Biospectra : ISSN: 0973-7057, Vol. 17(1), March, 2022, pp. 89-92 18(PS).
An International Biannual Refereed Journal of Life Sciences
Plant Science

ISSN : 0973-7057

Int. Database Index: 616 www.mjl.clarivate.com

Influence of low light and dark treatment on callus survival of *Elaeocarpus ganitrus*

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Received : 15th November, 2021 ; Revised : 16th December, 2021

Abstract- An attempt has been made to analyze the effects of low light conditions on callus cultures of *E. ganitrus* on full strength MS medium for long time preservation. For the study dark and light conditions were taken on the basis of time differences out of which low light conditions for 16h and dark conditions for 8h were selected to recapitalize the callus of *Elaeocarpus ganitrus* when supplemented with plant growth regulators and different kinds of antioxidants in growth medium.

Key words: *Elaeocarpus ganitrus*, Callus color, Dark treatment, Low light condition

INTRODUCTION

For efficient callus and shoot initiation, light plays crucial role which make it as one of the most important factor in an outcome of any tissue culture study.[1] The etiolation and de-etiolation mechanisms are important for knowing about the accession of photosynthetic capability at the situation of biogenesis of chloroplasts and response of plant with respect to light.[2] The influence of dark and light conditions was analyzed on callus cultures of *Citrus reticulata.* Under both kinds of treatment explants culture shown variations in the response. Under the dark and light incubation treatment it was observed that genetic variation regarding callus induction exists.[3]

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In a study it was observed that regeneration of shoot is light independent and no etiolation character was observed in the regenerated shoots in dark, except they were lacking chlorophyll which was further developed when 16 h light exposure was given.[4] To analyze the effects under various spectra of light rose plants were grown.[5]

In the tissue culture seedlings of *Cunninghamia lanceolata* the composite LED light effects on the antioxidant capacity and root growth and development was reported.[6] The tissue culture study on indirect regeneration of *Lilium ledebourii* Bioss was reported, in which all the stages were investigated under the dark conditions for the first time.[7]

For micropropagation study using nodal segments as explants of *Elaeocarpus sphaericus*, the cultures were

kept under 8h dark and 16h light conditions (using 1200 lux, 40 watt cool white fluorescent tubes).[8] To maintain the shoot cultures of *E. sphaericus* for *in vitro* propagation 40-50µmol $m^{-2}s^{-1}$ of irradiance was used for 16h light condition.[9] The calli of *E. sphaericus*[10] and *E. tuberculatus*[11] were developed and well maintained using *in vitro* tissue culture protocol which had shown positive outcome.

MATERIALS & METHODS

The callus samples were developed from immature fruits of *Elaeocarpus ganitrus* on MS medium earlier[12] in Plant tissue culture laboratory of School of Biological Engineering and Life Sciences, Shobhit Institute of Engineering and Technology, Modipuram, Meerut (UP) India were procured for the investigation purpose. The callus samples were sub-cultured on full strength MS[13] medium in conical flasks of 250 ml (Borosil), containing 100 ml of medium. The procedure of growth media preparation was similar to that which was applied earlier for callus initiation from immature fruit of *E. ganitrus* explants material.[12]

Sample treatment

The dark treatment was provided to the cultures for 10 days (Fig.2A). After 10 days of treatment the cultures were maintained on the growth medium and placed under the single tube light of 40 watt, 6 inches away on the rack separating the open area using black colored paper of A4 Size (Fig.2B).

During the initial first week after the sub-culturing of the callus, the cultures were maintained at a range of light and dark conditions depending on time variations in hours. The callus cultures treated under complete dark conditions (24 h) were taken as control.

Statistical Analysis

The experiments were performed with ten technical and three biological replicates. The analysis of variance was performed (one way ANOVA) for significant difference (Pd" 0.05) in between the control and treated experiments. The asterisk mark used for denoting significant difference.

RESULTS & DISCUSSIONS

It was observed that callus growth was very slow when the dark conditions were provided to the cultures. When complete dark conditions for 10 days were provided, the color of the callus turned from light green to dark green and then to white and then light yellow and then to dark yellow to yellowish brown (Fig.2A). It was observed that new callus tissues were started appearing during the 1st week after first sub-culturing which was performed just after dark treatment and further in later stages of treatment i.e., from 2nd to 4th week with similar low light and dark conditions (16 h /8 h) started becoming light green (Fig.2B). From 4th week to 6th week of exposure callus was completely appearing light green to dark green in color.

Fig 1: Trees of *Elaeocarpus ganitrus* located at SIET campus, Meerut (UP) India

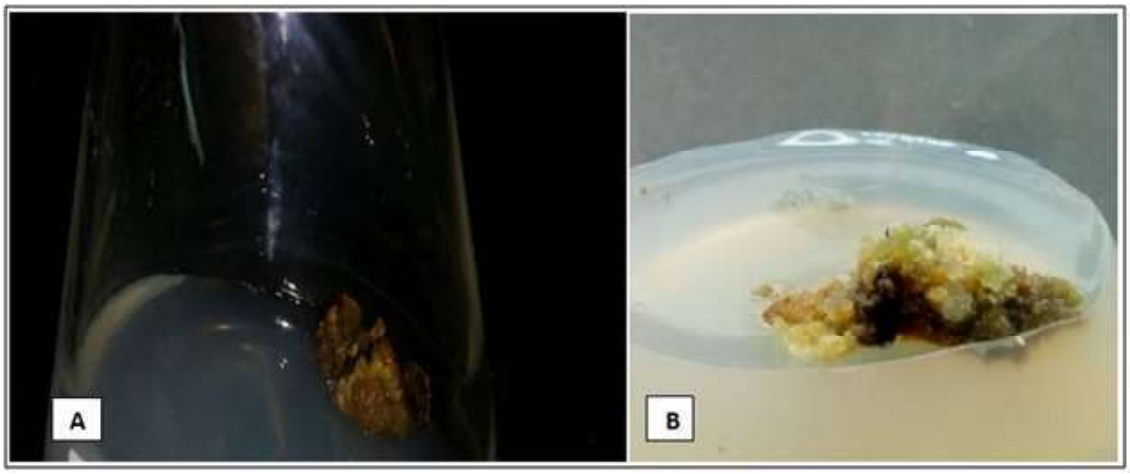

Fig 2: Callus culture under dark conditions of 10 days [A] and Callus culture re-multiplied during 21st day to 42nd day under treatment of 16h low light/ 8 h dark conditions [B].

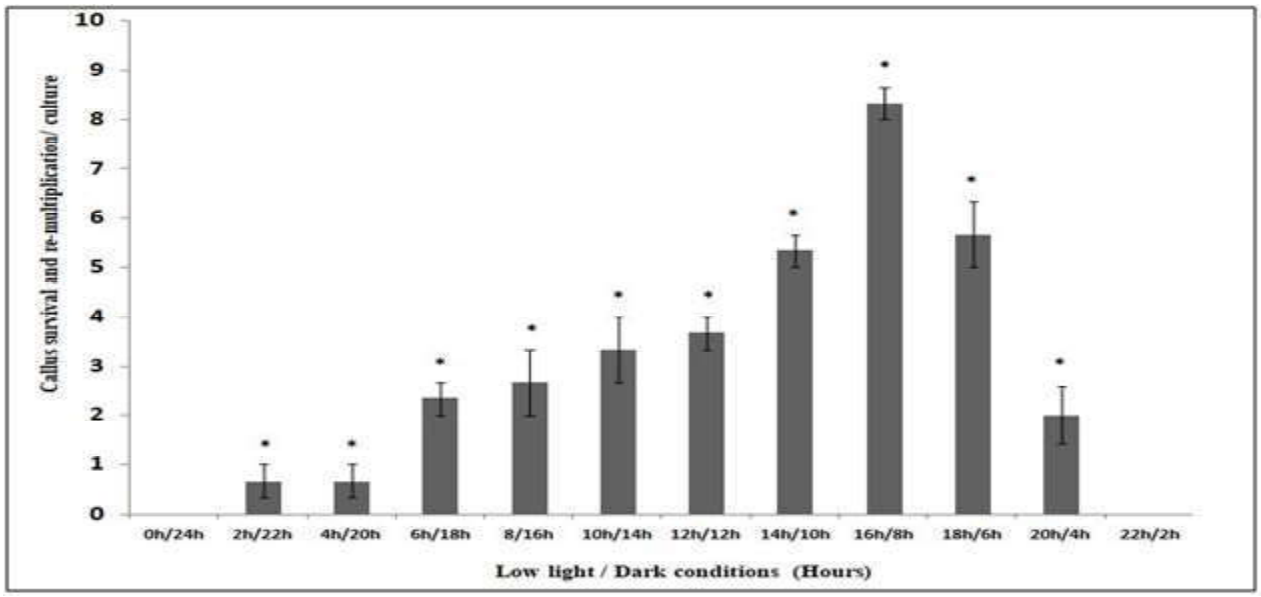

Fig 3: Callus cultures under 24 hours dark conditions were taken as control and under the treatment of light and dark conditions it was observed that low light for 16 h and 8 h dark environment provided better results.

Depending on the physical condition of callus the second sub-culturing may be performed in the 5th week otherwise it should be performed during the 6th week. The entire process may be repeated again keeping the same parameters to maintain the *in vitro* callus cultures. It was noted that low light was helpful for callus multiplication, and complete dark incubation for slow multiplication which was observed during the callus study of *E.ganitrus.*[14]

However, low light and dark environment based on a range of time variations in hours has shown the survival and re-multiplications of cultures (Fig.3). The use conical flasks for callus studies were previously reported.[14] The light and dark effects were studied for initiation and regeneration of callus for *Nicotiana tabaccum.*[15] During propagation of *Haworthia* through tissue culture for better organogenesis, best union of plant growth regulators and intensity of light was determined.[1] The effects of TDZ and dark conditions for callus formation from rose leaf explants were analyzed.[16]

CONCLUSION

The callus re-multiplications were observed higher under 16h low light and 8h dark environment. By using this protocol the number of sub-culturing for callus cultures of *Elaeocarpus ganitrus* may be reduced and

cultures can be preserved for long time period. Because the cultures can be remain available for a long time period several other investigations can be taken into consideration.

Many more investigations based on effects of light and dark conditions on *in vitro* cultures yet needed. The study may be helpful for investigating the effects of light and dark conditions on *in vitro* callus cultures survival and re capitalization for other endangered tree species.

ACKNOWLEDGEMENT

Authors are thankful to Department of Bio technology, School of Biological Engineering and Life Sciences [SIET, Meerut (UP) India].

COMPETING INTEREST

Declared none

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