# INDUCTION OF CALLUS FROM NODAL EXPLANT OF *ACACIA SENEGAL*

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## ABSTRACT

**Purpose:** *Acacia* tree species are undoubtedly important for the rural poor in the Sahel†, but propagation through seed is often limited by poor seed selection and storage, exacerbated by the high mortality of seedlings in the nursery. *In vitro* micro propagation techniques provide an alternative way of developing high yielding tree species.

**Design Methodology/Appproach:** A protocol for callus induction was developed on nodal explants of *Acacia Senegal*, cultured on full and half strengths of MS media supplemented with different concentrations of 2,4-D (1.0–2.5mg/l), alone and in combination with 0.5mg/l kinetin.

**Findings/Results:** Results indicate prolific callus formation on full strength MS medium containing 2,4-D at all the concentrations. The addition of kinetin to the auxin enhanced calli formation, especially at concentrations 2.0mg/l 2,4-D +0.5mg/l kinetin. Calli morphology was friable and yellow white in colour. Half strength MS medium seems to have a delaying effect on induction period, with moderate formation of calli that are hard and brownish white. Callus induction is a promising pathway for tree improvement programmes.

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†The Sahel is a transition zone between the wooded savannas of the south and the true Sahara Desert, stretching across Africa from Northern Senegal to Sudan

**Originality and Value:** This work would be the first attempt to induce callus from nodal explants of *Acacia Senegal* in Borno State of Nigeria, with the view of obtaining alternative methods for generating reproducible protocol for future mass propagation of the tree crop in the State.

**Keywords:** *Acacia Senegal*; callus induction; induction capacity; Murashige and Skoog (MS); 2,4-Dichlorophenoxyaceticacid (2,4-D); Kinetin (KN)

## INTRODUCTION

Gum arabic tree, *Acacia Senegal* (L.) Willd. (family - Fabaceae), is an ecologically and economically important tree that is native to semi-desert regions of sub-Sahara Africa. It is mostly found in the Sudano-Sahelian zone of Africa from Sudan to Senegal (Khalafalla and Daffalla, 2008). This tree crop has been found to improve soil fertility through symbiosis with Rhizobium and mycorrhiza (Badji et al., 1993; Singh and Pandey, 1998). The exudate of this tree (gum arabic) is highly prized for its use in the manufacturing industry as an emulsifier, and as a binding agent in the pharmaceutical industries. It also has wide ranging applications in the paint, ink and cosmetic industries.

*Acacia Senegal* has a remarkable adaptability to drought and frost (NAS, 1983). It contributes substantially to Nigeria's exports (Commodity Network Ltd, 2008), and, thus, to the revenues of the farming communities of the gum belt. Gum production is a pillar of family economy and considered as an income-generating source that requires only a low input of work after the rainy season (Mohamed, 2005). In addition to gum arabic production, the tree species has been used for desertification control, re-establishment of a vegetation cover in degraded areas, sand dune fixation and wind erosion control.

Conventionally, *A. Senegal* is propagated through seeds. However, poor germination and death of young seedlings in the natural habitat limit the scope of seed propagation (Khalafalla and Daffalla, 2008). Few studies have demonstrated the feasibility of cutting in vegetative propagation of this important tree (Badji et al., 1991; Danthu et al., 1992); however, the success of cuttings in producing plants with well-developed roots was found to be season dependent (Badji et al., 1991). Moreover, in the majority of trees, propagation by cutting is often characterised by a rapid loss of rooting capacity of the cutting with increasing age of the parent plant (Rai et al., 2010).

Tissue culture is considered as a very promising technique for both large-scale clonal propagation of plants and genetic engineering of plant germplasm. Therefore there is a great need to develop an efficient *in vitro* regeneration protocol, which not only fulfils the demand for healthy seedlings but also can be applied for future programmes of genetic transformation of this species.

The present study describes the work that was conducted to cover the aspect of callus induction potential under different exogenous factors; these were culture media

and plant growth regulator supplementation. The effects of those factors were studied to fulfil the aim of obtaining profuse callus production with friable morphology. Further study will determine the function of callus formation in the plant.

## MATERIALS AND METHODS

### *Surface Sterilisation of Explants*

Seeds of *A. Senegal* were acquired from a gum arabic tree plantation grown in Gubio Local Government Area of Borno State, Nigeria. Seedlings were raised on the experimental site of the Biotechnology Centre, University of Maiduguri (Gadzama et al., 2018).

Nodal segment explants with one axillary bud were excised from six month old seedlings growing in the nursery. The explants were washed under running tap water for 30 minutes to remove surface dust, and then soaked in a solution mixture of 100mg/l ascorbic and 150mg/l citric acid for 10 minutes. The explants were then immersed in 70% ethanol for 30 seconds, washed by several changes of sterilised distilled water. They were then immersed in 100ml Clorox solution of 10% and 15% mixed with two drops of Tween 20 (surfactant) for 10 minutes each, with continuous agitation. Explants were rinsed several times with sterile distilled water under a laminar airflow cabinet. Sterilised explants were cultured in culture bottles containing Murashige and Skoog (1962) basal medium.

### *Media Preparation*

Different concentrations and combinations of 2,4-dichlorophenoxyacetic acid (2,4-D) (1.0–2.5mg/l) and kinetin (0.5mg/l) were used in the study. Two strengths of Murashige and Skoog's (MS) medium were used (full and half strengths). The prepared media consisted of 30g/l sucrose (sigma) and 7g/l agar (sigma). The pH was adjusted to be within 5.6–5.8 by using 1mol/1 HCI and NaOH after the addition of plant hormones. The media were autoclaved at 121$^{o}$C under the pressure of 1.06kg/cm$^{2}$ for 15 minutes.

### In vitro *Callus Induction*

The sterilised nodal explants were further aseptically trimmed into small pieces approximately 1.0–1.5cm and cultured onto callus induction media. Ten replications of inoculated explants were prepared for each treatment, and the experiments were repeated thrice. The cultures were incubated at (25$\pm$2)$^{o}$C under photoperiod of 16/8 light and dark hours daily, with exposure to 1,000 lux, provided by LED lamps. These were sub-cultured onto fresh media after three weeks of culture, and observations were done on a weekly basis. At the end of six weeks, the data for callus induction

were recorded in which the morphology and percentage induction of callus in each treatment were taken.

The percentage of callus induction in each treatment was calculated using the following formula:

$$\text{Callus induction } \% = \frac{\text{Number of explants formed callus}}{\text{Total number of explants cultured}} \times 100\%$$

## RESULTS/FINDINGS

### *Callus Induction*

The induction of callus serves as a basis in plant biotechnology studies in which the development of various plant regeneration studies and somatic embryogenesis may be initiated from callus (Ikeuchi et al., 2013; Osman et al., 2013).

MS medium (full and half strength) was supplemented with various concentrations of 2,4-D-(1.0, 1.5, 2.0 and 2.5)mg/l, alone and in combination with 0.5mg/l KN (Tables 1 and 2). Results show that there was a difference in the degree of callus induction when different MS strengths were used. Full strength MS medium supplemented with all the concentrations of 2, 4-D used in this work produced moderate to profused calli, but augmentation of the above medium with 0.5mg/l kinetin yielded a more profuse and friable calli (Table 1 and Figures 1A, 1B, 1C and 1D). Reducing the nutrient strength of MS medium seems to have a delaying effect on the rate of callus formation (Table 2 and Figures 2E, 2F and 2G).

**Table 1 Effect of Different Concentrations of 2, 4-D, Alone and in Combination with Kinetin on Callus Induction from Nodal Explants of *Acacia Senegal* after Six Weeks Culture on Full Strength MS Medium**

| *Treatments* | *PGR concentration 2,4-D + KN(mg/l)* | *Callus induction (%)* | *Onset of callus induction (days)* | *Degree of callus induction* | *Morphology (callus appearance)* |
|---|---|---|---|---|---|
| Control | 0.0 | – | – | – | – |
| DI | 1.0 | 100 | 6 | ++++ | Friable yellow white |
| D2 | 1.5 | 90 | 6 | ++++ | Compact yellow white |
| D3 | 2.0 | 100 | 6 | +++ | Compact yellow white |
| D4 | 2.5 | 100 | 6 | ++++ | Friable yellow white |
| DIK | 1.0 + 0.5 | 100 | 5 | ++++ | Friable yellow white |
| D2K | 1.5 + 0.5 | 100 | 6 | ++++ | Friable yellow white |
| D3K | 2.0 + 0.5 | 100 | 5 | ++++ | Compact yellow white |
| D4K | 2.5 + 0.5 | 100 | 5 | ++++ | Friable yellow white |

+ + +: Moderate; + + + +: Profuse; –: no callus
*Source*: devised by authors

**Table 2 Effect of Different Concentrations of 2, 4-D, Alone and in Combination with Kinetin on Callus Induction from Nodal Explant of *Acacia Senegal* after Six Weeks of Culture on Half Strength MS Medium**

| *Treatments* | *PGR concentration 2,4-D + KN (mg/l)* | *Callus induction (%)* | *Onset of callus induction (days)* | *Degree of callus induction* | *Morphology (callus appearance)* |
|---|---|---|---|---|---|
| Control | 0.0 | – | – | – | – |
| DI | 1.0 | 100 | 12 | ++ | Hard, brown |
| D2 | 1.5 | 100 | 14 | ++ | Hard, brown |
| D3 | 2.0 | 80 | 14 | ++ | Hard, brown |
| D4 | 2.5 | 90 | 13 | + | Hard, brown |
| DIK | 1.0 + 0.5 | 70 | 12 | ++ | Hard, brown |
| D2K | 1.5 + 0.5 | 80 | 13 | + | Hard, brown |
| D3K | 2.0 + 0.5 | 90 | 13 | ++ | Hard, brown |
| D4K | 2.5 + 0.5 | 80 | 12 | ++ | Hard, brown |

+: Very weak; + +: Weak; –: no callus
*Source*: devised by authors

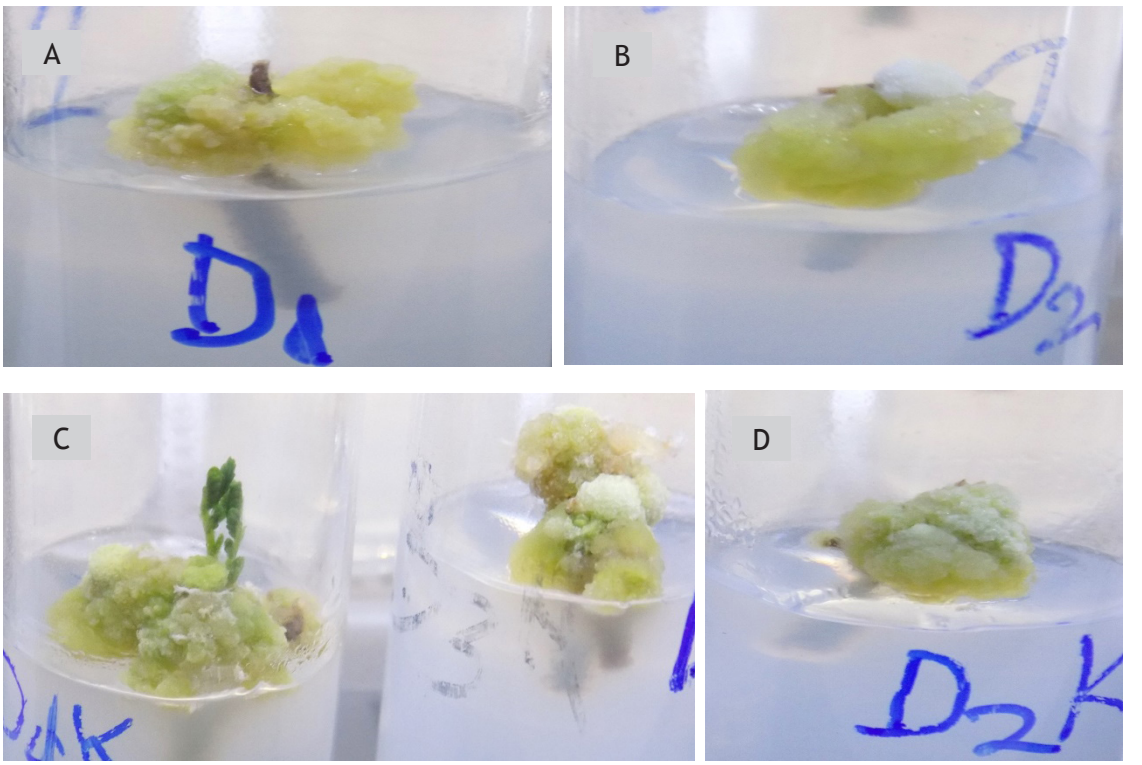


**Figure 1 Morphology of Callus on Full Strength MS Medium Supplemented with (A) 1.0mg/l 2,4-D (B) 1.5mg/l 2,4-D (C) 1.0mg/l 2,4-D + 0.5mg/lKN (D) 1.5mg/l2,4-D + 0.5mg/l KN**

*Source*: produced by authors

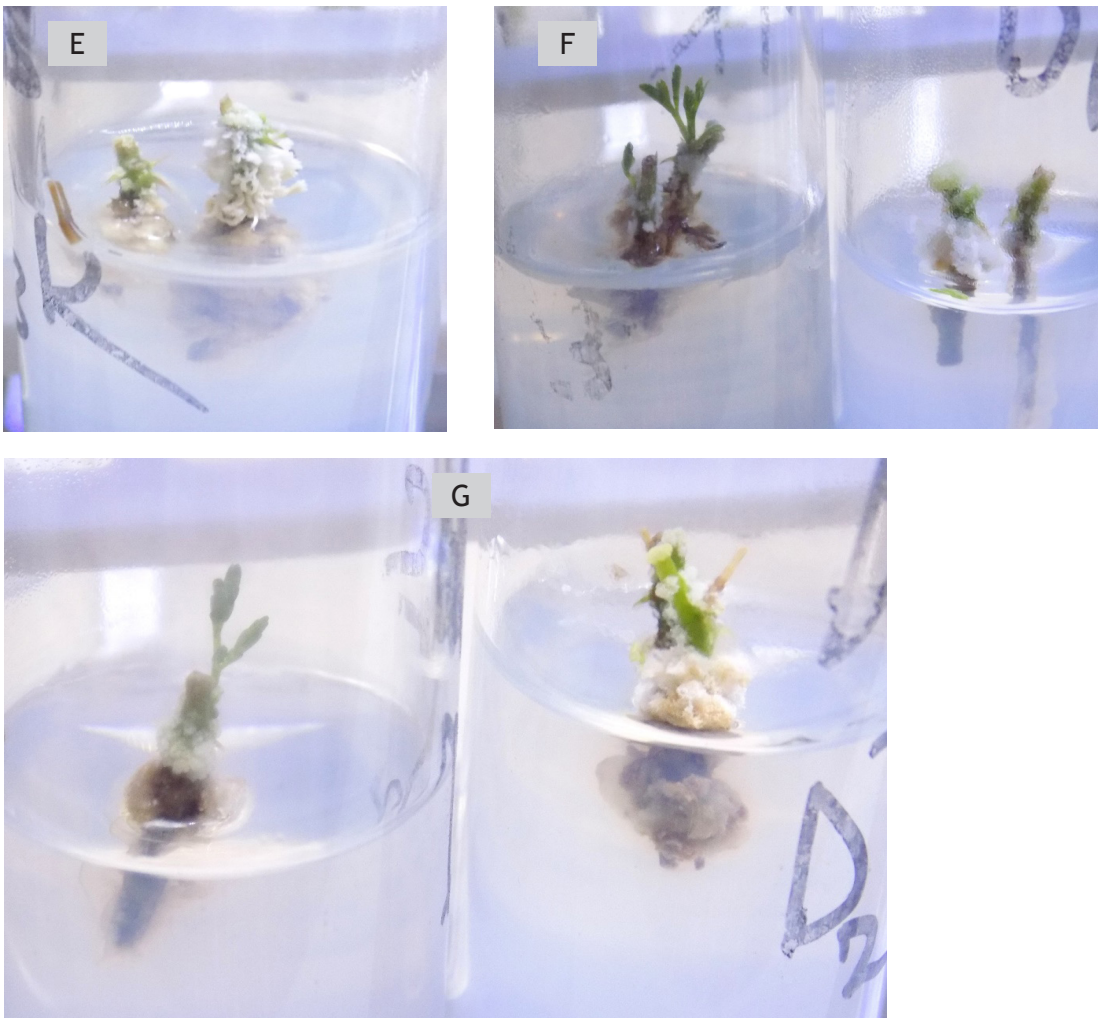


**Figure 2 Morphology of Callus on Half Strength MS Medium Supplemented with 2.0mg/l 2,4—D + 0.5mg/lKN (F) 1.0mg/12,4—D + 0.5mg/1 KN, and (G)1.5mg/12, 4—D alone**

*Source*: produced by authors

## DISCUSSION AND CONCLUSIONS

### *Effects of Hormonal Treatments on Callus Induction*

After a six week culture period, the results from this study revealed that the presence of 2,4-D in the culture media was essentially required to induce callus formation in the nodal explants of *Acacia Senegal*, even without the presence of cytokinin (Figures 1A and 1B). The effectiveness of 2,4-D in inducing callus formation is attributed to

its main characteristic, which can stimulate cell division of plant tissues and strongly suppress organogenesis (Osman et al., 2016). Callus formation was obtained at the basal end and then spread to the surface of the whole explant. It is noted that 2,4-D is considered to be the most potent among the other commonly used auxins (Staba, 1980). Nevertheless, in the current study, the formation of calli in MS medium supplemented with 2,4-D alone at the concentrations of 1.0mg/l–2.5mg/l was found to be delayed, whereby the calli only started to form after six days of culture (Table 1). However, the addition of kinetin in the culture media in combination with 2,4-D was fruitful in enhancing callus formation, especially at concentrations of 2.0mg/l 2, 4-D and 0.5mg/l kinetin (Table 2 and Figure 1C). This is in contrast to Rashid et al. (2009) where they found that the addition of kinetin affected the callus formation negatively in *Triticuma estivum* (Rashid et al., 2009). Therefore, the addition of kinetin is required to exert additional physiological effect. The findings revealed that hormonal combinations do have significant effect towards the formation of callus in this study.

### *Effects of MS Media Strength on Callus Induction*

The level of nutritional components in MS medium do affect the callus induction potential, hence the morphology of calli formed (Figures 2E, 2F and 2G). The observation in this study showed that the degree of callus formation and morphology of formed calli in half strength MS medium supplemented with 2, 4-D, alone and in combination with kinetin, was completely different from that formed on full MS medium (Table 2 and Figure 2). The calli were smaller and less profuse (weak) and were generally brown and hard (Figures 2E, 2F and 2G). In terms of time taken for the callus to be induced, generally, half strength MS medium slowed the induction response, which delayed the onset of callus formation to 12 days (Table 2).

The findings gathered in this study are useful for the production of calli, which is required for plant regeneration studies, and somatic embryogenesis. It may also function as a starting point for establishing cell suspension cultures, plant bioreactor and bioactive compounds studies in the species.

## CONCLUSIONS

The findings gathered in this work are useful for the production of calli, which is required for plant regeneration studies. The highest callus induction of nodal explants of *Acacia Senegal* was obtained on full strength MS medium containing a combination of 2,4-D (2.0mg/l) and kinetin (0.5mg/l). Half strength MS medium was not suitable as it delayed the formation of callus.

Therefore, further experiments are needed for the optimisation of callus induction and possible subsequent somatic embryogenesis.

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