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RESEARCH ARTICLE

EFFECT OF MONOCHROMATIC LIGHT TREATMENTS ON EMBRYOGENESIS FROM LEAVE EXPLANTS OF *CELASTRUS PANICULATUS*

[1,*]Sentiya Priti, [2]Sharma Tripti and [2]Rathore Pragya

[1]Department of Bio-Sciences, Pacific Academy of Higher Education and Research University, Udaipur 313001, India
[2]Altius Institue of Universal Studies, Indore 452001, India

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ABSTRACT

An efficient protocol has developed for induction, maturation and plantlet germination. In this study the leaf explant of C. paniculatus inoculated into Murashige and Skoog Medium supplemented with different concentrations and combination of 2,4-D, BAP and TDZ. Maximum percentage of callus induction was observed induction was 86.5% in 2,4-D and BAP (1.0+0.5mg/lt) under the influence of red light treatment and 83.3%, 81.6%, and 78% under the influence of white, blue and yellow light treatment respectively. Maximum percentage of somatic embryogenesis 92% was observed in MS medium fortified with 2, 4-D (1mg/lt) + BAP (0.5mg/lt) under the influence of red light treatment, 90%, 88% and 87% under the influence of blue, white and yellow light treatment respectively. Maximum average number of plantlets 38.0±3.4 was observed in MS medium fortified with 2, 4-D and BAP (1.0+0.5mg/lt) under the influence of red light treatment and 33.6±3.2, 31.3±4.1 and 27.6±0.5 under the influence of blue, yellow and white light treatment respectively. We report for the first time the use of higher wavelength for an efficient rapid propagation of this threatened medicinally important plant. This protocol will prove suitable in overcoming the demand and supply ratio of this plant for medicinal use. At the same time it will help in conserving C. paniculatus in its natural habitat.

INTRODUCTION

Celastrus paniculatus is an important medicinal plant belonging to the family Celastraceae also known as jyotismati, malkanganai and duhudu. It is a woody climber shrub mainly distributed to hilly regions of India and South East, Asia at an altitude of 1250 meters. The species is vulnerable in Western Ghat of South India (Rajesekharan and Ganeshan, 2002). This plant is mainly known for its valuable medicinal properties. Oil obtained from its seed is very beneficial for the treatment of psychiatric patient and also helpful for treatment of rheumatism, gout and neurological disorder. *Celastrus paniculatus* also know its ability to improve memory (Nandkarini, 1976). Pharmacological studies suggest that the oil obtained from seed posses sedative and anticonvulsant properties (Gatinode, 1957). Leaves are emmenagouge, appetizer, laxative, sex invigoration, various fever and antidote for opium poisoning. The bark is reported to be abortifacient, depurative and brain tonic taken internally for snake bite (Govil, 1993).

****Corresponding author: Sentiya Priti,***
Department of Bio-Sciences, Pacific Academy of Higher Education and Research University, Udaipur 313001, India.

Root bark extract also shows antimalarial activity (Rastogi and Mehrotra 1998). Roots are useful in the treatment of cancerous tumor (Parotta, 2001). Celapagin, celapanigin, celastrine and paniculatine are the alkaloids founds in seed oil, which are responsible making this plant medicinally highly potent (Parimala, 2009). Conventionally, *C.paniculatus* is propagated mainly through the seeds. However, the viability and germination (11.5 %) of the seeds are poor (Rekha, 2005). *C.paniculatus* has been prioritized by the National Medicinal Plant Board (Government of India) for conservation and cultivation emphasizing the need to develop agro-technology packages. Tissue culture techniques are being used globally for the conservation and utilization of genetic resources (Rao *et al.,* 1996). This leads to an urgent need for propagation and conservation of the plant species by plant tissue culture technology which offers a great potential for regeneration of these important medicinal plants.

MATERIALS AND METHODS

Collection of plant material : Mother plant of *Celastrus paniculatus* was collected from herbal garden of Rajmata Vijyarajae Scindia Krishi Vishwavidyalaya, Indore. The plant

was diseases free and showed good biomass yield. Juvenile leaf excised from healthy mother plant of *Celastrus paniculatus*. They were washed into running tap water for 15-20 minutes for the removal of dirt particles. Then after they were washed with tween20 liquid detergent for 10-15 minutes and then again washed with tap water to remove the traces of detergent. For surface sterilization explants were taken into laminar air flow, surface sterilization carried into sterile wash bottles. Explants firstly rinsed with 0.1% Mercuric chloride (Himedia) for 3-4 minutes, and then they were rinsed with 70% alcohol for 30 seconds. After that explants were rinsed with sterile distill water for 3-4 times to remove the traces of mercuric chloride and alcohol.

Medium and culture conditions

After surface sterilization leaf were trimmed about 1cm with the help of sterile scalpel and forcep. Trimmed leaf explants were inoculated into Murashige and Skoog medium supplemented with 3% sucrose, different concentrations and combinations of auxins and cytokinins. All media pH were adjusted to 5.8±2 by using 1N NaOH and 1N Hcl and 0.6% agar-agar powder (Himedia) was used for the solidification of medium. Media were autoclaved at 121^{o} C for 15-20 minutes. All cultures were kept at 22±2 ^{o} C and relative humidity was 70%. All the cultures kept under the influence of different monochromatic light treatments (white, blue, yellow and red).
Callus induction and proliferation. Leaf explants of *Celastrus paniculatus* were inoculated into MS Medium containing 3%sucrose, 0.6% agar-agar and fortified with different concentrations of plant growth regulators 2,4-D individually (1.0-2.0 mg/lt) and in combination with TDZ (0.5, 1 mg/lt), BAP (0.5, 1mg/lt) after 8 days of inoculation. For proliferation of callus, four week old callus were cut into small pieces and then transferred onto same medium for proliferation along with control (MS) medium without any plant growth regulators.

Somatic embryo induction and maturation

Selected callus with morphogenic character were transferred on Murashige and Skoog medium supplemented with 3% sucrose and fortified with different concentrations of 2,4-D (1.0-2.0 mg/lt), individually and in combination with TDZ (0.5 and 1 mg/lt), BAP (0.5 and 1mg/lt) for induction and maturation of embryos. Mature embryos were transferred on MS basal medium containing 3% sucrose and fortified with 2,4-D (1.0 mg/lt) in combination with TDZ (0.5 and 1 mg/lt), BAP (0.5 and 1mg/lt). They were maintained on same medium and subcultured regularly for 4 weeks. MS medium without plant growth regulators were used as a control.

Plantlet formation

In-vitro germinated plantlets with well developed shoot and root were transferred on subcultured on MS basal semisolid medium. The number of plantlets with shoot and root system was counted.

Hardening and Acclimatization

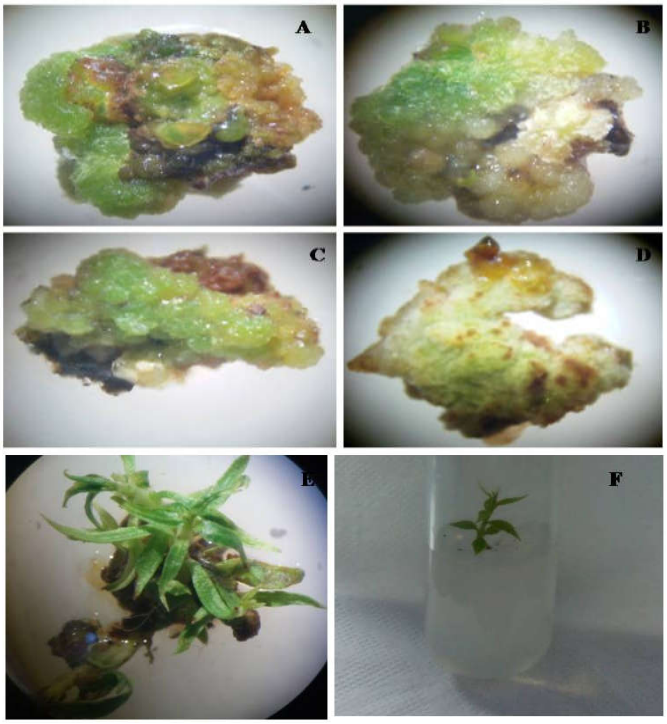
Well rooted plantlets were taken out from culture bottles with the help of forcep to prevent from any damage and then after they were washed with distill water to remove agar-agar. The regenerated plants were transferred into plastic pots containing soil, sand and manure in (2:1:1) ratio for hardening of plants. These plantlets were irrigated with ½ MS Medium without any growth regulators and sucrose and with sterile water. The plantlets were exposed to natural conditions daily for 2-3 hours for the hardening of plantlets for 10-20 days. Then after 30 days plants were transferred to bigger pots and kept into polyhouse for the acclimatization of plants where temperature and humidity were maintained.

Statistical analysis

Each treatment consisted of minimum 20 explants in triplicate. The percentage of callogenesis, embryogenesis and number of regenerated plantlets was counted. Mean and standard error were used for statistical analysis.

RESULTS AND DISCUSSION

The present study deals with induction, maturation and germination of embryos which has been successfully achieved as shown in (Fig-1). Murashige and Skoog medium devoid of plant growth regulators failed to induce the formation of callus and embryos. Supplementation of 2,4-D (1.0-2.0 mg/lt) in combination with TDZ (0.5 and 1 mg/lt), BAP (0.5 and 1mg/lt) was found efficient for callogenesis and embryogenesis. Callus induction with the symptoms of curling, stiffness and change in color of leaves was observed after 7-8 days of inoculation. Two different types of callus was observed type-i callus was non-embryogenic observed on MS medium supplemented with only 2,4-D (1.0-2.0 mg/lt) alone and type-ii callus was embryogenic observed on MS medium supplemented with 2,4-D (1.0-2.0 mg/lt) in combination with TDZ (0.5 and 1 mg/lt), BAP (0.5 and 1mg/lt) (Fig-1 a, b, c, d, e and f). The highest percentage of callus induction was 86.5% in 2,4-D and BAP (1.0+0.5mg/lt) under the influence of red light treatment and 83.3%, 81.6%, and 78% under the influence of white, blue and yellow light treatment respectively (Table 1, 2, 3 and 4). The primary sign of somatic embryogenesis was swelling of explants tissue. After 20 days of inoculation shiny globular green translucent embryos were observed. The highest percentage of embryogenesis 92% was observed in MS medium fortified with 2, 4-D (1mg/lt) + BAP (0.5mg/lt) under the influence of red light treatment, 90%, 88% and 87% under the influence of blue, white and yellow light treatment respectively (Table 5, 6, 7 and 8). The callus was friable and white green in 2, 4-D and BAP (1.0+0.5mg/lt) under the influence of white, blue, yellow and red light treatment, compact light green in 2,4-D and TDZ (2.0+0.5mg/lt), friable yellow green in 2,4-D and TDZ in (1.0+0.5mg/lt) and compact white brown green in 2,4-D and TDZ (1.0+1.0mg/lt). Somatic embryogenesis is mostly promoted by auxins either alone or in combination with cytokinin (Fei *et al*., 2002; Shahana & Gupta 2002; Anthony *et al*., 2004; Shu *et al*., 2005). Its look like that this combination is suitable environment for progression of totipotent somatic cells into somatic embryos. Embryos development reached on completion after 4 week of inoculation and maturation of embryos were observed when they were subcultured on MS medium containing 2, 4-D and BAP (1.0+0.5mg/lt) embryos development and maturation were noticed.



A- Globular shaped embryo; B- Early and late globular shaped embryo; C- Heart shaped embryo; D- Nodular shaped embryo; E- Germination from embryo; F- Germinated plantlets

Fig. 1. Differnet shapes of somatic embryo development in *celastrus panicualtus*

Plantlets were germinated from matured embryo after 65 days of growth period (Fig 1f). The hormone 2, 4-D has been reported to promote somatic embryogenesis and also play an important role in dedifferentiation and cell division in rice. Organogenesis and somatic embryogenesis both triggered by auxins and cytokinins. Further cytokinin, particularly BAP played a vital role in induction, maturation and germination of maximum number of somatic embryos in *Abies nordamanniana* (Norgaard *et al*., 1991). In this study 2, 4-D and BAP (1.0+0.5mg/lt) were found most effective for germination of plantlets. Germination is characterized by cotyledonary expansion and chlorophyll formation with vegetative leaf formation (Fig 1e). Germinated embryos developed into complete plantlets on MS medium fortified with different concentration of 2, 4-D, BAP and TDZ. Maximum average number of plantlets 38.0±3.4 was observed in MS medium fortified with 2, 4-D and BAP (1.0+0.5mg/lt) under the influence of red light treatment and 33.6±3.2, 31.3±4.1 and 27.6±0.5 under the influence of blue, yellow and white light treatment respectively (Table 5, 6, 7 and 8). The regenerated shoots with well developed root plantlets were taken out from culture bottles and then washed with distill water to remove the patches of agar and remaining agar patches were removed with the help of forcep and holder. Then after they were transferred into plastic pots containing soil, sand and manure in (2:1:1) ratio for hardening of plants and also they were irrigated with MS basal medium without any plant growth regulators for 10-15 days.

Table 1. Effect of white light and different concentration of 2,4-D, BAP and TDZ on callus induction

Light treatment	PGR Treatment		Callus Induction %	Morphology of callus
White light	2,4-D	BAP		
	1	0.5	83.3	Friable, white green
	2	0.5	73.6	Compact, light green
	1	1	80	Compact, white green
	2	1	78	Compact, white green
	2,4-D	TDZ		
	1	0.5	80.2	Friable, yellow green
	2	0.5	71	Compact, white green
	1	1	76	Compact, white green
	2	1	71.5	Compact, white green

Table 2. Effect of blue light and different concentration of 2,4-D, BAP and TDZ on callus induction

Light treatment	PGR Treatment		Callus Induction %	Morphology of callus
Blue light	2,4-D	BAP		
	1	0.5	81.6	Friable, green white
	2	0.5	76.2	Friable, green white
	1	1	79.1	Friable, green white
	2	1	74	Compact, green white
	2,4-D	TDZ		
	1	0.5	77.6	Friable, green yellow
	2	0.5	72.7	Compact, green white
	1	1	75.3	Compact, green white
	2	1	73	Compact, green white

Table 3. Effect of yellow light and different concentration of 2,4-D, BAP and TDZ on callus induction

Light treatment	PGR Treatment		Callus Induction %	Morphology of callus
Yellow light	2,4-D	BAP		
	1	0.5	78	Friable, white green
	2	0.5	71	Compact, white green
	1	1	77.1	Compact, white green
	2	1	69	Compact, white green
	2,4-D	TDZ		
	1	0.5	75.3	Friable, yellow green
	2	0.5	65.4	Compact, green white
	1	1	71.3	Compact, green white
	2	1	70.8	Compact, green white

Table 4. Effect of red light and different concentration of 2, 4-D, BAP and TDZ on callus induction

Light treatment	PGR Treatment		Callus Induction %	Morphology of callus
Red light	2,4-D	BAP		
	1	0.5	86.5	Friable, white green
	2	0.5	80	Compact, white green
	1	1	83.3	Compact, white green
	2	1	81.2	Compact, white green
	2,4-D	TDZ		
	1	0.5	79	Compact, brown green
	2	0.5	75	Compact, brown green
	1	1	80	Compact, white brown green
	2	1	73.3	Compact, brown green

Table 5. Effect of white light and different concentration of 2,4-D, BAP and TDZ on percentage of embryogenesis and No. of plantlets germinated

Light treatment	PGR Treatment		Percentage of embryogenesis	No. of Plantlets
	2,4-D	BAP		
White light	1	0.5	88	27.6±0.5
	2	0.5	45	15.3±2.0
	1	1	30	10.0±1.0
	2	1	20	7.0±1.0
	2,4-D	TDZ		
	1	0.5	56	21.6±1.5
	2	0.5	47	22.3±2.5
	1	1	23	8.6±0.5
	2	1	18	7.3±0.5

Table 6. Effect of blue light and different concentration of 2, 4-D, BAP and TDZ on percentage of embryogenesis and No. of plantlets germinated

Light treatment	PGR Treatment		Percentage of embryogenesis	No. of Plantlets
	2,4-D	BAP		
Blue light	1	0.5	90	33.6±3.2
	2	0.5	48	16.3±1.5
	1	1	32	12.0±2.6
	2	1	23	9.3±2.0
	2,4-D	TDZ		
	1	0.5	61	23.6±4.0
	2	0.5	50	24.0±3.6
	1	1	25	10.6±0.5
	2	1	20	9.0±1.0

Table 7. Effect of yellow light and different concentration of 2, 4-D, BAP and TDZ on percentage of embryogenesis and No. of plantlets germinated

Light treatment	PGR Treatment		Percentage of embryogenesis	No. of Plantlets
	2,4-D	BAP		
Yellow light	1	0.5	87	31.3±4.1
	2	0.5	46	16.0±3.0
	1	1	35	13.6±1.1
	2	1	22	8.0±1.0
	2,4-D	TDZ		
	1	0.5	55	22.0±3.6
	2	0.5	49	21.0±1.7
	1	1	24	10.0±1.0
	2	1	21	7.6±1.1

Table 8. Effect of red light and different concentration of 2, 4-D, BAP and TDZ on percentage of embryogenesis and No. of plantlets germinated

Light treatment	PGR Treatment		Percentage of embryogenesis	No. of Plantlets
	2,4-D	BAP		
Red light	1	0.5	92	38.0±3.4
	2	0.5	51	18.6±4.0
	1	1	38	15.0±2.0
	2	1	26	10.6±1.5
	2,4-D	TDZ		
	1	0.5	63	27.3±2.5
	2	0.5	52	24.6±4.7
	1	1	27	11.0±1.0
	2	1	24	10.3±2.0

Plants were transferred to bigger pots and kept into polyhouse and irrigated regularly with water for the acclimatization of plants where temperature and humidity were maintained for the hardening and acclimatization of plantlets.

Conclusion

In conclusion, the present study reports the successful protocol for somatic embryogenesis and plantlets regeneration. In this study callogenesis, somatic embryogenesis and plantlet regeneration was achieved by using leaf explants. This is the first time when we are studying the impact of different monochromatic light treatments on *in-vitro* somatic embryogenesis of *Celastrus paniculatus.* This study concludes that among all different monochromatic lights, blue and red light was found to be most suitable light for maximum plantlet regeneration of *Celastrus paniculatus*. The results obtained in this study showed that lower and higher wavelengths of the visible spectrum (Blue and Red light) influence percentage of somatic embryogenesis and plantlet regeneration. In addition, the results of this study suggest that the light quality emitted by red and blue lights were both beneficial for vegetative propagation of *Celastrus paniculatus*. The Blue and Red light receptors cryptochromes, phytochrome A and phytochrome B appears to regulate growth of *Celastrus paniculatus* cultures. Blue- light photoreceptors absorb wavelengths of blue light and trigger a number of reactions in plants. Blue wavelengths affect phototropism, the opening of stomata (which regulates a plant's retention of water), and chlorophyll production. Phytochrome absorbs mostly red light. Red wavelengths set off a variety of responses in plants as well. They initiate seed germination and root development. Hence a strong possibility of combinatorial effect of Blue and Red light treatment for high frequency regeneration can be explored. This protocol will help in regeneration and conservation and also bears the potential to accomplish the demand and supply ratio for pharmaceutical industry.

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Conflict of interest

The authors declare that they have no conflict of interest in the publication.

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