 mg/l 2,4-D + 2.5 mg/l BAP) and callus was friable in nature and whitish green in color. At the similar concentration, axillary bud also produced friable and whitish green callus with 90.62 % frequency.

Effect of 2, 4-D with KN

Hypocotyl induced spongy and pale white callus at lower concentration (2 mg/l KN+ 0.5 mg/l BAP) and spongy and whitish brown at higher concentration (2 mg/l KN + 2.5 mg/l BAP). Apical bud and axillary bud produced maxi-

mum callus at 2,4-D (2.0mg/l) and BAP (2.0mg/l). Minimum percentage of callus was observed from apical bud at 2,4-D (2.0mg/l) with BAP (0.5mg/l). Highest frequency of callus was observed in apical bud (93.7%), followed by axillary bud (87.5%) and hypocotyl (84.3%). Increase the concentration of BAP with 2,4-D induced lower frequency of callus.

In the present study, the callus growth was fast in the first 12-14 days of culture, later slowed down, dark brown. This was due to phenolic exudation. Similar results showing variations among explant types with respect to callus induction have also been reported in other plants like *Albizia lebbek* (Lakshmana Rao and De, 1987); *Psoralea corylifolia* (Pandey et al., 2013); *Lonicera japonica* (Georges et al., 1993) and *Holarrhena antidysenterica* (Raha and Roy, 2003). Among the various concentration, 2,4-D with BAP was more effective for callus induction. Liu et al., (2006) reported that auxin by itself or in combination with cytokinins has been widely used to enhance callus induction. The medium supplemented with either 2, 4 -D alone or in combination with BAP/Kn induced friable callus. The induction of callus from other members of Menispermaceae viz. *Stephania cepharantha* Hayata (Suzuki et al., 1992), *Coscinium fenestratum* (Nair et al., 1992) and *Tinospora cordifolia* (Chintalwar et al., 2003) were reported when cultured on MS medium supplemented with 2, 4 - D + Kn/BAP.

CONCLUSION

The important part of present study was induction of callus in *Oroxylum indicum* (L.) Vent. by different explants. This study showed that response to callus induction depended on explant type and different concentration of plant growth regulators which may boost in vitro development.

Table-1 : Effect of hormones on callus formation of *Oroxylum indicum* (L.) Vent.

Sr.No	Hormones	Hypocotyl explant		Apical bud		Axillary bud	
		Nature of callus	% frequency	Nature of callus	% frequency	Nature of callus	% frequency
2,4-D (mg/l)							
1	Control	-	-	-	-	-	-
2	0.5	Spongy, Pale white	56.25	Friable, pale white	62.5	Friable, pale white	65.62
3	1.0	Spongy, Pale white	62.5	Friable, pale white	68.75	Friable, pale white	71.87
4	1.5	Spongy, Pale white	71.87	Friable, pale white	75	Friable, pale white	84.37
5	2.0	Spongy, Pale white	87.5	Friable, pale white	81.25	Friable, pale white	90.62
6	2.5	Spongy, Pale white	78.12	Friable, pale white	87.5	Friable, pale white	87.5
2,4-D + BAP							
7	2 + 0.5	Spongy, Pale white	71.87	Friable, Whitish green,	68.75	Friable, Whitish green,	59.37
8	2 + 1	Spongy, Pale white	84.37	Friable, Whitish green,	71.87	Friable, Whitish green,	65.62
9	2 + 1.5	Spongy, Pale white	90.62	Friable, Whitish green,	84.37	Friable, Whitish green,	78.12
10	2 + 2	Spongy, Pale white	100	Friable, Whitish green,	90.6	Friable, Whitish green,	90.62
11	2 + 2.5	Spongy, Pale white	93.75	Friable, Whitish green,	96.8	Friable, Whitish green,	75
2,4-D + Kn							

12	2 + 0.5	Spongy, Pale white	59.3	Friable, Pale white	56.25	Friable, Pale white	59.37
13	2 + 1	Spongy, Pale white	65.6	Friable, Pale white	68.75	Friable, Pale white	71.8
14	2 + 1.5	Spongy, Pale white	75	Friable, Pale white	78.12	Friable, Pale white	81.25
15	2 + 2	Spongy, whitish brown	81.25	Friable, Pale white	93.7	Friable, whitish brown	87.5
16	2 + 2.5	Spongy, whitish brown	84.3	Friable, whitish brown	84.3	Friable, whitish brown	78.12

Plate-1: Callus formation from different explant



(A- from hypocotyl, B- from apical bud, C – from Axillary bud)



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