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Embryogenic Callus Induction from Mature Embryos of Sorghum Genotypes

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Abstract: Sorghum bicolor is the fifth most important cereal crop in the world. Sorghum is considered to be one of the most recalcitrant species among the cereals for in vitro response and genetic transformation. In this study four sorghum genotypes were examined for their ability for callus induction. The cultures were initiated from mature embryos. The highest number of somatic embryos was obtained on MS medium supplemented with 2mgl^{-1} 2, 4 D. Highest frequency of embryogenic callus formation was observed in the genotype Wad Ahmed.
Keywords: Sorghum; Tissue culture; 2, 4 D; MS media.

1. Introduction

Sorghum bicolor L. Moench (2n=20) is an economically important cereal plant belonging to the family Poaceae, it is one of the most significant crops in Asia, Africa and Latin America. Sorghum is the fifth most important cereal crop in the world after wheat, rice, maize and barley. Sorghum is stable food crop for millions of poor people in the semi arid and arid Tropics [1]. It is used as food in Asia and Africa and is employed as feed for animals in developed countries such as USA. It has been reported that the tolerance of sorghum to drought and high temperature is higher than that of corn wheat and other crops and sorghum has the unique ability to grow under a wide array of harsh environmental conditions [2]. Cereal crop improvement through genetic transformation requires establishment of an efficient and reproducible plant regeneration system [3]. Genetic engineering of Sorghum has emerged as an alternative tool for conventional breeding for the introduction of desirable traits into elite varieties. On the other hand Sorghum is considered to be the one of the most recalcitrant species among the cereals for in vitro response and genetic transformation Nguyen , *et al.* [4], Sudhakar, *et al.* [5].Therefore, it is necessary to establish a high efficiency regeneration system for genetic transformation of sorghum. Mature seeds are the most preferred explants for in vitro protocols as they can be stored, available round the year and can easily handled [6]. A limited number of studies has been carried out on in vitro culture of mature embryos of Sorghum, Bhaska, *et al.* [7], obtained sodium chloride tolerant callus derived from mature embryo. Bhaska, *et al.* [7], reported plant regeneration from mature embryos derived callus on aluminum selected media. Waskom, *et al.* [8] and Miller, *et al.* [9], also studied in vitro culture of mature embryos in mature embryos Sorghum.

Objective of this study was to establish a simple and efficient callus induction system from mature embryo, which can be used as source material for in vitro culture of Sorghum in any season.

2. Material and Methods

2.1. Plant Material

Mature seeds of four popularly grown Sudanese sorghum (*Sorghum bicolor* L .Moench) cultivars, namely Tabat, Wad Ahmed, Dwarf white Milo and Aros Al -remal were used in this study.

2.2. Method

2.2.1. Callus initiation from mature embryos

a) Surface sterilization of Explants

The materials studied were consisted of Four Sudanese grain sorghums (*Sorghum bicolor* (L.) Moench) genotypes namely, Wad Ahmed, Tabat, Dwarf Milo, and Aros- alremal, seeds were obtained from Agricultural Research Corporation (ARC) Wad Medani. Mature sorghum seeds were used as explants. Selected healthy seeds of each genotype were washed thoroughly under running tap water for 15 min. Seeds were then transferred to 70 % ethanol for 5 min under the laminar air-flow cabinet, then treated with 15% sodium hypochlorite (Clorox) plus one to two drops of tween 20 for 20 min and then rinsed with sterile distilled water three to six times. Seeds were spread separately to sterilized petri plates having filter papers for drying.

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b) Callus Induction and Subculture

For callus formation, mature embryo explants were transferred to culture bottles containing MS [10] medium. The MS medium was fortified with 3% sucrose (w/v), solidified with 0.6% agar and supplemented with growth regulators such as 2,4D (2.5 mg L-1) , Kinetin (0.2 mg L-1) and proline (0.6 g L -1). The pH was adjusted to 5.8 (Table 1). Mature embryos were cultured for 4 weeks on callus induction medium and sub cultured biweekly onto fresh media. All callus cultures were maintained in darkness at 25± 2°C. The calli induced were planted on the same media for subculture. The calli which showed a watery and friable appearance was identified as non- embryogenic and the shiny yellow compact nodular calli as embryogenic calli.

The percentage of callus induction efficiency (CIE) and percentage of primary callus induction were scored after 4 weeks as follow:

Callus induction efficiency percentage

$$= \frac{\text{Number of calli obtained}}{\text{Total number of seeds}} \times 100$$

Percentage of primary callus induction

$$= \frac{\text{Callus number}}{\text{Non-contaminated germinating seed number}} \times 100$$

3. Results

3.1. Callus Induction

A wide range of organs, which include meristematic cells, such as mature seed [11], immature embryo [12], have been reported to be good resources for production of embryogenic calli. Detectable callus formation was obtained within five days culture in MS media which supplemented by 2,4 D (2.5 mg l^{-1}). . In the second week, two types of calli were observed in the culture. A soft and friable callus that was yello wish and mucilaginous in nature and another one is a hard, nodular, compact and white embryogenic callus as shown in (Figure 1). In some genotypes such as Arose Al- remal., purple pigments were released from necrotic of the callus into the media. Also browning was observed in the genotype Dwarf Milo. These pigments were described as phenolic compounds.

On the other hand the obtained results showed that the culture responses were greatly influenced by the genotype in all types of embryo cultures (Table 1). The percentage of Callus induction efficiency was ranged from 80 to 25 and the percentage of primary callus induction was ranged from 90.1 to 46.6. Mature embryos from the genotype Wad Ahmed had an excellent callus induction frequency (80%) and a high primary callus induction (91.1%). In contrast genotypes Dwarf Milo, Tabat and Arose Al- remal had a high primary callus induction (80%, 71.1%, 46.6 respectively) but a low callus induction efficiency (25 % , 52.5 %, 35% respectively) as shown in (Figure 2).

From these results we conclude that the genotype Wad Ahmed showed high ability of callus formation. Also we concluded that 2.5 mg L^{-1} was optimum concentration to obtain high frequency of embryogenic calli.

4. Discussion

Tissue culture system is fundamental for successful regeneration of transgenic plants after genetic transformation. Also we observed that sorghum explants are rich in phenolic compounds especially from cultivars Dwarf Milo and Aros Al- remal and these compounds are recalcitrant during tissues culture and this agree with different results presented by Cai and Butler [13]; Casas, *et al.* [14].

Previous studies in cereals showed that, the use of 2, 4-D to induce callus formation from mature embryos was a critical factor. In general, auxins usually 2,4-D in the range of 1–3 mg l^{-1} , is essential for the formation of embryogenic callus from cereal embryos [15].

Pandey, *et al.* [16] worked with matured rice seeds using different levels of 2, 4-D in nutrient medium and they concluded that seed response for callusing was the best on 2 mg/l. Other workers like Islam, *et al.* [17] obtained good number of calli on the MS medium when 2, 4-D 1 mg/l was applied. Furthermore they reported that best embryogenic calli were recovered from 2, 4-D supplied media than the media supplemented with other organic supplements.

5. Conclusion

Efficient plant regeneration is a prerequisite for a complete genetic transformation protocol in cereals. The transfer of foreign genes by genetic engineering techniques requires the development of efficient *in vitro* regeneration systems, such as mature embryo culture, which may provide enough material for direct gene transfer studies

Mature seeds are most readily available and free from seasonal limits. The present study may afford a base for in vitro study of sorghum.

Also the genotype Wad Ahmed may be good genotypes for sorghum *in vitro* studies.

Figure-1. Types of callus obtained from sorghum genotypes (A to D) : Embryogenic calli , (E and F): non-embryogenic calli Types

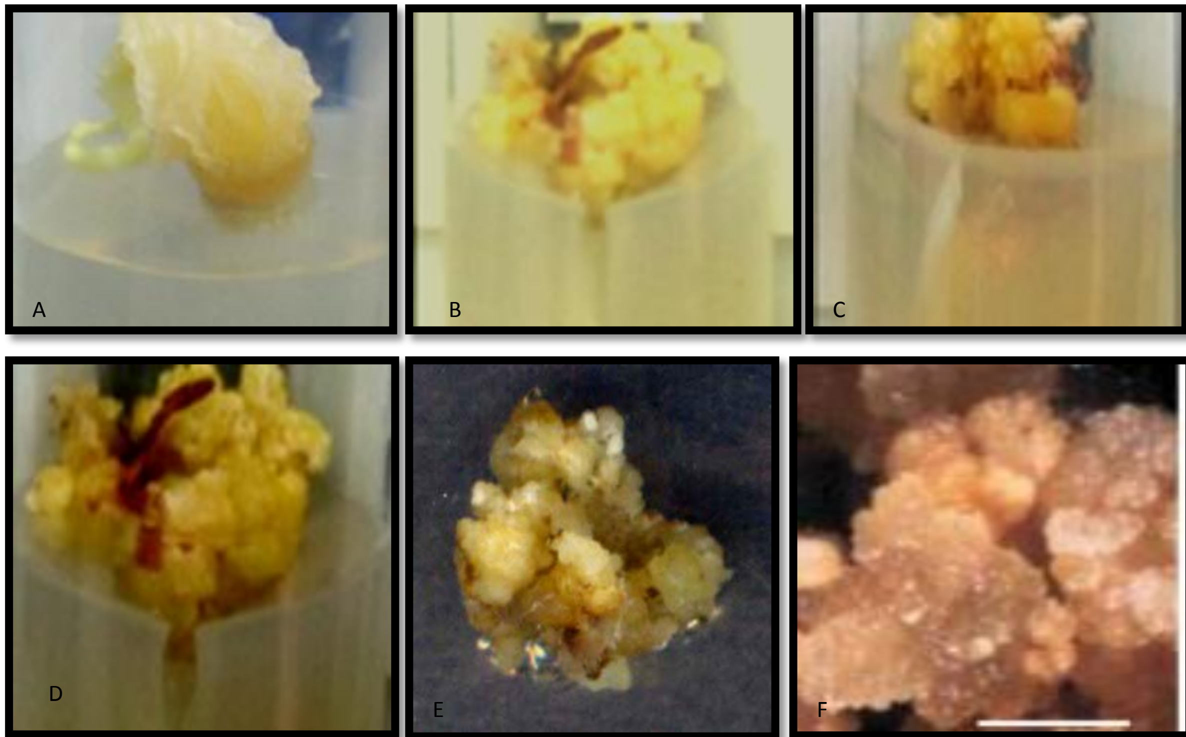
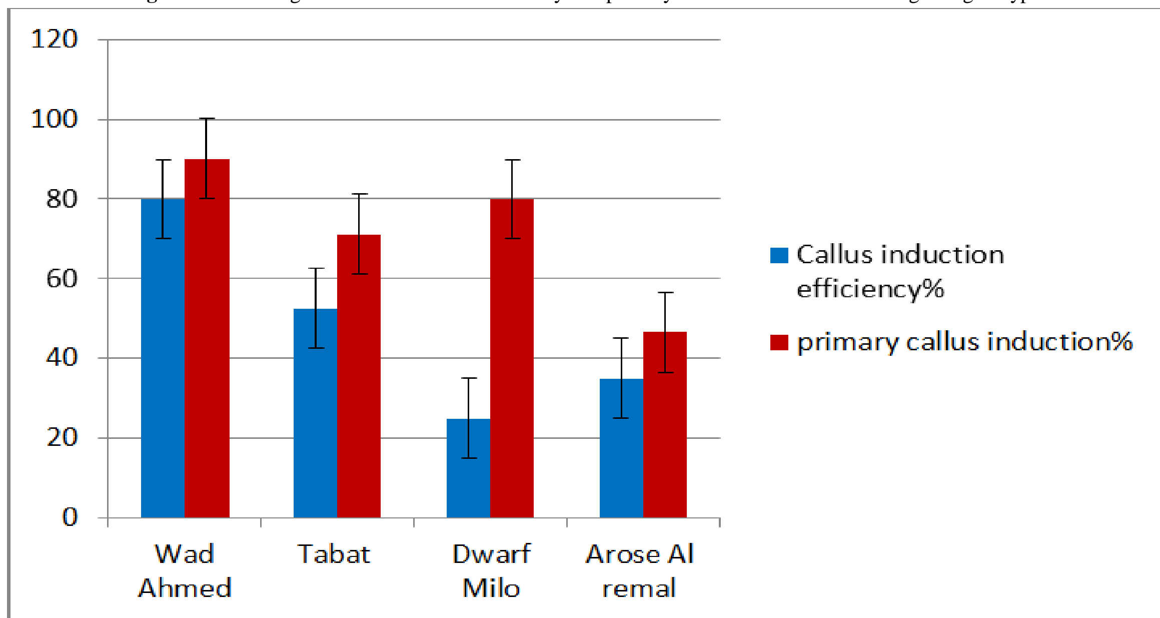


Table-1. Percentage of callus induction efficiency, primary callus induction and morphology of calli from four sorghum genotypes.

Varieties	Callus induction efficiency%	primary callus induction%	Morphology of callus
Wad Ahmed	80	90.1	Yellowish-white, compact , Or Whitish green, compact callus (Embryogenic type)
Tabat	52.5	71.1	Yellowish-white, compact (Embryogenic type).
Dwarf Milo	25	80	Soft, granular appearance and loose textured callus (Non-embryogenic type)
Arose Al- remal	35	46.6	loose texture, watery with dark spots (Non-embryogenic type)

Figure-2. Percentage of callus induction efficiency and primary callus induction in four sorghum genotypes



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