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# *In vitro* Callus Induction in Guggul [*Commiphora wightii* (Arnott)]- An Endangered Medicinal Plant

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**ABSTRACT**

*Leaf explant and nodal segment explants of guggul were placed on Murashige and Skoog Medium, supplemented with different concentration of cytokinins and auxins alone or in combination for callus induction. In leaf explants maximum callus induction was observed on a medium containing 2.0 mg/l 2, 4-D with 100 per cent frequency followed by 5.0 mg/l Kn+0.5 mg/l NAA with 100 per cent frequency. Whereas, maximum callus induction from nodal segment explant was observed on a medium containing 5.0 mg/l BAP+5.0 mg/l NAA with 100 per cent frequency.*

***Key words:*** *Guggul, Callus induction, Tissue culture, In vitro.*

## INTRODUCTION

*Commiphora wightii* (Arnott) is a medicinally important plant which is now considered as critically endangered species of the family Burseraceae and having the chromosome number 2n = 26[1]. The name *Commiphora* originated from the Greek words kommi (meaning 'gum') and phero (meaning 'to bear'). In Indian languages, it is known by various names like guggul in Hindi, gukkulu and maishakshi in Tamil, guggulu in Sanskrit and Indian bdellium in English. The genus *Commiphora* is widely distributed in tropical regions of Africa, Madagascar, Asia, Australia and the Pacific islands[2]. In India, it is found in arid, rocky tracts of Rajasthan and Gujarat, Maharashtra and Karnataka[3]. In Rajasthan it is found in the districts namely Jaisalmer, Barmer, Jodhpur, Jalore, Sirohi, Ajmer, Sikar, Churu, Jhunjhunu, Pali, Udaipur, Alwar (Sariska Tiger Reserve), Jaipur (Ramgarh, Jhalana area), Bhilwara and Rajsamand. *Commiphora wightii* is a small tree or shrub. It is a slow growing plant and takes 8 to 10 years to reach to a height of 3 to 3.5 meters. The plant is dimorphic, one having bisexual and male flowers and the other having female flowers with staminodes. A third category of plant with only male flowers has also been reported[4].

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The fruits are green berry like drupe. Size of the fruit varies from 6 to 8 mm and 5 mm in diameter. Fruit parts exposed to sun develop pinkish tinge. Seeds show polyembryonic nature[5].

Guggul is considered endangered in India and is listed as 'Data Deficient' in the IUCN Red Data list[6]. because of a lack of knowledge regarding its conservation status as well as excessive and unscientific tapping methods to increase yield of oleo-gum resin causes mortality of plants leading to the extinction danger of the species. Over the past 84 years (three generation lengths) there has been a decline of more than 80 per cent in the wild population as a result of habitat loss and degradation, coupled with unregulated harvesting and tapping of oleo-gum resin. The majority of the species yield a fragrant oleo-gum-resin following damage to the bark[7]. This species is therefore assessed as critically endangered[8]. Over-exploitation, a narrow extent of occurrence, small area of occupancy, severe fragmentation of populations, very low regeneration and invasion of alien species mean that *Commiphora wightii i*s facing a high extinction risk[9,10].

## MATERIAL AND METHODS

The present research work was conducted on *Commiphora wightii* (Arnott). Leaves were used as explant and obtained from healthy trees grown at Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner. Leaf explant was sterilized by using different surface sterilization agents. Explant was 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, explant was again 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 for 1-2 minutes.

### INDUCTION OF CALLUS

Leaf and nodal segments exolants were placed on MS medium supplemented with different concentration of cytokinins (BAP/Kn 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 4.5 and 5.0 mg/l and auxins (NAA/ 2, 4-D 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 4.5 and 5.0 mg/l) alone and BAP (0.5 and 5.0 mg/l) + NAA/2, 4-D (0.5 and 5.0 mg/l) and Kn (0.5 and 5.0 mg/l) + NAA/2, 4-D (0.5 and 5.0 mg/l) in combination for callus induction.

## RESULTS

### CALLUS INDUCTION

When leaf explant was inoculated on medium supplemented with different concentration of plant growth regulators, it responded differently. Maximum semi-compact, light green callus proliferation (0.90 g) was observed at 2.0 mg/l 2, 4-D with 100 per cent frequency (Fig. 1 and Table 1 ) followed by (0.87g) friable, light green callus unduction at 5.0 mg/l Kn +0.5 mg/l NAA with 100 per cent frequency (Fig. 2 and Table 2).

Nodal segment explant was inoculated on Ms media supplemented with different concentration of plant growth regulators. Maximum compact, light brown callus (0.80 g) proliferation was observed when medium supplemented with higher level of plant growth regulators (BAP, 5.0 mg/l + NAA, 5.0 mg/l) with 100 per cent frequency (Fig. 3 and Table 3). Profuse callus with shoot bud induction was observed at 1.0 mg/l Kn levels with 100 per cent frequency (Fig. 4 and Table 4).

## DISCUSSION

In the present investigation auxins evoked significant different response in different explants of guggul as cytokinins. Auxins (NAA/2, 4-D) induced callus and shoot bud in nodal segment whereas, only callus proliferation was observed in leaf explants. Maximum profuse callusing proliferation was observed at 2.0 mg/l 2, 4-D in leaf explant. These results are in close agreement with observation of Harikrishan[11] in *Plumbago rosea*, Zeng *et al.*[12] in *Ixora coccinea,* Singh *et al.*[13] and Singh *et al.*[14] in *Commiphora wightii*,. Presence of 2, 4-D has been shown to be essential for callus formation in *Vernonia cinerea* Baig and Shahzad[15] and *Momordica*

*charantia* Agrawal and Kamal[16], while NAA played an important role in callus formation in *Actinida deliciosa* Kumar *et al*.[17] and *Withania somnifera Ka*nnan *et al*[18].

Callus initiated from the cut end of the leaf explants and finally whole surface of the explants was observed. Similar observations have been made by Guo *et al.*[19], in *Saussurea involucrate* and also by Singh and Lal[20]. in *Leucaena leucocephala.* This may be obviously due to the production of endogenous auxin from the damaged cells of cut surface which triggered the cell division as found in *Ornithogallum* Hussey[21] where active cell division was observed at cut ends of tissue.

Profuse callus was observed on 5.0 mg/l Kn + 0.5 mg/l NAA with 100 per cent frequency. Similar results were reported by Singh *et al*.[13], in *Commiphora wightii* that best callus proliferation and growth reported at 2.0 mg/l Kn + 1.0 mg/l NAA. Fougat *et al.*[22], observed callus induction from cotyledon and leaf explants on MS media supplemented with 4.0 mg/l NAA + 2.0 mg/l Kn in pomegranate cv. Ganesh. Jarzina *et al*.[23], reported callus induction in leaf explants in five different varieties of hemp on MS medium supplemented with 1.0 mg/l Kn + 0.5 mg/l NAA.

This observation was contrary with the findings of Thirupathy *et al.*[24] for callus induction in *Tefrosia hookeriana* from leaf, node and internode explants in MS medium supplemented with 0.25 mg/l BAP+ 2.0 mg/l 2,4-D this is might be due to difference in genera and kind of explants used in the particular study.

**Fig. 1: Callus induction in leaf explant on MS medium supplemented with 2.0 mg/l 2, 4-D.**

**Fig. 2: Callus induction in leaf explant on MS medium supplemented with 5.0 mg/l Kn and 0.5 mg/l NAA.**

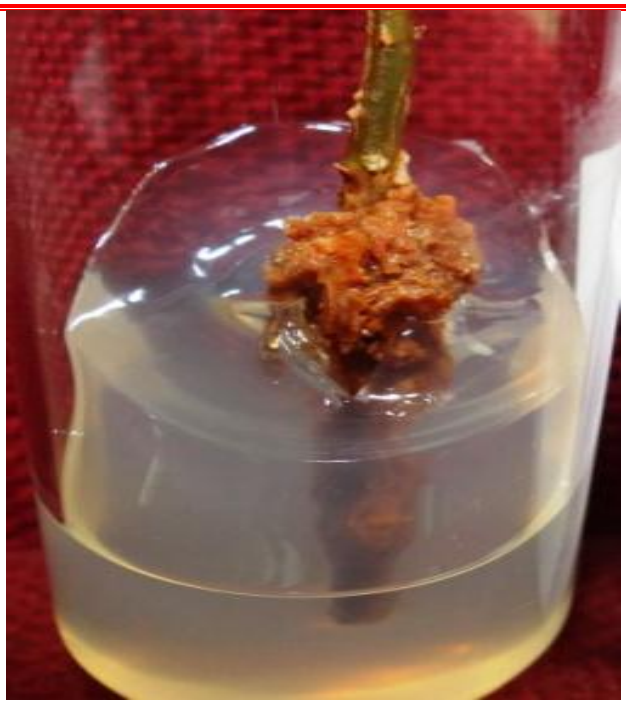

**Fig. 3 Callus induction in nodal segment explant on MS medium supplemented with 5.0 mg/l BAP and 5.0 mg/l NAA.**

**Fig. 4 Callus induction with shoot bud in nodal segment explant on MS medium supplemented with 1.0 mg/l 2,4-D.**

**Table 1: Morphogenetic effect of various concentration of auxin (2, 4-D) in the MS medium on leaf explant**

| Leaf explants | | | | | | |
|---|---|---|---|---|---|---|
| **PGR** | **Days taken in callus initiation** | **Fresh weight of callus (mg)** | **Visual growth** | **Colour** | **Texture** | **Response** |
| 0.10 | 19.30 | 442.20±46.3 | + | Yellow | Friable | 40 |
| 0.25 | 17.80 | 501.20±52.3 | + | Yellow | Semi compact | 70 |
| 0.50 | 17.90 | 556.00±46.3 | + | Light green | Friable | 70 |
| 0.75 | 17.10 | 578.00±57.2 | + | Light green | Friable | 70 |
| 1.0 | 19.00 | 604.60±45.2 | ++ | Pale yellow | Semi compact | 100 |
| 1.5 | 19.20 | 703.00±35.6 | ++ | Pale yellow | Friable | 100 |
| 2.0 | 18.50 | 905.40±14.9 | +++ | Light green | Semi compact | 100 |
| 2.5 | 19.80 | 704.60±47.8 | ++ | Yellow | Semi-compact | 100 |
| 3.0 | 18.60 | 632.90±62.3 | ++ | Yellow | Semi- compact | 100 |
| 4.0 | 17.70 | 595.10±70.2 | + | Light green | Friable | 70 |
| 4.5 | 16.60 | 453.70±52.3 | + | Pale yellow | Friable | 50 |
| 5.0 | 17.20 | 432.20±46.3 | + | Pale yellow | Friable | 20 |

+=Slight callus, ++=Medium callus, +++=Profuse callus

**Table 2: Morphogenetic effect of various concentration of cytokinin (Kn) and auxin (NAA) added in combination in the MS medium on leaf explant**

| Concentration (mg/l) | Callus | | | | | Shoot multiplication | | |
|---|---|---|---|---|---|---|---|---|
| | Response (%) | Days taken for callus initiation | Texture of callus | Colour | Fresh callus weight (mg) | Days taken for sprouting | Number of shoot buds /explants | Response (%) |
| **Leaf explants** | | | | | | | | |
| **Kn** | **NAA (0.5 mg/l)** | | | | | | | |
| 0.5 | 90 | 19.20 | Friable | Light green | 730.10 | - | - | - |
| 5.0 | 100 | 19.10 | Friable | Light green | 870.20 | - | - | - |
| **Kn** | **NAA (5.0 mg/l)** | | | | | | | |
| 0.5 | 60 | 17.30 | Friable | Light green | 590.50 | - | - | - |
| 5.0 | 70 | 18.10 | Friable | Light green | 690.70 | - | - | - |

# = Transformed value, (-) = No response

# = Transformed value, (-) = No response

**Table 3: Morphogenetic effect of various concentration of cytokinin (BAP) and auxin (NAA) added in combination in the MS medium on nodal segment explant**

| Concentration (mg/l) | Callus | | | | | Shoot multiplication | | |
|---|---|---|---|---|---|---|---|---|
| | Response (%) | Days taken for callus initiation | Texture of callus | Colour | Fresh callus weight (mg) | Days taken for sprouting | Number of shoot buds /explants | Response (%) |
| **Nodal segment explants** | | | | | | | | |
| **BAP** | **NAA (0.5 mg/l)** | | | | | | | |
| 0.5 | 70 | 19.40 | Semi-compact | Brown | 550.60 | 18.20 | $0.9^{\#}$±0.16 | 40 |
| 5.0 | 80 | 19.80 | Semi-compact | Brown | 650.10 | 18.60 | 0.8±0.15 | 30 |
| **BAP** | **NAA (5.0 mg/l)** | | | | | | | |
| 0.5 | 90 | 18.70 | Compact | Light brown | 765.10 | 17.60 | 0.6±0.15 | 10 |
| 5.0 | 100 | 19.20 | Compact | Light brown | 805.00 | - | - | - |

**Table 4: Morphogenetic effect of various concentration of auxin (2, 4-D) in the MS medium on nodal segment explant.**

| Nodal segment explants | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **PGR** | **Days taken in callus initiation** | **Fresh weight of callus (mg)** | **Visual Growth** | **Colour** | **Texture** | **Response** | **Shoot induction** | **Response** |
| 0.10 | 17.00 | 425.90±24.3 | + | Brown | Semi-compact | 20 | $1.1^{\#}$±0.11 | 60 |
| 0.25 | 17.70 | 476.80±42.3 | + | Pale green | Compact | 20 | 0.8±0.13 | 30 |
| 0.50 | 17.80 | 489.70±23.4 | + | Brown | Compact | 40 | 0.8±0.14 | 30 |
| 0.75 | 18.00 | 564.10±27.9 | + | Brown | Semi-compact | 60 | 1.1±0.12 | 60 |
| 1.0 | 18.50 | 704.40±21.0 | ++ | Brown | Semi-compact | 100 | 1.4±0.14 | 90 |
| 1.5 | 18.20 | 648.70±25.6 | ++ | Pale green | Semi-compact | 100 | 1.1±0.14 | 60 |
| 2.0 | 17.70 | 638.40±21.0 | ++ | Brown | Compact | 100 | 1.2±0.13 | 70 |
| 2.5 | 17.20 | 593.70±27.9 | + | Brown | Semi-compact | 70 | 0.9±0.11 | 40 |
| 3.0 | 17.10 | 565.60±52.0 | + | Brown | Compact | 50 | 0.8±0.13 | 30 |
| 4.0 | 16.70 | 493.80±51.0 | + | Pale green | Compact | 40 | 0.6±0.15 | 10 |
| 4.5 | 16.50 | 426.00±44.3 | + | Brown | Semi-compact | 40 | 0.7±0.14 | 20 |
| 5.0 | 16.20 | 365.00±22.1 | + | Brown | Semi-compact | 30 | 0.6±0.13 | 10 |

+++ = Profuse callus, ++=Medium callus, +=Slight callus, # =Transformed value, (-) = No response

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