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ORIGINAL ARTICLE

Effect Of Photoperiod On *In Vitro* Culture of Pomegranate Cv. Sindhuri

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ABSTRACT
Present investigation was carried out the effect of different photoperiod regimes on callus induction, shoot bud induction and shoot regeneration from callus culture in leaf and shoot apex explants of pomegranate. Standard protocols [standard callus induction (1.0 mg/l BAP + 1.0 mg/l NAA), micropropagation protocol (2.5 mg/l BAP for nodal segment and 2.5 mg/l BAP for shoot apex explant) and regeneration protocol (1.0 mg/l BAP + 2.0 mg/l NAA)] 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 hour's photoperiod regime was found best for shoot bud induction, callus differentiation and de novo shoot development among all the tested photoperiod regimes.
Keywords: *Shoot apex, photoperiod, callus, In vitro, pomegranate.*

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INTRODUCTION

Pomegranate (*Punica granatum* L.) var. Bhagwa belonging to the family Punicaceae is one of the favorite table fruit of tropical and subtropical countries. In India, pomegranate is commercially cultivated in Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Telangana, Madhya Pradesh, Tamil Nadu and Rajasthan. At present, Maharashtra is the leading state in acreage covering about 68.7 per cent of the area and 70.2 per cent of total production under pomegranate [1]. In India, it is cultivated over 2.16 lakh ha with a production of 27.95 lakh tones and productivity of 12.94 tones/ha [2]. In Rajasthan, it is cultivated over 2857 ha area with production of 10379 tones and productivity of 3.63 tones/ha. Jalore, Chittorgarh, Barmer, Bhilwara and Jodhpur are major pomegranate producing district in Rajasthan. Out of these districts Jalore is leading district with 847 ha area, 3134 tones production and 3.7 tones/ha productivity [3].

The usage of pomegranate is deeply embedded in human history with references in many ancient cultures for its use as food and medicine. It is commercially cultivated for its delicious fruits. A fully matured fruit is highly nutritive and rich source of protein, fat, fibre, carbohydrate *etc*. The fruits are rich in Fe, Ca, and antioxidant component like phenol, pigments and tannins. Apart from its demand for fresh fruits and juice, the processed products like pomegranate wine, pomegranate tea and candy are also gaining importance in world trade. The pomegranate fruit juice is a good source of sugars, vitamin C, vitamin B, pantothenic acid, potassium, antioxidant polyphenols and a fair source of iron. In addition, the tree is also valued for its pharmaceutical properties. It is used for treating dyspepsia and considered beneficial in treating leprosy. The rind of the fruit and the bark of pomegranate tree are used as a traditional remedy against diarrhea, dysentery and intestinal parasites. Fruit rind, bark of stem and root are widely used for tannin production [4].

In vitro regeneration systems in pomegranate have been established basically for two factors i.e. a need for an inexpensive and efficient method for clonal propagation of elite genotypes and for application of modern genetic improvement methods for efficient *de novo* recovery of plants from cell cultures. Pomegranate cell and tissue culture is not easy though regeneration from existing meristems (shoot tip and nodal bud), vegetative and reproductive plant parts have been attempted with some noteworthy success.

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

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 research work was conducted on *Punica granatum* cv. Sindhuri. Leaves and Shoot apexes were used as explant and obtained from healthy trees grown at Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner.

Culture medium

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

Explant preparation and sterilization

To obtain sterilized explants is difficult because in the process of sterilization living material should not lose their biological activity; only bacterial and fungal contaminant should be eliminated. All the explants were sterilized by using different surface sterilization agents. Explants were washed thoroughly in running tap water for 20 minutes, these were again washed with liquid detergent (RanKleen) for ten minutes with vigorous shaking. After washing with detergent, explants were washed with running tap water to remove any trace of detergent for 5 minutes. Finally explants were surface sterilized with 0.1 per cent $HgCl_2$ in a laminar air flow cabinet. Shoot apex sterilized for 3-4 minutes and leaves sterilized for 1-2 minutes.. These were thoroughly washed four to five times with sterilized double distilled water and inoculated on the culture media supplemented with various concentrations of plant growth regulators.

Inoculation of explant

After sterilization the explants were inoculated on culture media aseptically. For inoculation, explants were transferred to large sterile glass Petri plates 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 vertically 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, the mouth of phyta jars, test tubes and borosil flasks were quickly flamed then test tubes and borosil flasks were closed with non adsorbant cotton plug and phyta jars with cap.

Culture conditions

All cultures were incubated at 25 ± 2^0C 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

Photoperiod is the physiological response of organism to the length of day and night. It occurs in plants and animals. Photoperiodism can also be defined as developmental responses of plant to the length of day and night. Hence it should be emphasized that photoperiodic effects related 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 (1.0 mg/l BAP + 1.0 mg/l NAA), micropropagation protocol (2.5 mg/l BAP for nodal segment and 2.5 mg/l BAP for shoot apex explant) and regeneration protocol (1.0 mg/l BAP + 2.0 mg/l NAA) in pomegranate cv. Sindhuri. Standard protocols were subjected to different photoperiod regimes (16:8, 14:10, 12:12 and 8:16).

When leaf explant incubated on MS medium supplemented with 1.0 mg/l BAP + 1.0 mg/l NAA 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. The 8:16 hours photoperiod was insufficient to induce callus on cut surface of the leaf explant further swelling (Table 1).
In present investigation maximum shoot bud induction (2.3) 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 except enhancement in shoot growth. Significant difference was observed in shoot bud induction from shoot apex explants at different photoperiod regimes (Table 2).
Perusal of Table 3 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 12:12 and 8:16 hour's photoperiod, the response with other photoperiod being similar. Differences in organogenesis were significant at different photoperiod regimes.

DISCUSSION

The ability to co-ordinate certain developmental processes to particular times of the year when environmental conditions are likely to be more favorable confer distinct advantages. Timing reproduction to spring time so that vulnerable young offspring have the maximum possible time to develop before experiencing the harsh conditions of winter, for example, would result in a greater survival rate of the offspring. There is thus a selective advantage for plants and animals that have acquired mechanisms enabling them to sense seasonal differences through the detection and response to changes in photoperiod. The photoperiod is the amount of light and darkness in a daily cycle of 24 h. 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 [5].
Several aspects such as wavelength (quality), intensity (quantity) and duration of light are important factors affecting plant growth [6]. Although light is an important factor in micropropagation, reports on the effect of artificial light intensities on plant growth, particularly of orchids, are rather scarce. This is chiefly because the higher light intensity necessary for some plants to mature are difficult to achieve and because of the space required by some plants at this stage. For relatively short time periods, plant performance probably reflects the photosynthetic process.
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 [7].
Photoperiodism is the physiological reaction of organisms to the length of day or night. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of the light and dark periods. Hence, it should be emphasized that photoperiodic effects relate directly to the timing of both the light and dark periods.
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 hour's photoperiod followed by 16:8 hour's. Similar results were also observed by Jakhar *et al*., [8], Kumawat, [9] in *Aloe vera*, Nagar, [10] and Burdak *et al*., [11] in fenugreek. Shortest light hour's (8:16) were insufficient to proliferation of callus in leaf, shoot bud induction and *de novo* shoot regeneration. These finding were also in close to the findings of the Choudhary *et al.,* [12] and Aparna *et al*., [13] in Gliricidia.
Zakizadeh *et al.* [14] reported significant differences among various photoperiod through increasing bulblets diameter, leaf length and shoot length in Amaryllis. Photoperiod 16:8 and 14:10 hour's increased leaf length in comparison to 12:12 hour's photoperiod regimes. There were no significant differences between photoperiod of 16:8 and 14:10. Current study revealed that maximum shoot length was also observed at 16:8 and 14:10 hour's photoperiod. However, the differences at these photoperiod regimes were significant in present investigation, indicating role of plant type and level of plant growth regulators also important under *in vitro* conditions.
However, these results were quite different to the finding of Burger *et al*., [15] in *Rosa hybrida,* Goyal *et al.,* [16] in Ber, Tyagi *et al.,* [17] in Ginger, Aasim *et al*., [18] in *Urginea maritime* and Gurjar, [19] in *Aloe vera*, where 16:8 hours photoperiod gave better regeneration. This might be due to difference in explants

and plant type. The present investigation revealed that the medium supplemented with responsive level of plant growth regulators differed significantly by the influence of photoperiod under *in vitro* conditions.

Table 1: Effect of different photoperiod regimes on callus induction in leaf explant supplemented with 1.0 mg/l BAP + 1.0 mg/l NAA

S.No.	Photoperiod regime	Days taken in callus induction	Callus weight (mg)	Morphogenetic response (%)
1	16 : 8	26.8	639.6	100
2	14 : 10	23.4	874.9	100
3	12 : 12	32.7	462.1	70
4	8 : 16	-	-	-

-= No callus induction

Table 2: Effect of different photoperiod regimes on shoot bud induction in shoot apex supplemented with 2.5 mg/l BAP.

S.No.	Photoperiod regime	Number of shoot bud induction	Shoot length (cm)	Morphogenetic response (%)
1	16 : 8	1.4# (1.5)	2.24# (5.12)	80
2	14 : 10	1.7# (2.3)	2.60# (6.32)	100
3	12 : 12	1.1# (0.9)	1.45# (2.44)	40
4	8 : 16	0.7# (-)	0.7# (-)	-
Mean sum of squares due to treatment		4.93**	12.43**	
Mean sum of squares due to error		0.07	0.26	
CD at 5%		0.37	0.46	

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

Table 3: 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	35.5	1.14# (1.1)	40
2	14 : 10	30.1	1.81# (3.2)	70
3	12 : 12	-	0.70# (-)	-
4	8 : 16	-	0.70# (-)	-
	Mean sum of squares due to treatment		4.80**	
	Mean sum of squares due to error		0.15	
	CD at 5%		0.35	

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

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