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ON INCIDENCE OF CALLUS INDUCTION IN THE DEVELOPMENT OF MICROPROPAGATION OF *ACACIA SENEGAL* IN BORNO STATE OF NIGERIA

ABSTRACT

BACKGROUND

Acacia senegal is a drought resistant tree that is important for environmental protection, community livelihood and a sustained reforestation programme. However, propagation through seed is often limited by poor seed selection and storage, and exacerbated by the high mortality of seedlings in nurseries. This work highlights the potential of developing an alternative method of raising healthy seedlings of *Acacia senegal* in Borno State of Nigeria, with a future view of the mass propagation of the tree crop.

DESIGN/METHODOLOGY

Six month old Nodal explants of *Acacia senegal* were cultured on full and half strength MS media, supplemented with different concentrations of 2,4-D (1.0–2.5mg/l), alone and in combination with 0.5mg/l kinetin.

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The gum arabic tree, *Acacia senegal* (L.) Willd., family – Fabaceae, is an ecologically and economically important tree that is well adapted to the arid and semi-arid conditions of the Sahel. It is mostly found in the Sudano-Sahelian zone of Africa from Sudan to Senegal (Khalafalla and Daffalla, 2008). The Sahel region of Africa extends from Senegal eastwards to Sudan, and forms a transition zone between the arid Sahara (desert) to the north and the belt of humid savanna to the south. In Nigeria, 350,000m^2 of arable land is lost to desertification at a rate of 0.6km per annum (Gadzama, 1995). This has displaced and impoverished the affected communities.

RESULTS/ FINDINGS

Results indicate prolific callus formation on full strength MS medium containing 2,4-D at all concentrations. Kinetin was added 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 media had a delaying effect on the induction period, with a moderate formation of calli that were hard and brownish white. Callus induction may provide a promising pathway for the mass propagation of the *A. senegal* tree for reforestation and other programmes.

ORIGINALITY / VALUE

This is considered to be the first attempt to *in vitro* propagate *A. senegal* and generate reproducible protocol for the possible future propagation of the tree crop in the Sahel of Borno State of Nigeria, with callus induction in the process.

Acacia senegal is a hardy tree that is drought resistant (NAS, 1983). This under-utilised legume tree is a potential solution to land degradation and soil nutrient depletion as it improves soil fertility through nitrogen fixation (Badji et al., 1993; Singh and Pandey, 1998). It also provides vegetative cover, and can be used for erosion control as well as sand dune aggregation. The exudate of this tree (gum arabic) is highly prized for its use in the manufacturing industry as an emulsifier; it is also used in the pharmaceutical industry for encapsulating and binding agents. It has a wide range of applications in the paint, ink and cosmetic industries.

Acacia senegal contributes substantially to Nigeria's exports (Commodity Network Ltd, 2008), and, therefore, to the revenues of the farming communities of the gum belt. Gum production is a critical means of livelihood for the Sahelian rural community. The rural populace comprises of women and youths who are mostly unemployed and depend on subsistence farming for survival. As an income-generating natural resource that requires only a low input of work after the rainy season (Mohamed, 2005), providing healthy *Acacia senegal* seedlings to these communities would not only boost their economic livelihood but also protect the environment and preserve biodiversity. In addition to gum arabic production, the tree species has been used for afforestation programmes. Women and youths play a major role in the sustainable management of *Acacia senegal* trees and, as such, are key participants in achieving the Great Green Wall initiative (GGW).

Conventionally, *Acacia senegal* is propagated through seeds; however, this has been limited by poor germination and the low survival rate of the young seedlings in their natural habitat (Khalafalla and Daffalla, 2008). Vegetative propagation of this important tree has been unsuccessful (Badji et al., 1991; Danthu et al., 1992). Moreover, propagation using cuttings is often characterised by a rapid loss of rooting capacity of the cutting with the increasing age of the parent plant (Rai et al., 2010). Therefore, there is a need to develop new ways of raising healthy seedlings of this important tree crop that will enhance the reforestation programme.

KEYWORDS

Callus induction; Nodal explant; *Acacia senegal*; Auxin; 2,4 dichlorophenoxy-acetic acid (2,4-D); Kinetin (KN); Murashige and Skoog (MS)

Tissue culture is a promising tool that can be harnessed for the large-scale clonal propagation of plants and genetic engineering of plant germplasm. The need to develop an efficient *in vitro* regeneration protocol will not only fulfil the demand for healthy seedlings but can also be applied for future programmes of the genetic transformation and mass propagation 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 these 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 the 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 (KN) (0.5mg/l) were used in the study. Two strengths of Murashige and Skoog's (MS) medium (full and half strengths) were used. 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 1N HCl and 1N NaOH after the addition of plant hormones. The media were autoclaved at 121°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 ± 2) °C under photoperiod of 16/8 light and dark hours daily with exposure to 1000 lux light intensity, 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 recorded.

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

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

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 strengths) 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 profuse calli. However, augmentation of the above media with 0.5mg/l kinetin yielded a more profuse and friable calli (Table 1 and Figures 1A, 1B and 1C). Generally, the calli formed were yellowish white to yellowish green in colour.

Reducing the nutrient strength of MS medium seems to have a delaying effect on the rate of callus formation (Table 2 and Figures 2A, 2B, 2C and 2D).

DISCUSSION AND CONCLUSION

Effects of hormonal treatments on callus induction

After a six week culture period, the percentage of callus induction and the morphology of calli formed were all recorded. The results show that the presence of 2,4-D in the culture medium was essentially required to induce callus formation in the nodal explants of *Acacia*

TABLE 1 **Effect of 2,4-D and 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)*	*Callus induction*	*Morphology (callus appearance)*
Control	0.0	0	–	–	No calli formation
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 2,4-D and 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	0	–	–	No calli formation
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

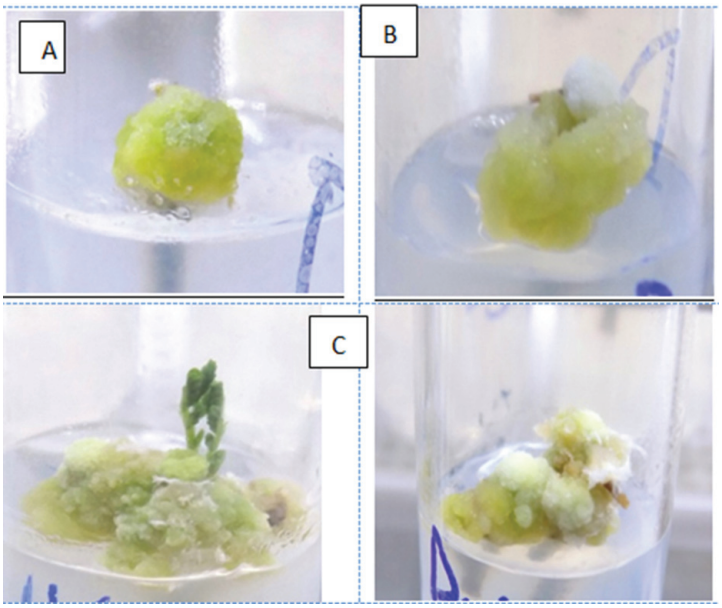

FIGURE 1 **Proliferation of calli formed from nodal explant of *A. senegal* cultured on full strength MS medium supplemented with (A) 1.0 mg/l 2,4-D; (B)1.5mg/l 2,4-D; (C)2.0mg/l2,4-D + 0.5mg/l KN**
Source: Produced by authors

senegal, even without the presence of cytokinin (Table 1, Figures 1A and 1B). The effectiveness of 2,4-D in inducing callus formation is attributed to its main characteristic that 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 whole explant surface. It is noted that 2,4-D is considered to be the most potent among the other

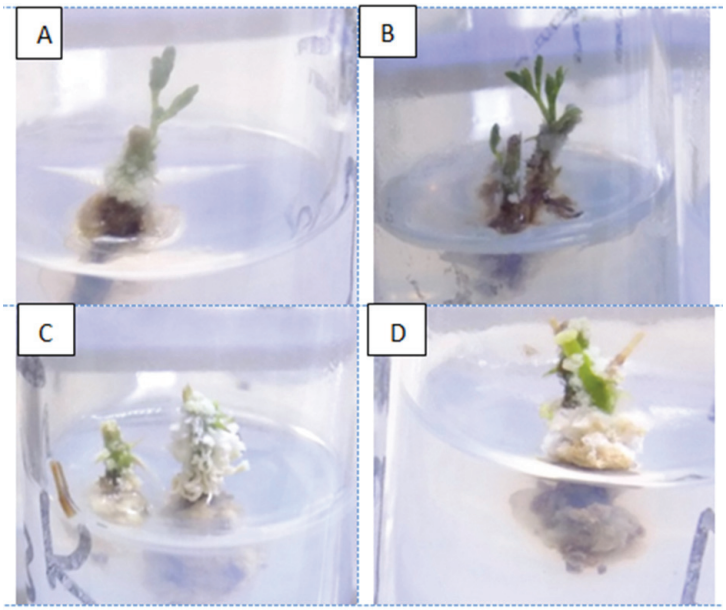

FIGURE 2

Proliferation of calli formed from nodal explant of *A. senegal* cultured on half strength MS medium supplemented with (A) 1.5mg/l 2,4-D; (B) 1.0mg/l 2,4-D; (C) 2.0mg/l2,4-D +0.5mg/l KN; and (D)1.5mg/l 24-D +0.5mg/l KN
Source: Devised by authors

commonly used auxins (Staba, 1980). In the current study, it was observed that the degree of callus induction was found to vary from moderate to profuse at concentrations of 1.0mg/l to 2.5mg/l of 2,4-D (Table 1 and Figures 1A and 1B). However, the addition of kinetin (0.5mg/l) in the culture media in combination with 2,4-D was fruitful in enhancing callus formation. The positive effect of kinetin was noted to have enlarged the calli formation, especially at concentrations of 2.0mg/l 2,4-D and 0.5mg/l kinetin (Table 1 and Figure 1C). Moreover, the morphogenic response was identified to be more friable in culture media containing kinetin (Figure 1C). This is in contrast to Rashid et al. (2009) where they noted that the addition of kinetin affected the callus formation negatively in *Triticum aestivum* (Rashid et al., 2009). Therefore, the addition of Kinetin is required to exert an additional physiological effect. The findings revealed that hormonal combinations do have an effect towards the formation of callus in this study.

Effects of MS media strength on callus induction

The nutritional components in medium do affect the callus induction potential and therefore the morphology of calli formed (Figures 2A, 2B, 2C and 2D). The observations in this study indicate 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 strength MS medium (Table 1 and Figures 1A, 1B and 1C).It was clearly observed that the calli formed on half strength MS media were smaller and less profuse (weak) (Figures 2A, 2B, 2C and 2D) than those cultured on full strength MS media (Figures 1A, 1B and 1C) and are generally brown and hard.

In terms of time taken for the callus to be induced, generally full strength MS medium exerted a faster induction response in which the onset of callus formation was as early as the first week of culture (Table 1). In contrast, half strength MS medium, at all the treatments of 2,4-D alone and in combination with kinetin, resulted in a delayed response in the onset of callus formation, which started two weeks after the first culture (Table 2).

Further studies should be conducted to examine the impacts of medium components towards callus morphogenic response.

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 function as a starting point for establishing cell suspension cultures, plant bioreactor and bioactive compounds studies in the species.

CONCLUSION

The results of this work indicate that various concentrations of 2,4-D influence calli induction on nodal explant of *Acacia senegal*. The addition of kinetin to the MS medium enhanced calli proliferation. 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). Calli produced are friable and yellow-white in colour. Half strength MS medium seemed unsuitable as it delayed the formation of callus.

Further experiments are needed for the optimisation of callus induction and possible somatic embryogenesis for the tree crop. Uyoh et al.'s (2016) method of inducing rooting development in yam vine cuttings by exposing tissues to natural (neem leaf ash and coconut water) and synthetic (2,4-D and IBA) hormones, will also be tried. Developing an in-house protocol for *Acacia senegal* using modern technology may, however, provide opportunity for sustained plantlet production for the needs of commercial and rural farmers, afforestation programmes and the Great Green Wall (GGW) initiative in the future.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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