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OPTIMUM PROTOCOL FOR SHOOT FORMATION IN SORGHUM (*SORGHUM BICOLOR* (L) MOENCH) THROUGH ORGANOGENESIS

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ABSTRACT

A protocol for shoot formation in Sorghum bicolor (L) Moench was developed using immature embryo. Murashige and Skoog (MS) basal medium supplemented with varying concentration of 6- benzyl aminopurin (0, 1, 2 and 3mg/l) was used for shoot initiation. After one week of inoculation the primary leaves of the shoot obtained were removed and meristems were cultured for shoot multiplication using MS medium fortified with 3mg/l BAP (6-benzylaminopurine) alone or in combination with 1mg/l NAA (Naphthalene Acetic Acid). For shoot initiation the media fortified with 2mg/l BAP recorded the highest percentage shoot formation (86.67%), the least was obtained from hormone free media (46.67%). The average shoot number per plant was more in the media fortified with 3mg/l BAP + I mg/l NAA (2.4) but this is not significantly different from what was obtained from the media fortified with 3mg/l BAP alone

KEYWORDS: A Protocol, Phytochemicals, *Sorghum bicolor*, (L) Moench

INTRODUCTION

Sorghum bicolor belongs to the family poacea and it is an important cereal crop worldwide. It is the fifth most important cereal crop after rice, maize, wheat and barley ((ICRISAT, 2010). Sorghum bicolor is used as food for human consumption and as feeds for animal. Sorghum bicolor is rich in different phytochemicals and these important bioactive compounds are used by pharmaceutical industries for the development of various drugs for the treatment of different kinds of ailments such as cancer, sickle cell anaemia, tubercular swellings, and measles among others. Tissue culture technique has been used as an alternative method of genetic improvement in sorghum because the use of conventional breeding method may take many years to be realized and it has also been used by pharmaceutical industries for the production of different types of secondary metabolites. According to Sadia *et al.* (2010) genetic engineering allows for the generation of novel varieties of a certain crop by introducing genes from sources outside that crop. To achieve efficient genetic transformation in sorghum, the development of an *in vitro* regeneration system specific to this crop is required. The aim of this work is to develop efficient protocol for shoot formation in sorghum bicolor through organogenesis

MATERIALS AND METHODS

Karandafi seeds collected from farmers' fields in Sokoto and Katsina States were used for this study. The seeds were planted in the green house in order to obtain immature seeds. The immature seeds of sorghum containing immature embryo were harvested from main spikes of caryopsis of 15-17 days after anthesis according to Sudhakar *et al*. (2008), the immature seeds were washed under running tap water for 20 minutes using detergent and surface sterilized in 95%(v/v) ethanol for 2 minutes and further sterilized in 20% (v/v) commercial bleach for 20minutes. The seeds were rinsed three times using double sterilized distilled water. The immature embryos were aseptically excised from immature seeds. Immature embryos obtained were used for shoot initiation using MS media supplemented with varying concentration of BAP (0, 1, 2 and 3mg/l), the pH of the media was adjusted to 5.8 using 0.5M NaOH and 0.5M HCl and gelled

with 8g/l agar. Phenolic secretion was controlled using activated charcoal. Fifteen explants were used per treatment and percentage shoot formations were recorded after 1 week of inoculation. Afterwards the primary leaves were removed from the shoot apices and the meristems were cultured for multiple shoots formation in the media fortified with 3mg/l BAP alone or in combination with 1mg/l NAA for shoot multiplication using ten test tubes per treatment. The number of shoots per treatment, number of shoots per explant and average plant height per treatment were recorded. The data collected were subjected to analysis of variance and their means were separated using Duncan multiple range test

RESULTS AND DISCUSSIONS

Shoot formation was observed third day after inoculation in all the treatments tested. Percentage shoot formation was recorded after one week of culture, media supplemented with 2mg/l BAP recorded the highest shoot formation (86.67%) followed by the media fortified with 1mg/l BAP (73.30%). Both root and shoot formations were observed on hormone free media and it recorded the lowest percentage shoot initiation (46.67%) (Table 1 and Figure 1).

The primary leaves were removed from the shoot apices and the meristems were cultured for multiple shoots formation. 1-4 shoots per explant were obtained from the apical meristem cultured after 2 weeks of inoculation. The mean shoot number per explant was more in the media supplemented 3.0mg/l BAP combined with 1mg/l NAA (2.4) and this not significantly different from what was obtained from the media supplemented with 3mg/l BAP alone (2.0) (Table 2). The plant height in the media fortified with 3.0mg/l+1NAA was 7.2cm and this is not significantly different from the media supplemented with 3BAP (6.2) (Table 2). The plantlets obtained from hormone free media were acclimatized after one week of regeneration (Figure 1).

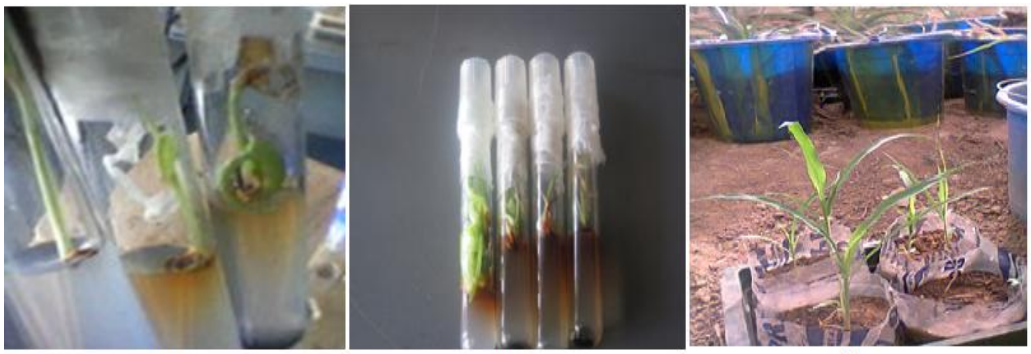

Figure 1: Plant Regeneration through Organogenesis (a) Shoots Initiated from Immature Embryo, (b) Shoot Multiplication Stage, (c) Graduated Plantlets in the Green House

Table 1: Effect of BAP on Shoot Initiation Using Immature Embryo

PGR Used	No of Explants Inoculated	No of Explants with Response	Frequency of Shoot Formation (%)
BAP (mg/l)			
0.0	15	7	46.67b
1.0	15	11	73.3ab
2.0	15	13	86.67a
3.0 CV (%) SE±	15	8	53.33b 23.50 12.4

Means followed by the same letter (s) within a column are not significantly different using DMRT (*significant atP<0.05, **highly significant at P<0.01)

Table 2: Effect BAP and NAA on Shoot Multiplication

PGR Used		No of Explants Inoculated	No of Shoots/ Treatment	No of Shoot/Explant	Average Plant Height (Cm)
BAP (mg/l)	NAA (mg/l)				
3.0	0.0	10	20	2.0a	6.2a
3.0	0.1	10	24	2.4a	7.2a
CV (%)				50.71	25.21
SE±				0.49	0.75

Means followed by the same letter (s) within a column are not significantly different using DMRT (*significant at P<0.05, **highly significant at P<0.01)

From the result of this study, it was observed that 2mg/l BAP is sufficient for shoot initiation in sorghum using immature embryo while 3mg/l combined with 1mg/l NAA recorded more number of shoot per meristem cultured. Anju verma and Anandkumar (2005) reported multiple shoot induction by using 2 mg/L of BAP in the culture medium. Sudhakar *et al*. (2008) reported that shoot regeneration was proficient at a concentration of 2mg/l BAP. Prathibha *et al*. (2001) reported that 2mg/l BAP+ 0.5mg/l 2, 4-D were found to be suitable to obtain maximum percentage of regeneration in sorghum. Pola *et al.* (2009) reported multiple shoot formation using 2mg/l BAP + TDZ (Thidiazuron). Sadia *et al.* (2010) reported multiple shoot formation using 3mg/l BAP combined with 1mg/l TDZ.

Formation of root and shoots were was also observed from this study when hormone free media was used which shows that it is possible to obtain both organs at a time using hormone free media but the limitation is that only one plantlet can be obtained from an explant but these plantlets are pathogen free. It was observed from this study that this contains high amount of phenolic compounds and its usage for the management of sickle cell anaemia may be attributed to high amount of phenols it contains because of the high antioxidant properties of phenolic compounds such as anthocyanin and flavonoids

CONCLUSIONS

From the result of this study it was observed that the use of BAP in shoot initiation gave better result because more percentage shoot initiation was obtained from the media fortified with BAP while the least was obtained from the media with zero hormones. In conclusion, the protocol described here is optimum for shoot formation in sorghum but more varieties need to be subjected to tissue culture technique to see whether the response is genotype dependent.

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