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Effect of Photoperiod on *In vitro* Culture of Guggul [*Commiphora wightii* (Arnott)] – A Medicinal Plant

Rajani Verma[1*], M.L. Jakhar[2] and Ravi Kumar[1]

Department of Plant Breeding and Genetics, SKN College of Agriculture, Jobner, India

**Corresponding author*

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A B S T R A C T

Present investigation was carried out the effect of different photoperiod regimes on shoot bud induction, callus induction and shoot regeneration from callus culture in leaf explants of guggul. Standard protocols micropropagation protocol (1.5 mg/l BAP) for nodal segment and (2.0 mg/l Kn) for shoot apex explant and callus induction (2.0 mg/l 2,4-D) and regeneration protocol (1.5 mg/l Kn+ 1.0 mg/l 2,4-D)] were subjected to different photoperiod regimes (16:8, 14:10, 12:12 and 8:16). The cultures were incubated at 25±2°C with a light intensity of 3000 lux. 14:10 hours photoperiod regime was found best for shoot bud induction, callus differentiation and *de novo* shoot development among all the tested photoperiod regimes.

Introduction

Commiphora wightii (Arnott) is a medicinally important plant which is now considered as critically endangered species of the family Burseraceae having the chromosome number 2n = 26 (Sobti and Singh, 1961). The name *Commiphora* originates from the Greek words kommi (meaning 'gum') and phero (meaning 'to bear'). *Commiphora wightii* is a small tree/shrub, grow very slowly and takes 8 to 10 years to reach to a height of 3 to 3.5 meters. Guggul mainly grow in arid regions, hillock and terrains and also considered as a drought and salinity resistant plant. Guggul grow well with mean annual rainfall of 225-500 mm and temperature ranging from 20-35 ^{0}C. It prefers loams to sandy loam soils with basic pH ranging from 7.5 to 9.0. The genus *Commiphora* is widely distributed in tropical regions of Africa, Madagascar, Asia, Australia, Pacific islands (Good, 1974) and arid areas of India, Bangladesh, and Pakistan. In India, it is found in arid, rocky tracts of Rajasthan, Gujarat Maharashtra and Karnataka (Kumar and Shankar 1982). In Rajasthan it is found in many districts *viz.*, Jaisalmer, Barmer, Jodhpur, Jalore, Sirohi, Ajmer, Sikar, Churu, Jhunjhunu, Pali, Udaipur, Alwar (Sariska Tiger Reserve), Jaipur (Ramgarh, Jhalana area), Bhilwara and Rajsamand.

Guggul is very much used in Ayurvedic system of medicine as astringent, anti-septic, expectorant, aphrodisiac, carminative, anti-spasmodic, anti-inflammatory, hypoglycemic, aperitif, sedative, stomachic, diaphoretic, diuretic, expectorant, anti-helminthic, emmenagogue, depurative, vulnerary, demulcent, aphrodisiac, liver tonic and lithonotriptic (Watt, 1972). It is widely used for obesity and it is also known as fat burning agent all over the world. It helps to lower cholesterol and triglycerides level. Guggul is very effective in rheumatoid arthritis, gout and sciatica. In addition it treats sluggish liver, stimulates libido, nervous diseases, bronchial congestion, cardiac and circulatory problems, weak digestion, wounds, abscess, foetid ear, fractures, gynaecological problems and various skin diseases. Guggul is a very important and trustworthy herb in Ayurvedic medicine. Basically it is used almost in every kind of illness due to its amazing treating power (Singh *et al.,* 2015).

Guggul is considered as an endangered plant in India and listed as 'Data Deficient' in the IUCN Red Data list (IUCN, 2010) because of a lack of knowledge regarding its conservation status as well as excessive, unscientific tapping methods to increase yield of oleo-gum resin causes mortality of plants leading to the extinction danger of the species. Now considered a critically endangered species (IUCN, 2015). The conventional methods of propagation by seeds are very slow. Fruit set and yield of fruits per plant are very low in natural conditions. the plant is slow growing. Normally it is propagated vegetatively by stem cutting and air layering. However, such methods are not suitable for large scale multiplication as stock material with sufficient quantity is not available further, response of cuttings/ air layering is variable and affected by seasons. Therefore, there is an urgent need to conserve this species *ex situ* through *in vitro* method and to develop reliable and rapid protocol for its micropropagation (Singh *et al.,* 2010).

Thus the present investigation has been undertaken to establish reliable protocol for study the effect of different photoperiod regimes on shoot bud induction, callus induction and shoot regeneration from callus culture in leaf explants of guggul.

Materials and Methods

The present investigation was carried out at the Department of Plant Breeding and Genetics, S. K. N. College of Agriculture, Jobner.

Plant material

The present investigation was carried out in Tissue Culture Laboratory, Department of Plant Breeding and Genetics, S. K. N. College of Agriculture, Jobner. The plant material for this investigation was obtained from Horticulture farm, S. K. N. College of Agriculture, Jobner. Three explants *viz.*, nodal segments, shoot apex and leaves were used as explant in the present investigation.

Culture medium

All chemicals used in the present study were of analytical grade. Murashige and Skoog Medium were used throughout the course of investigation.

Explant preparation and sterilization

Various explants like shoot apex, nodal segment and leaf explants were used. All the explants were washed with liquid detergent under running tap water for 20 minutes to remove dust particles. These were again washed with liquid detergent (Rankleen) for ten minutes with vigorous shaking. After washing with detergent, explants were again

washed with running tap water to remove any trace of detergent for 5 minutes. After it were sterilized with bavistin for 5-10 minutes and then washed with double distilled water 4-5 times. In laminar air flow it were surface sterilized with 90 per cent ethanol for 30 seconds, then with 0.1 per cent $HgCl_2$ for 2-5 min depending upon the nature of explants. Thereafter, the explants were washed 4-5 times with autoclaved distilled water.

Inoculation of explant

After sterilization the explants were inoculated on culture media aseptically. For inoculation, explants were transferred to large sterile glass petriplates with the help of sterile forceps under strict aseptic conditions. Here the explants were further trimmed to desired sizes with sterile scalpel blade. After cutting explants of suitable size, these were transferred to culture test tubes, phyta jars and borosil flasks containing MS medium supplemented with different plant growth regulators. After vertically inoculating the explants in culture phyta jars, test tubes and borosil flasks, the mouth of phyta jars, test tubes and borosil flasks were quickly flamed, test tubes and borosil flasks were closed with non adsorbent cotton plug and phyta jars with cap.

Culture conditions

All cultures were incubated at 25+20C with a light intensity of 3000 lux.

Effect of photoperiod

To see the effect of different photoperiod regimes on *in vitro* cultures, especially in relation to direct shoot proliferation, callus induction and organogenesis, the following photoperiod regimes were tested on responsive cultures.

Photoperiod regimes

Light (hrs)	Dark (hrs)
16	8
14	10
12	12
8	16

Results and Discussion

Photoperiod is the length of time for which a plant is exposed to light in 24 hours. Photoperiodism can also be defined as developmental responses of plant to the length of day and night. Photoperiod of tissue culture grown room is dependent on type of culture. Hence it should be emphasized that photoperiodic effects relate to the timing of both the light and dark periods.

In the present investigation different photoperiod regimes were assessed for morphogenetic effect with standard callus induction (2.0 mg/l 2, 4-D), direct shoot proliferation (1.5 mg/l BAP for nodal segment and 2.0 mg/l Kn for shoot apex explant) and regeneration protocol (1.5 mg/l Kn + 1.0 mg/l 2,4-D) in guggul. Standard protocols were subjected to different photoperiod regimes (16:8, 14:10, 12:12 and 8:16).

When nodal segment explants incubated on MS medium supplemented with 1.5 mg/l BAP with different photoperiod regimes, Maximum shoot bud induction (1.53) was observed at 14:10 hours photoperiod followed by 16:8 hours photoperiod with 100 per cent frequency. Frequency of shoot bud

differentiation reduced with decreasing hours of light. 8:16 hours photoperiod was not sufficient to induce shoot buds in nodal segment explants (Table 1). Significant differences were observed among different photoperiod regimes for shoot bud induction in nodal segment explants.

In present investigation maximum shoot bud induction (1.50) was observed in shoot apex explants incubated at 14:10 hours photoperiod followed by 16:8 hours photoperiod. 8:16 hours photoperiod was not sufficient to induce shoot buds in shoot apex explant even on responsive level of plant growth regulators (Table 2). As like nodal segment, frequency of bud differentiation also reduced at all other photoperiod regimes in shoot apex explants.

When leaf explant incubated on MS medium supplemented with 2.0 mg/l 2, 4-D with different photoperiod regimes. Maximum callus induction from cut ends of leaf explant was observed at 14:10 hours photoperiod followed by 16:8 hours photoperiod. Frequency of callus differentiation on cut ends of explants ranged from 50–100 per cent at different photoperiod regimes (Table 3). Perusal of Table 4 indicated that *de novo* shoot regeneration from callus cultures exhibited significant differences at different photoperiod regimes. The response was best when the dark period was shorted and the reverse when the dark period was longer. In case of organogenesis from callus cultures, regeneration was not observed in cultures incubated at 8:16 hours photoperiod, the response with other photoperiod being similar.

Photoperiod controls many developmental responses in animals, plants and even fungi. The response to photoperiod has evolved because day length is a reliable indicator of the time of year, enabling developmental events to be scheduled to coincide with particular environmental conditions (Jackson, 2009). Photoperiodism is one of the most significant and complex aspects of the interaction between plants and their environment. It is defined as plant responses to day length, enabling living organisms to adapt to seasonal changes (Zuoli *et al.*, 2004).

Table.1 Effect of different photoperiod regimes on shoot bud induction in nodal segment supplemented with 1.5 mg/l BAP

S.No.	Photoperiod regime	Number of shoot bud induction	Shoot length (cm)	Morphogenetic response (%)
1	16 : 8	$1.29^{\#}$(1.3)	$1.30^{\#}$(1.36)	80
2	14 : 10	$1.53^{\#}$(1.9)	$1.55^{\#}$(1.92)	100
3	12 : 12	$0.98^{\#}$(0.6)	$1.02^{\#}$(0.71)	40
4	8 : 16	$0.70^{\#}$ (-)	$0.70^{\#}$ (-)	-
Mean sum of squares due to treatment		**1.31****	1.32**	
Mean sum of squares due to error		**0.07**	0.09	
CD at 5%		**0.24**	0.27	

** Significant at p= 0.01,
(#) = Transformed values,
(-) = No response,
() = Value in parenthesis represents mean number of shoot bud

Table.2 Effect of different photoperiod regimes on shoot bud induction in shoot apex supplemented with 2.0 mg/l Kn

S.No.	Photoperiod regime	Number of shoot bud induction	Shoot length (cm)	Morphogenetic response (%)
1	16 : 8	1.38# (1.1)	1.21# (1.16)	60
2	14 : 10	1.50#(1.8)	1.54# (1.88)	100
3	12 : 12	0.94#(0.5)	1.01# (0.66)	40
4	8 : 16	0.7# (-)	0.70# (-)	-
Mean sum of squares due to treatment		**1.40****	1.22**	
Mean sum of squares due to error		**0.05**	0.09	
CDat5%		**0.21**	0.27	

** Significant at p= 0.01,
(#) = Transformed values,
(-) = No response,
() = Value in parenthesis represents mean number of shoot bud

Table.3 Effect of different photoperiod regimes on callus induction in leaf explant supplemented with 2.0 mg/l 2,4 D

S.No.	Photoperiod regime	Days taken in callus induction	Callus weight (g)	Morphogenetic response (%)
1	16 : 8	24.2	0.71	100
2	14 : 10	24.4	0.96	100
3	12 : 12	30.7	0.52	70
4	8 : 16	35.2	0.42	50

Table.4 Effect of photoperiod regimes on de novo shoot regeneration in callus culture

S.No.	Photoperiod regime	Days taken in regeneration	Number of *de novo* regenerated shoots	Morphogenetic response (%)
1	16 : 8	41.8	0.91# (0.3)	20
2	14 : 10	36.7	1.00# (0.5)	30
3	12 : 12	42.5	0.73# (0.1)	10
4	8 : 16	-	0.70# (-)	-
Mean sum of squares due to treatment			0.09**	
Mean sum of squares due to error			0.06	
CD at 5%			0.23	

** Significant at p= 0.01,
(#) = Transformed values,
(-) = No response,
() = Value in parenthesis represents mean *de novo* developed shoots

Photoperiodism is essential for the maintenance of plant and animal fitness in temperate and arctic climates (Bradshaw and Holzapfel, 2008). Everyday plants absorb definite amount of light for flowering. The effect of light which initiates flower is known

as photoperiodic induction. The leaves of plants receive this photoperiodic induction and pass it to the region of flowering through phloem tissue.

Generally, plant growth and development are affected by both internal factors including genotype and plant hormones and external factors such as light, duration, temperature and moisture supply. This result may be due to the interaction between light intensity and internal factors which directly affect plant growth. The suitable light intensity and duration will give the best result of product (Soontornchainaksaeng *et al.,* 2001). In the present investigation different photoperiod regimes (16:8, 14:10, 12:12 and 8:16) were assessed for shoot bud, callus induction and *de novo* shoot regeneration in MS medium supplemented with different responsive levels of plant growth regulators. Maximum shoot bud induction, callus proliferation and *de novo* shoot regeneration was observed at 14:10 hours photoperiod followed by 16:8 hours. Similar results were also observed by Yadav (2008), Jakhar *et al.,* (2012), Kumawat (2013) in *Aloe vera*, Nagar (2017) Burdak *et al.,* (2017) in fenugreek and Kumar *et al.,* 2018 in pomegranate. However, Yadav (2008) reported maximum shoot induction in micro shoot explant of *Aloe vera*, when cultures were incubated at 14:10 hours photoperiod followed by 12:12 hours photoperiod.

Longest light hours (16:8) promoted shoot bud induction, callus development and direct regeneration as reported by Singh *et al.,* (2010) in *Commiphora mukul*, Kant *et al.,* (2010) and Soni (2010) in *Commiphora wightii*. These results are contradictory to the result of present investigation. This might be due to difference in explants, plant type and different concentration of growth regulator.

Current study revealed that maximum shoot length was also observed at 14:10 followed 16:8 hours photoperiod (Table 1 and 2). Zakizadeh *et al.,* (2013) reported significant differences among various photoperiods through increasing bulblets diameter, leaf length and shoot length in Amaryllis. Yadav and Singh 2012 reported highest bud break, longest shoot length and maximum number of shoots in Liquorice (*Glycyrrhiza glabra* L.) at 16:8 hours photoperiod. These results are contradictory to the finding of current investigation due to differences in plant species.

Shortest light hours (8:16) were insufficient for shoot bud induction and *de novo* shoot regeneration. These finding were also in close to the findings of the Gurjar (2009) in *Alove vera*, Choudhary *et al.,* (2017) and Aparna *et al.,* (2017) in Gliricidia.

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