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Effect of various plant growth regulators and light intensity (complete and diffused light) on the callus proliferation of the carraluma plant

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Abstract:

The experiment was carried out at the Biotechnology Laboratory, Agricultural research institute (ARI), Tarnab, Peshawar, Pakistan. The effect Of various plant growth regolators and light intensity (complete and diffused light) on the callus proliferation of the carraluma plant the effect of varying concentrations (0, 0.5 and 1 mg/l) of GA_3 hormones showed non-significance effect while the

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cytokinins i.e. BA, BAP, and kinetin in varying concentrations (1, 2 and 3 mg/l) showed significant effect for callus proliferations. The largest callus (3.10) was observed when BAP 3mg/l were applied in the media, while lowest callus (2.21) was given by BA when applied as 1 mg/l in the culture media. Although varying concentrations of GA_3 showed non-significant effect on callus proliferation but its interaction with cytokinins effected mainly the amount of callus an increase in the amount of callus. The best calluses (3.75) were produced when GA_3- 0.5 mg/l combines with BA- 3 mg/l, followed by the callus amount 3.12, produced the combination of GA_3-0.5 mg/l and BA- 3 mg/l. The least amount of callus proliferation (1.77) were given by the interaction of BAP 1mg/l and GA_3 0.5mg/ respectively. The fig.1 showed the interaction of two light intensities i.e. Normal light (NL) at intensity 2000- 2500 Lux and Diffused light (DL) at intensity 500- 1000 Lux with varying concentrations (1, 2 and 3 mg/l) of BA, BAP, and kinetin for the callus proliferations .It is clear from the fig.1, that the light intensity at the range of 2000-2500 encourages the callus proliferation.

Key words: Plant growth regulators, light intensity, Hormones, Carraluma plant

INTRODUCTION

Caralluma (Caralluma *tuberculata* L.) is a genus of flowering plants in a dogbane family (Apocynaceae) consisting of about 120 species**.** The generic name is derived from the Arabic word qahr al-luhum**,** meaning "wound in the flesh" or "abscess," refraining **to** the floral odor**.** Most of the species occur in Africa**,** including several people used it for their medicinal properties. Species are found in Middle East, Africa, and other countries (Ansari *et al.,* 2005).

Caralluma tuberculata grows abundantly on the mountains in the FATA and its surrounding districts in Pakistan. It is also used to reduce sugar levels in diabetes patients. Peoples eat their raw fleshy leaves either as salad or

cook with meat which removes its bitter taste (Sattar *et al.,* 2007).*Caralluma* is a succulent plant (cactus) from Pakistan. In country it grows wild and is often used as a border in gardens and as a roadside shrub. It is also found in the wild in Africa, Saudi Arabia, Afghanistan, and Southern Europe. *Caralluma* is cooked as a vegetable and is used in preserves such as chutneys and pickles. *Caralluma tuberculata* is a very important medicinal plant with a range of anti-diabetic and weight reduction properties. This high-valued medicinal plant is now a days considered as endangered due to its unsustainable elimination from wild (Zamir *et al.,* 2016). And Plants of *Caralluma tuberculata* have used for paralysis and joints pain and fever (Khan and Khatoon *et al.,* 2008).The indiscriminate and destructive harvesting of many of these plants continues unabated despite increased governmental regulation, resulting in many species (especially those with slow growth) becoming endangered. Natural populations of these plants are declining because of increase in demand in pharmaceutical market. There is no agro technology available for controlled cultivation. We have developed protocol for their micro propagation and conservation. (Raj *et al*., 2005)Through Cutting Propagation can done round a year. Cutting with root and without roots germinate 100% Plant 30 gm cutting in a pot of (10x3 inch). Method of Sowing If through seed, seed depth not more than one inch. Cutting to be planted ½ to 1 inch deep. After planting light watering, Irrigation, in winter after 15 days interval in summer after 7 days interval. Shoots with 5 cm in height were separated and individual shoots were transferred for rooting to half-strength MS medium containing different concentrations of NAA (α-naphthalene acetic acid), IAA (indole-acetic acid) and IBA (Indole-3-butyric acid). The cultures were incubated under 16h photoperiod for 30 days until the micro shoots developed the roots (Qiu *et al.,* 1997).

In vetro propagation of *Caralluma tuberculata* (Chungah) was developed from shoot tip and meristem explants. *C. tuberculata* is an imperative medicinal plant comprising antidiabetic and anticancer properties. The explants were inoculated on Murashige and Skoog (MS) medium containing different plant growth regulators. Presence of BA or Kin alone in the MS medium did not favor regeneration of shoot from both explants. However, addition of 2,4-dichlorophenoxy acetic acid (2,4- D), gibberellic acid (GA_3) and thidiazuron (TDZ) along with 6-benzyl amino purine (BA) or kinetin (Kin) in the medium exhibited significant percentage response, number of shoots per explants and shoot length. Maximum shooting response (53.3±5.77% from meristem and shoot tip explants each) with highest number of shoots per explants (5.33±2.08 and 5.6±2.52 from meristem and shoot tip explants, respectively) were observed at 13.32 µmol BA along with 2.26 µmol 2,4-D, 2.89 µmol GA_3 and 9.08x10-3 µmol TDZ. Replacing BA with kin showed less shoot regeneration response and number of shoots per explant, however, shoots length markedly increased in the presence of Kin. The regenerated plants were successfully rooted and acclimatized in ex vitro condition. The protocol described here can be used for fast multiplication of this endangered herb and genetic transformation (Rehman *et al.,* 2014).The basic nutrient medium used was Murashige and Skoog (MS) medium (7) with 3% sucrose. The medium was supplemented with various concentrations of BA (0.44 – 35.5µM), KN (0.46-37.17µM), 2iP (0.49 – 39.4µM) and Zeatin (0.46 – 36.49µM). Numerous complex nutritive mixtures of undefined composition like CH (10 – 50 mg l-1), coconut milk (CM) (5 – 20%), and YE (10 – 50 mg l-1) and Antioxidants such as CA (50 – 200 mg l-1), PVP (50 – 200 mg l-1) and AC (10 – 50 mg l-1) were added individually or in combination to culture medium to improve shoot multiplication (Murashige T & Skoog F, *Physiol Plant,* 15.*,et al* 1962.

MATERIALS AND METHODS

The experiment was carried out at the Biotechnology Laboratory, Agricultural research institute (ARI), Tarnab, Peshawar, Pakistan.

Experimental design and statistical analysis

The experiment was carried out in factorial experiment in completely randomized design (CRD). Each treatment consists of three test tubes, repeated three times and number of days to callus initiation, the results are expressed as mean SD of three experiments. The data were subjected to analysis of variance-ANOVA (Gomez and Gomez, 1984) and analyzed statistically using SPSS ver. 16.0 software. The significance of differences among mean values was calculated using Duncan's Multiple Range Test (DMRT) at $p< 0.05$ (Duncan, 1995).

The amount of callus was determined by a 0-4 scale scoring system. (Gurel *et al*., 2001).

Table 1. A '0-4 scale' scoring system developed for measuring the amount of callus

Score	Description
0	No visible callus
1	Small proliferation at cut ends only
2	5 mm callus
3	5-10 mm callus
4	10-15 mm callus
5	More than 15 mm callus

Callus induction media:

The MS (Murashige and Skoog, 1962) medium was used as the basal culture medium. The media was supplemented with 3% (w/v) sucrose, solidified with 8 g L^{-1}agar (Mearck) along with two plant growth regulators namely 6-Benzyladenine (Fluka BioChemika) 3.0mg $^{-1}$either alone or in combinations with 6-Benzyladenine. The pH was adjusted at 5.8 using HANNA,

H122u pH/ORP pH meter with 1N NaOH or 1N HCL before autoclaving.

Culture conditions:

For callus induction, the callus was inoculated in 30 ml test tube containing 10 ml standard MS media with 3% (w/v) sucrose, 0.7 % (w/v) agar and various combinations of plant growth regulators. To maintain aseptic condition, precautions were taken in every step of works. All inoculations and aseptic manipulations were carried out in a laminar airflow cabinet. During operation, hands and cabinet base were rubbed with 70% ethyl alcohol frequently for maintaining clean condition. To obtain possible contamination free condition in clean bench, proper care was taken during explant inoculation. The ex-plant was cut into about 5 mm pieces inside the laminar airflow cabinet using a fine sterile forceps and scalpel. The excised pieces were then inoculated on to the culture test tubes containing plant growth regulators in various combinations.

The physical conditions for growth and development of cultures were maintained at the temperature of 25 ± 1^0C. Light intensity of 2000-2500 lux was maintained for normal light intensity and 500-1000lux for diffused light intensity at 16 light photoperiod. For dark treatment, inoculates were kept in complete dark. The diffused light and dark treatments were transferred to normal light intensity after 10 days of initial inoculation. The light was provided by tube light 40 W tubes (Philips) and the light intensity was measured using lux meter (LX-101 Lutron). The relative humidity was maintained at 60-70% .In addition to the effect of various light intensities in the presence of different plant growth regulators on callusing in *Caralluma*, two types of culture tubes sealing i.e. normal plastic cap and cotton plug were also employed.

RESULT AND DISCUSSION:

The experiment was carried out at the Biotechnology Laboratory, Agricultural research institute (ARI), Tarnab, Peshawar, Pakistan, during July-September 2017.

Table-1 Mean results for callus proliferation of *Carallumatuberculata*by varying concentrations of BA, BAP, Kinetin and GA3 after 10 days and 49 days of inoculation

	Cytokinine									
GA3 (mg/l)	BA (mg/l)			BAP (mg/l)			Kinetin (mg/l)			Mean
	1	2	3	1	2	3	1	2	3	
0	2.50b	3.30bc	2.31ab	1.77a	2.56b	2.94bc	2.50b	2.37ab	3.50c	2.60A
0.5	3.12c	2.69bc	3.75d	2.37ab	2.18ab	1.94a	2.75bc	2.12ab	2.44ab	2.59A
1.0	2.50a	2.50a	3.25b	2.50a	2.06a	2.37a	2.31a	3.31b	2.06a	2.54A
Mean	2.21A	2.25AB	2.41ABC	2.70CD	2.73CD	3.10D	2.52ABC	2.60ABC	2.67BC	

Table-1. Showed that the effect of varying concentrations (0, 0.5 and 1 mg/l) of GA_3 hormones showed non-significance effect while the cytokinins i.e. BA, BAP, and kinetin in varying concentrations (1, 2 and 3 mg/l) showed significant effect for callus proliferations. The largest callus (3.10) was observed when BAP 3mg/l were applied in the media, while lowest callus (2.21) was given by BA when applied as 1 mg/l in the culture media. Although varying concentrations of GA_3 showed non-significant effect on callus proliferation but its interaction with cytokinins effected mainly the amount of callus an increase in the amount of callus. The best calluses (3.75) were produced when GA_3- 0.5 mg/l combines with BA- 3 mg/l, followed by the callus amount 3.12, produced the combination of GA_3-0.5 mg/l and BA- 3 mg/l. The least amount of callus proliferation (1.77) were given by the interaction of BAP 1mg/l and GA_3 0.5mg/respectively.

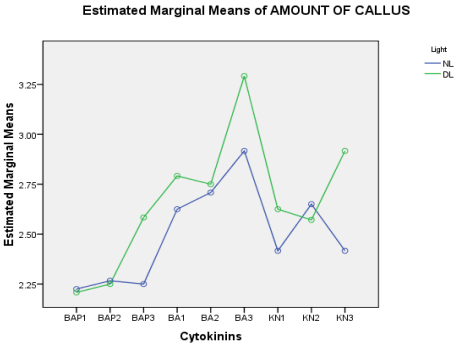

Fig-1. Interaction of two light intensities i.e. Normal light (NL) at intensity 2000- 2500 Lux and Diffused light (DL) at intensity 500- 1000 Lux with varying concentrations (1, 2 and 3 mg/l) of BA, BAP, and kinetin for the callus proliferations after 49 days of inoculation

The fig.1 showed the interaction of two light intensities i.e. Normal light (NL) at intensity 2000- 2500 Lux and Diffused light (DL) at intensity 500- 1000 Lux with varying concentrations (1, 2 and 3 mg/l) of BA, BAP, and kinetin for the callus proliferations .It is clear from the fig.1, that the light intensity at the range of 2000-2500 encourages the callus proliferation.

Recommendation

The largest callus (3.10) was observed when BAP 3mg/l were applied in the media, It's recommend that the concentration of cytokinin i.e. BA, BAP and kinetin in the concentration of (1, 2 and 3mg/l) show the significant result on the proliferation of callus.

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