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Influence of Growth Hormone and Additives on *In vitro* Shoot Initiation from Apical and Axillary Meristems of *Commiphora wightii*

Manisha Bhardwaj* and Aparna Alia

Department of Botany, Rajeev Gandhi College, Trilanga, Bhopal-462039, (M.P), India.

ABSTRACT

Commiphora wightii is an important plant species of immense medicinal value and pharmaceutically used for the treatment of various diseases. The present study was designed to develop an efficient protocol for micropropagation of *Commiphora wightii* from apical and nodal meristems and to study the influence of additives on the enhancement of bud breaking, number of shoot per explant and shoot length. MS (Murashige and Skoog, 1962) basal media supplemented with various concentrations of 6- Benzyl amino purine (BAP: 0.5-5.0 mg/l) alone or in combinations with kinetin (KN: 0.5-1.0 mg/l) and naphthalene acetic acid (NAA: 0.5-1.0 mg/l) and additives like ascorbic acid (AA), citric acid (CA), adenine sulphate (AS) and activated charcoal (AC). The highest shoot initiation was reported in T13 (MS + 4.0BAP + 1.0 NAA mg/l + Additives) i.e. 0.80 ± 0.07 with highest number (1.57 ± 0.15) of shoots per explants. Treatment T12 (MS + 3.0BAP + 1.0 NAA+1.0 KN mg/l + Additives) highest shoot length (1.13) was recorded followed by T13 (MS + 4.0BAP + 1.0 NAA mg/l Additives) and T14 (MS + 5.0BAP + 1.0 KN) having mean value 1.07 and 0.90 respectively. MS basal medium (control) devoid of growth regulators did not support the shoot induction. The shoot induction protocol developed in current study provides a basis for germplasm conservation and for further investigation of medicinally active constituents of the elite medicinal plant

Keywords: *Commiphora wightii,* 6- Benzyl amino purine, kinetin, naphthalene acetic acid, additives.

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***Address for Correspondence:**

Manisha Bhardwaj, Department of Botany, Rajeev Gandhi College, Trilanga, Bhopal-462039, (M.P), India.

INTRODUCTION

Commiphora wightii, commonly known as Guggal, is an imperative plant species of immense medicinal value belonging to family Burseraceae. It is slow growing, highly branched and threatened indigenous plant species of arid and semi-arid regions of India and Pakistan and extended to Saudi Arabia. In India it is found in Rajasthan, Gujarat and Maharashtra[1]. It is used in the Allopathic, *Ayurvedic* and *Unani* systems of medicines due to its anti-inflammatory, anti-rheumatic, hypocholesteremic and anti-fertility activities[2]. It yields guggul, an important oleo-gum resin which is used as incense, fixative in perfumery and in medicine. Modern therapeutic uses of guggul include nervous diseases, hemiplegia, leprosy, marasmus, muscle spasms, neuralgia, ophthalmia, pyelitis, pyorrhea, scrofula, skin diseases, spongy gums, ulcerative pharyngitis, hypertension, ischemia, hypertension, hemorrhoids, and urinary tract disorder[3]. *Commiphora wightii* has become endangered and depleted from its natural habitat because of its slow growing nature, poor seed setting, and lack of cultivation, poor seed germination rate and excessive and unscientific tapping for its gum resin by the pharmaceutical industries and religious purposes. There by, widening the gap between demand and supply, further putting pressure on this species. Owing to these factors, the species is at verge of extinction and will extinct soon if proper steps are not taken for its conservation. It is essential for the conservation of *C. wightii,* to encourage the ex situ multiplication of this species using in vitro techniques. It seems to be a viable approach for its germplasm conservation and biomass utilization. As the domestication of the plant using conventional techniques has not yet been successfully employed, so the present studies aim to develop a protocol for the rapid propagation of this commercially important medicinal plant. Therefore, the investigation has to carry out to ascertain the most appropriate basal culture medium and growth hormones for in vitro shoot initiation of *C. wightii* by means of apical and axillary meristem.

MATERIALS AND METHOD

Plant material

Healthy plants of *C. wightii* were collected from Guggal Herbal Farm (G.H.F.), Mangliawas (Ajmer) Rajasthan, India and were propagated in the botanical garden of Rajeev Gandhi College, Bhopal, Madhya Pradesh, India. Axillary and apical meristem were taken from 2 year old clonally propagated plants and used as explants.

Explants and surface sterlization

Explants were cut and reduced to length of 2 cm and retaining the apical dome (1 cm). Firstly the explants were washed in running tap water for 20-30 minutes. Then to avoid contamination, they were soaked in a solution of fungicide (Bavistin) for an hour followed by three to four times rinsing with plain water and then with liquid soap solution followed by washing with tape water. Further surface –sterilization treatment was conducted in a laminar – flow chamber where explants were pre sterilized with single dip in 70% ethanol for half minute and washed with sterile distilled water. Explants were dipped into 0.1% freshly prepared mercuric chloride solution for 5 minutes, and then washed with 4-5 times in sterile double distilled water.

Effect of growth hormones on shoot induction

Surface-sterilized explants were inoculated aseptically on MS modified[4] basal media supplemented with various concentrations of 6- Benzyl amino purine (BAP: 0.5-5.0 mg/l) alone or in combinations with kinetin (KN: 0.5-1.0 mg/ l) and naphthalene acetic acid (NAA: 0.5-1.0 mg/l) and additives ascorbic acid (AA), citric acid (CA), adenine sulphate (AS) and activated charcoal (AC). All media contained 0.8% agar and 30g sucrose. The pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1 N HCl before autoclaving at 121°C for 15 min. the cultures were at (25±2) °C and 70% - 80% relative humidity under a 16-h photoperiod. In this study, 14 different hormonal treatments were assessed for shoot induction from meristems of *C. wightii.* The experiment was replicated three times. Each treatment per replication consisted of 30 explants. Medium lacking growth regulators served as control. The data on percentage of bud showing response, number of shoots initiated per explant and shoot length was recorded after four weeks. The experiment was factorial arranged in Completely Randomized Design (CRD). Data were subjected to analysis of variance (ANOVA) using the SPSS software. Treatment means were compared using the Duncan's Multiple Range Test (DMRT) at P > 0.05 where the F- value was significant.

RESULTS

In this study, growth regulators in different types and different concentrations were used to determine optimal culture conditions to shoot initiation in *C. wightii in vitro*. Explants were taken from 2 year old clonally propagated (through cuttings) potted plants, surface sterilized and aseptically cultured on solidified medium containing cytokinins i.e. BAP@ 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l; KN @ 0.5 and 1.0 mg/l and auxins i.e. NAA @ 0.5,1.0 and 2.0 mg/l respectively, supplemented with additives. All the explants remained fresh and green indicating the positive signs of establishment even after 10 days. The morphogenetic response of explants to various cytokinins(BAP) alone and in combination (BAP and KN) with auxin (NAA) and additives are summarized in Table1

Shoot induction response

Results revealed that MS basal medium (control) devoid of growth regulators did not support the shoot induction. MS supplemented with 3.0 BAP mg/l (0.30 ± 0.09) was much effective for bud breaking response as comparison to cytokinins alone or in combinations. No significant difference was reported in MS supplemented with BAP, KN and NAA in combinations i.e. all the treatment had almost same effect even with the change in BAP concentrations. The interaction of among all the combination of MS, BAP, NAA, KN and additives with regard to shoot induction response was significant (Fig 1A). The highest shoot initiation was reported in T13 (MS + 4.0BAP + 1.0 NAA mg/l + Additives) i.e. 0.80 ± 0.07 followed by (0.70 ± 0.09) in T12 (MS + 3.0 BAP + 1.0 NAA + 1.0 KN mg/l + Additives) and (0.63±0.09) in T14 (MS + 5.0 BAP + 1.0 KN mg/l + Additives).

Number of shoots per explant.

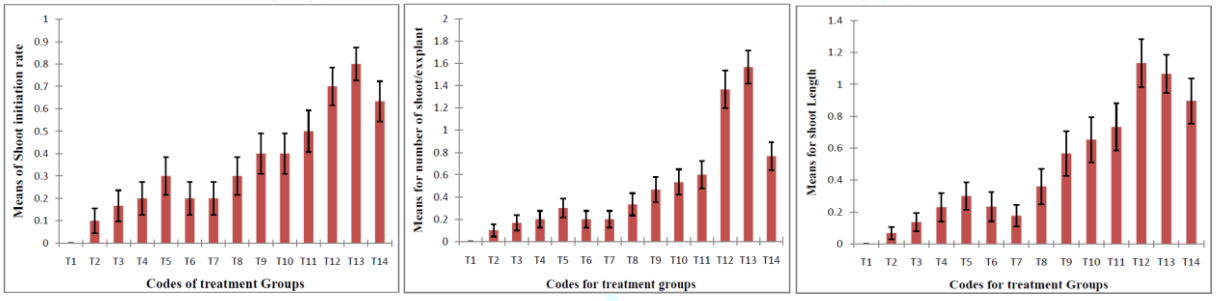
It is evident from the figure 1B that MS medium fortified with cytokinins, auxin and additives had better effect and their supremacy was absolute for all the other treatments. However in all applied concentrations of BAP, KN and NAA no significant difference was observed. Among all the treatments, T13 (MS + 4.0BAP + 1.0 NAA mg/l + Additive) was reported highest number (1.57 ± 0.15) of shoots per explant which subsequently fall down in case of treatment T14 i.e. MS + 5.0BAP + 1.0 KN mg/l + Additives having 0.77±0.12 shoots. The minimum number of shoots was obtained in medium supplemented with 0.5 BAP mg/l (0.10± 0.06).

Shoot length

For shoot length, the response varied with the type of growth hormone used. Out of different combinations and concentrations of growth hormone tried, it was explicit that in treatment T2 (MS + 0.5 BAP mg/l) shoot length was reported 0.10. Moreover in case of treatment T12 (MS + 3.0BAP + 1.0 NAA+1.0 KN mg/l + Additives) highest shoot length (1.13 ± 0.15) was recorded which subsequently fall down in case of treatments T13 (MS + 4.0BAP + 1.0 NAA mg/l Additives) and T14 (MS + 5.0BAP + 1.0 KN) having mean value 1.07± 0.12 and 0.90 ± 0.14 respectively. Among all the combinations of MS and BAP alone as well as the combinations of MS, BAP and KN almost same effect was reported on shoot length. However treatment T5 i.e. MS + 4.0 BAP mg/l was much effective as compare to other concentrations. While treatment T12 (MS + 3.0BAP + 1.0 NAA + 1.0 KN mg/l + Additives) was reported most effective as compare to all above stated other treatments for shoot length (Fig. 1 C).

Table 1 Effect of MS medium and growth regulators and additives on shoot induction from axillary and apical meristem of *Commiphora wightii*

Treatments	Medium	Mean Shoot Induction Response	Mean Number of Shoots/Explants ±SE	Mean Length of Shoots In (Cms) ±SE
T1	Control	0.00 ±0.00	0.00	0.00
T2	MS + 0.5 BAP mg/l	0.10 ±0.06	0.10 ± 0.06	0.07 ± 0.04
T3	MS + 1.0 BAP mg/l	0.17 ±0.07	0.17 ± 0.07	0.14 ± 0.06
T4	MS + 2.0 BAP mg/l	0.20 ±0.07	0.20 ± 0.07	0.23 ± 0.09
T5	MS + 3.0 BAP mg/l	0.30 ±0.09	0.30 ± 0.09	0.30 ± 0.09
T6	MS + 3.0 BAP + 0.5KN mg/l	0.20 ±0.07	0.20 ± 0.07	0.23 ± 0.09
T7	MS + 4.0BAP + 0.5 KN mg/l	0.20 ±0.07	0.20 ± 0.07	0.18 ± 0.07
T8	MS + 5.0 BAP + 1.0 NAA mg/l	0.30 ±0.09	0.33 ± 0.10	0.36 ± 0.11
T9	MS + 5.0 BAP + 0.5 NAA mg/l	0.40 ±0.09	0.47 ± 0.12	0.57 ± 0.14
T10	MS + 4.0 BAP + 0.5 NAA mg/l	0.47 ±0.09	0.53 ± 0.12	0.65 ± 0.14
T11	MS + 3.0 BAP + 0.5 NAA mg/l	0.50 ±0.09	0.60 ± 0.12	0.73 ± 0.15
T12	MS + 3.0BAP + 1.0 NAA + 1.0 KN mg/l + Additives (AA+ CA +AS + AC)	0.70 ±0.09	1.37 ± 0.17	1.13 ± 0.15
T13	MS + 4.0BAP + 1.0 NAA mg/l Additives (AA+ CA +AS + AC)	0.80 ±0.07	1.57 ± 0.15	1.07± 0.12
T14	MS + 5.0BAP + 2.0 NAA mg/l + Additives (AA+ CA +AS + AC)	0.63 ±0.09	0.77 ± 0.12	0.90 ± 0.14



A B C

Fig.1: Comparative study of different *in vitro* shoot initiation treatments of *Commiphora wightii* (A): Effect of different concentrations of growth regulators on shoot initiation response; (B): Effect of different concentrations of growth regulators on number of shoots per explants; (C): Effect of different concentrations of growth regulators on shoot length.

Fig.2. Shoot initiation from apical and axillary meristem of *Commiphora wightii* in MS media supplemented with 4.0BAP mg/l , 1.0 NAA mg/l and Additives.

DISCUSSION

Tissue culture technology offers an alternative method for the conservation of germplasm as well as micropropagation of medicinally important plant resources[5,6] .The present study is an effort for *in vitro* shoot initiation of highly valuable endangered medicinal plant using different plant growth regulators. For shoot induction of *Commiphora wightii* both apical shoot buds and meristems were used. Propagation from existing meristems & apical shoot buds yields plants that are genetically identical with donor plants[7]. The medium devoid of growth regulators failed to induce *in vitro* shoot induction responses. Similar results were obtained in *Crataeva nurvala*[8], *Nyctanthus arbor-tristis*[9] and *Celastrus paniculatus*[10]. Various workers reported the role of cytokinins in shoot bud formation[11-13]. This study showed that cytokinins were essential for shoot bud Induction and multiplication. BAP alone at three concentrations still remained well than that of its combination with KN and the combination consisting of BAP and NAA showed good improvement over combination of cytokinins alone for shoot bud induction response. However in the present study different concentration of BAP and NAA supplement with additives revealed that addition of the additives in the medium resulted in significant enhancement in the percentage of shoot induction and shoot length. The results of the present study supported by the findings of [14-16]. However, in the present study, effect of cytokinins on shoot length remained more or less similar to that observed individually and in combinations. Hence, during the initial stages of culture establishment stunted shoot growth was observed. Over a period of time the shoot length attained an average length of 2 to 2.5 cm in four weeks' time. This can be ascribed to the fact that explants get acclimatized to *in vitro* conditions. We can also attribute the lack of elongation in *in vitro* cultures partially to the fact that *C. wightii* is a slow growing plant. Further, In vitro induced shoot of will be tried for best shoot multiplication and in vitro rooting. The present study is important in terms of providing an efficient and reproducible protocol for enhanced bud initiation and elongation of shoots from apical and nodal meristems of *C. wightii.* Micropropagation is a possible alternative for *C. wightii* which is difficult to regenerate by conventional methods and its population have decreased due to over exploitation by destructive harvesting and. This method can effectively be used to meet the growing demand for clonally elite plants. Thus the present protocol for shoot initiation of *C. wightii* offers a potential system for improvement, conservation and mass propagation of this medicinal plant.

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