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Regeneration of Sorghum through Tissue Culture Techniques

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Abstract This is the first study in East and Central Africa for optimization tissue culture protocol of sorghum. The effect of hormones on callus induction of sorghum and finally to assess the regeneration response of sorghum through tissue culture techniques. Callus induction and regeneration of four sorghum varieties (Tabat, Elwafer, Yrwasha and Dwarf white - Mylo) was evaluated using mature embryos as source of explants and different concentration of the hormone 2,4-D (0, 1, 2, 4, 6, mg/L) supplemented with 0.2 mg/L Kinetin. The highest callus induction frequency was observed in 2 mg/L for Elwafer and D.W. Milo while 6 mg/L of 2,4-D level gave higher callus induction frequency for Tabat and Yrwasha. The lowest callus induction frequency was observed in 0 and 1 mg/L of 2,4-D level for all the varieties. The highest embryogenic callus induction frequency was observed in Tabat at 4 mg/L and 6 mg/L of 2,4-D while the lowest embryogenic callus induction frequency was observed in 0 and 1mg/L of 2,4-D. Regeneration efficiency was observed higher in the variety Tabat at 4 mg/L of 2,4-D level.

Keywords 2,4,D, Sorghum, Mature embryos, Callus

1. Introduction

Sorghum (*Sorghum bicolor* L.) it is the fifth important cereal crop grown in the world (US Grains Council). In the years 2012/2013, production of sorghum was reported to be 59.3 million metric tons worldwide (USDA Grain: World Markets and Trade, Feb. 2012/2013).

Sorghum used as staple food for over 500 million people in the world, especially in African and Asian countries. Sorghum is rich in antioxidants and it is grain free of gluten and for this reason provides an attractive grain replacement for people suffer from celiac disease. Sorghum is drought-resistant crop addresses global climate change and limited water resources issues, sorghum used as source of food, feed and fiber, is also (Kargi *et al*., 1985; Gnansounou *et al*., 2005; Laopaiboon *et al*., 2007; Rooney *et al*., 2007). There are several reports indicating the tolerance of sorghum to drought and high temperature than corn, wheat and other cereal crops (Gnansounou *et al*., 2005). Sorghum has the unique ability to grow under a wide range of harsh environmental conditions (House, 1995).

Modifications of tissue culture protocols of sorghum have been reported (2010). In these reports, based on callus induction experiments, the highest callus induction reported were 4.5% (Shridhar *et al*., 2010). Transformation of sorghum via *Agrobacterium*-mediated method was reported unsuccessful due to it is recalcitrant for tissue culture and genetic engineering (Shrawat and Lorz, 2006). Regeneration of transgenic sorghum was successfully reported using *Agrobacterium*-mediated transformation (Zhao *et al.*, 2000). This was followed by reports from other researchers using *Agrobacterium*-mediated transformation method (Gurel *et al.*, 2009; Howeet *et al.*, 2006; Lu *et al.*, 2009; Nguyen *et al.*, 2007). There are several factors that affect transformation efficiency, including the sensitivity of sorghum explants to *Agrobacterium* infection, type of explant, composition of the culture media. Addition of coconut water to the co-cultivation medium together with the use of vigorous and actively growing immature embryos as explants for infection and the removal of excess *Agrobacterium* significantly improved the survival rate of explants and were critical for the success of transformation (Hiei *et al.*, 1997).

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2. Material and Methods

Plant materials

Seeds of four sorghum varieties (Tabat, Elwafer, Yrwasha and Dwarf White Milo (D.W. Milo)) were sterilized by first soaking in 70% ethanol for 30 seconds. The seeds were then soaked in 2.5% sodium hypochlorite for one hour before washing with sterile water three times under sterilized conditions. The sterile seeds were used as sources of mature embryos as explants for callus induction.

Callus induction and culture conditions

All callus induction experiments were performed on callus induction medium (CIM). The CIM comprised of MS salts and vitamins supplemented with 0.7 g/l L-Proline, 0.5 g/l MES, 0.5 g/l casein hydrolysate, 0.2 mg/L Kinetin and 3% (w/v) sucrose. The pH of the medium was adjusted to 5.8 with 1M NaOH or 0.1M HCL. 0.65% (w/v) gelrite was added to solidify the medium. CIM was sterilized by autoclaving. Five levels of 2,4-D (0, 1, 2, 4, and 6 mg/l) were tested to establish their efficacy in establishing callus from four different varieties of sorghum via mature embryos.

The Mature embryo explants were obtained from sterile seeds washed in distilled and autoclaved water. Mature embryos were cultured using a sterile forceps on CIM containing different 2,4-D levels (0, 1, 2, 4, 6, mg/L). The embryos were orientated with the embryo axis in contact with the medium. Twenty embryos were cultured in a 90 x 15 mm Petri dish for each 2,4-D level. The cultures were completely covered with aluminium foil and incubated in the dark at 25±1℃ for two weeks. Callus induction frequency was recorded after four weeks of culture on the CIM.

After six weeks of callus induction all calli were transferred to MS medium for 14 days to initiate shoots. For induction of shoots the embryogenic calli were transferred to shoot induction medium (RII) comprising MS basal salts and vitamins (Murashige and Skoog, 1962), supplemented with 30 g/L sucrose, casein hydrolysate and proline. The number of regenerated plantlets per calli was evaluated.

Statistical analysis

The experiments were designed in randomized complete block design (RCBD) with four replications per treatment. Callus induction frequency were calculated on number callus induced from the total number of explants were cultured, these data were used to compute callus induction frequency. Analyses of variance (ANOVA) were done by using statview statistical software to test the statistical significance of differences among explants source and 2,4-D levels. Mean separation was done using least significance difference (LSD) test at 5% probability level.

3. Results and Discussion

Clear differences in callus induction frequencies were observed among the four varieties used in this project, the variety Elwafer gave the lowest callus induction frequency among all the varieties. While the variety Tabat gave the highest callus induction frequency among all the varieties. Callus induction frequency from variety Tabat was highest at 2,4-D concentrations of 4 and 6 mg/L with callus induction frequencies of 85.0 and 93.8 respectively (Table 1). This was significantly higher than at other concentrations but there was no significant difference in callus induction between the concentrations 2 and 4 mg/L (Figure 2C). There was no significant difference in callus induction frequency for all the treatments for the variety Elwafer the concentrations 2 and 4 mg/L gave the same and the highest callus induction (1.250 and 1.250) (Table 1) with no significant differences between them. The variety Yrwasha gave the highest callus induction frequency at concentrations 2, 4 and 6 mg/L of 2,4-D and there were no significant differences in callus induction for those three concentrations but there were significant differences between the other 2,4-D level (Figure 2B). For the variety Dwarf Milo the highest callus induction frequency was obtained at 2,4-D concentration of 2 and 4mg/L with means of 8.750 and 6.250. This was not significant than that of other 2,4-D concentrations but there was significant differences between 0 mg/Land 2 mg/L of 2,4-D (Table 1).

Table 1. Callus induction frequency from mature embryos

2,4-D levels (mg/L)	Tabat	Elwafer	Yrwasha	D.W Milo
0	0.00	0.00	0.00	0.00
1	55.00	0.00	51.25	5.00
2	75.00	1.25	88.75	8.75
4	85.00	1.25	88.75	6.25
6	93.75	0.00	91.25	5.00
LSD		2.383	10. 833	7. 149
P-Value	12.112	0.573	<.0001	0.1803

Mature embryos gave the lowest callus induction frequencies from the variety Elwafer while the highest callus induction from mature embryos was observed from the variety Tabat because Elwafer produced phenolic compound on callus induction medium which affect tissue culture response.

The hormone 2,4-D is important for cereal callus induction and callus formation from mature and immature embryos. Mature embryos from dry seeds are more readily available and can be used as an effective and alternative explants source in tissue culture (Figure 2A).

The variety Tabat gave the highest embryogenic callus induction from mature embryos at 2,4-D concentrations of 4 mg/L and 6 mg/L (Figure 1A and 1B). The varieties Yrwasha and D.W. Milo were producing non embyogenic callus non friable, compact callus which was not able to regenerate (Figure 1C and 1D).

Table 2. Embryogenic callus induction frequency from mature embryos of Tabat

2,4-D levels (mg/L)	R1	R2	R3	R4
0	0	0	0	0
1	0	5	15	5
2	35	25	35	30
4	50	30	40	45
6	30	40	25	35

Table 3. Number of shoot from mature embryos of Tabat

2,4-D levels (mg/L)	R1	R2	R3	R4
0	0	0	0	0
1	0	0	0	0
2	7	0	1	0
4	5	5	5	0
6	0	0	0	4

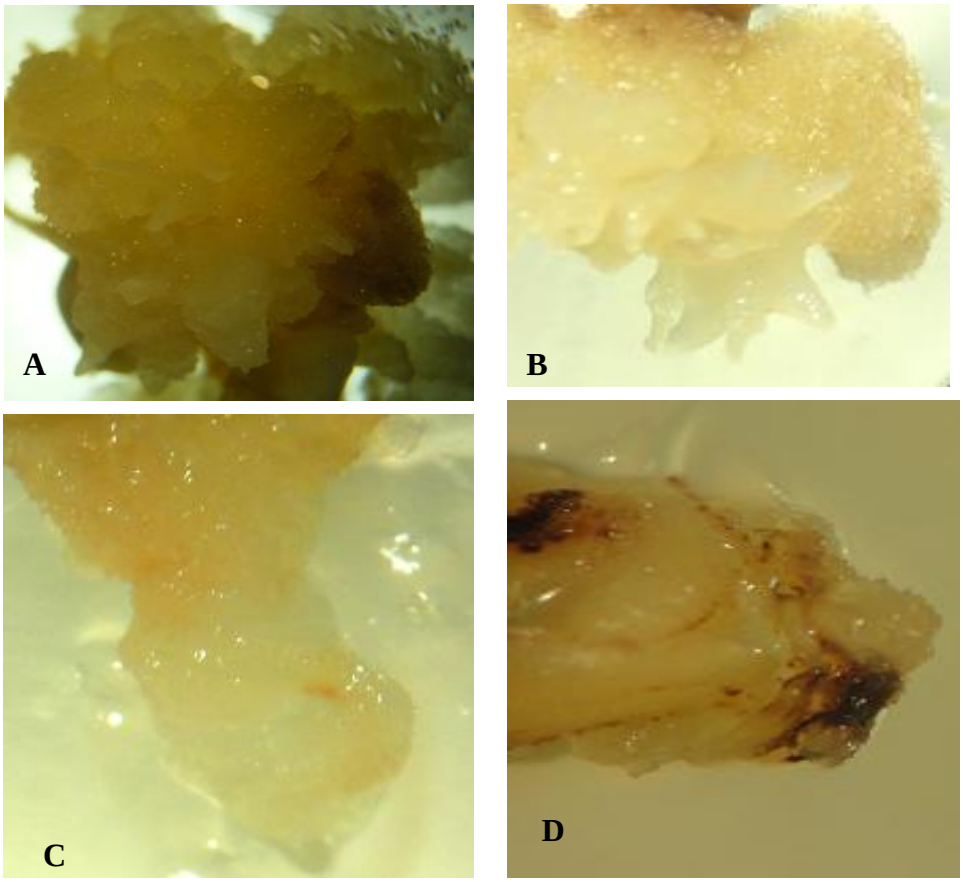

Figure 1. Callus induction from mature embryos of sorghum achieved using 4 mg/l of 2,4,D. A and B: Embryogenic callus induced from the variety Tabat at 4 mg/L2,4-D. C: non embryogenic callus induced from mature embryos of Yrwasha. D: Callus contain phenolic compound induced from mature embryos of Elwafer

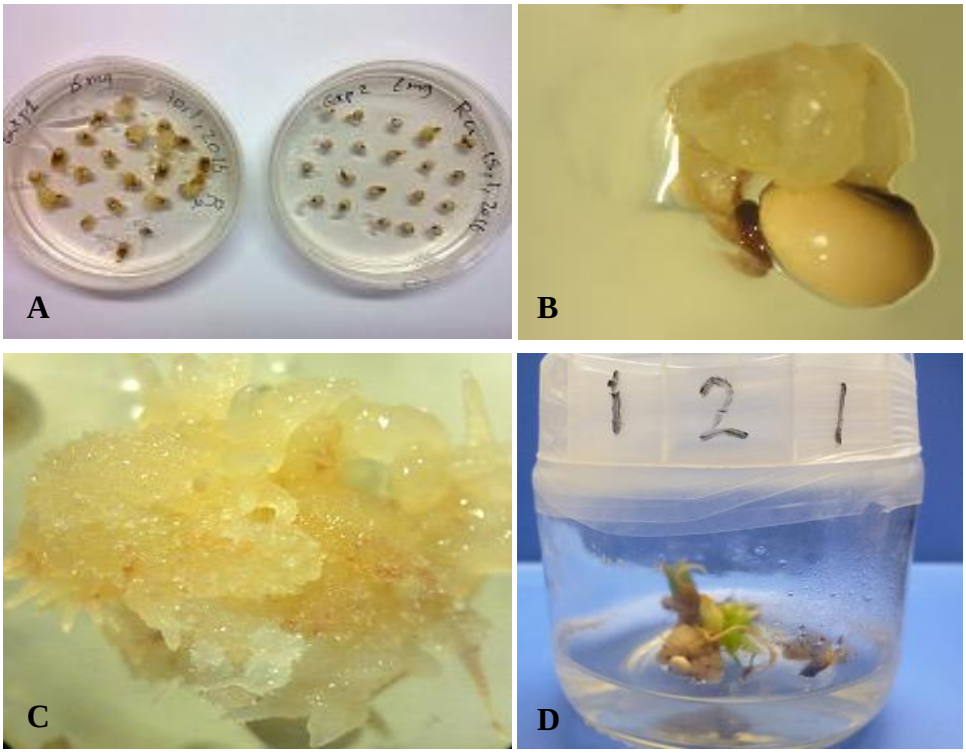

Figure 2. Callus induction and regeneration from mature embryos of sorghum achieved using different concentrations of 2,4,D

The highest shoot induction from mature embryos was observed in the variety Tabat only, the variety Tabat was able to produce higher number of shoots at 4 mg/L of 2,4-D it gave shoots on shoot induction medium (Figure 2D) while the lowest number of shoots was observed at 2 and 6 mg/L of 2,4-D (Table 3). All the other varieties used in this study they were not able to produce shoots. The variety Tababt it is the most promising variety for tissue culture among all the varieties used in this study.

This research focuses on developing protocols for callus induction and regeneration of sorghum through tissue culture techniques. There are many factors that affect the response of crops to tissue culture, including the source of explants, composition of the culture media and size of explants were critical for the success of tissue culture techniques.

Tropical germplasm have been reported to be recalcitrant to tissue culture manipulation and plant regeneration methods, making the regeneration of sorghum a challenging issue. Tissue culture is a popular techniques for improvement of germplasm for tolerance to drought and salinity through somaclonal variation (Matheka *et al*., 2008).

There are several reports indicating that the responses of sweet sorghum to callus induction depending on the concentrations of Auxin used in the callus induction medium (Liming *et al*., 2010) in our experiments we indicated that callus formation was highly stimulated by addition of 4 or 6 mg/L of 2,4-D for all the four verities. The auxin 2,4-D is important for callus induction and embryogenic callus induction from mature and immature embryos in monocotyledon plants Rakshit 2010 and Jogeswar *et al*., 2007). The output of this research has indicated that the presence of 2,4-D could induce callus formation from seeds. However, high concentrations of 2,4-D made callus subculture easy and reduce regeneration frequency for the other three varieties (Mendoza and Kaeppler, 2002).

Mature embryos are ready and available all the season and for this reasons can be used as an effective and alternative source of explants for tissue culture of crops (Jia *et al*., 2008). The varieties Yrwasha and Elwafer induced callus which become watery and non-embryogenic it is reported that callus induction and regeneration of cereal crops is genotypic dependent (Armstrong and Green, 1985). Also there was report indicated that the addition of proline promoted the embryogenic callus production and enhancement of plant regeneration during culture of maize and sorghum (Rao *et al*., 1995) and for this reason we used proline in our tissue culture medium. Induction of callus from mature embryos of Tabat and Yrwasha increased with increasing the concentrations of 2,4-D in the range of 4 and 6 mg/L with addition of 0.2 mg/L Kinetin, the induction percentage of embryogenic callus has negative effect of KT on callus culture was noted in sorghum and wheat (Lazar *et al*., 1983).

In conclusion, in this research we have optimized a protocol for tissue culture and regeneration from mature embryos of sorghum. This report shows that it possible to improve tissue culture conditions by optimizing the compositions of callus induction and plant regeneration media for specific varieties, because of reproducibility and the easy accessibility of mature seeds, the optimization of tissue culture protocols of sorghum provides foundation for genetic transformation of for improving important traits.

4. Conclusions

This work has formed the base for transformation of sorghum there for transformation can be achieved because we have the infrastructure and protocols for sorghum at Agricultural Research Corporation of Sudan.

The variety Tabat it is the most promising sorghum genotype and shall be used for tissue culture and transformation of sorghum.

Tissue culture research has been optimized in sorghum there for protocols for tissue culture of millet and finger millet can be establish for enhancement of cereal crops.

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Appendices

Appendix 1. Analysis of varieance for callus induction from mature embryos for the varieties Tabat, Elwafer, Yrwasha and D.W Milo

Sources of variation	Tabat	Elwafer	Yrwasha	D.W Milo
Degrees of freedom	4	4	4	4
Mean Squares	5598.75	1.875	6226.25	40.625
F- value	86.690	0.750	120.508	1.806
Residual	64.583	2.500	51.667	22.500
P- value	<0.001	0.573	<0.001	0.180

REFERENCES

[1] Armstrong, C.L. and Green, C.E. (1985). Establishment and maintenance of friable, embryogenic maize callus and the involvement of L-proline. Planta. 164(2): 207-214.

[2] Gnansounou, E., Dauriat, A., Wyman, C.E. (2005). Refining sweet sorghum to ethanol and sugar: Economic trade-offs in the context of north china. Bioresour. Technology 96: 985-1002.

[3] Gurel. S., Gurel. E., Kaur. R., Wong. J., Meng. L., Tan. H.Q. and Lemaux. P.G. (2009). Efficient, reproducible Agrobacterium-mediated transformation of sorghum using heat treatment of immature embryos. Plant Cell Report 28: 429-444.

[4] Hiei, Y., Komari, T. and Kubo, T. (1997). Transformation of rice mediated by Agrobacterium tumefaciens. Plant

Molecular Biology 35: 205-218.

[5] House, L.R. (1995). Sorghum: One of the world's great cereals. African Crop Science 3: 135-142.

[6] Jia, X., J. Zhang, H. Wang and W. Kong. (2008). Efficient maize *(Zea mays L.)* regeneration derived from mature embryos in vitro. Maydica 53: 239-248.

[7] Jogeswar, G., Ranadheer, D., Anjaiah, V., Kishor, P.B.K. (2007). High frequency somatic embryogenesis and regeneration in different genotypes of *Sorghum bicolor* (L.) Moench from immature inflorescence explants. *In Vitro* Cell Development Biology Plant 43: 159-166.

[8] Kargi, F., Curme, J.A., Sheehan, J.J. (1985). Solid-state fermentation of sweet sorghum to ethanol. *Biotechnol. Bioeng*. 27: 34-40.

[9] Laopaiboon, L., Thanonkeo, P., Jaisil, P. and Laopaiboon, P. (2007). Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. World J. Microbiol. Biotechnol. 23: 1497-1501.

[10] Lazar, M,, Collins, G. and Vian, W. (1983). Genetic and environmental effects on the growth and differentiation of wheat somatic cell cultures. J. Hered. 74: p.353.

[11] Liming Z.Shujun L and Songquan S (2010). Optimization of callus induction and plant regeneration from germinating seeds of sweet sorghum (*Sorghum bicolor* Moench). African Journal of Biotechnology 9(16): 2367-2374.

[12] Lu. L., Wu. X., Yin. X., Morrand. J., Chen. X., Folk. W.R. and Zhang. Z.J. (2009). Development of marker-free transgenic Sorghum [*Sorghum Bicolor* (L.) Moench] using standard binary vectors with bar as a selectable marker. Plant Cell Tissue Organ Culture 99: 97-108.

[13] Mendoza MG, Kaeppler HF (2002). Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of wheat (*Triticum aestivum* L.). *In Vitro* Cell Development Biology Plant 38: 39-45.

[14] Nguyen. T.V., Thu. T.T., Claeys. M. and Angenon. G. (2007). *Agrobacterium* mediated transformation of sorghum [*Sorghum Bicolor* (L.) Moench] using an improved in vitro regeneration system. Plant Cell Tissue Organ Culture 91: 155-164.

[15] Rakshit, S., R. Zerka, J. Sekhar, T. Fatma and D. Sain, 2010. Callus induction and whole plant regeneration in elite maize (*Zea mays L*.) inbred. Plant cell tissue organ culture 100: 31-37.

[16] Rao AM, Sree KP, Kishor PBK (1995). Enhanced plant regeneration in grain and sweet sorghum by asparagine, proline and cefotaxime. Plant Cell Report 15: 72-75.

[17] Rooney, W.L., Blumenthal, J., Bean, B. and Mullet, J.E. (2007). Designing sorghum as a dedicated bioenergy feedstock. Biofuels, Bioproducts and Biorefining 1: 147-157.

[18] Shrawat, A.K. and Lörz, H. (2006). *Agrobacterium*-mediated transformation of cereals: a promising approach crossing barriers. Plant Biotechnol. J. 4(6): 575-604.

[19] Shridhar, J. Bhat, R. S. Bhat, S. and Kuruvinashetti, M. S. (2010). *Agrobacterium* mediated transformation studies in sorghum using an improved gfp reporter gene. Journal statistic Agriculture Research 8: 1-5.

[20] Zhao, Z. Y., Cai, T., Tagliani, L., Miller, M., Wang, N., Pang, H., Rudert, M., Schroeder, S., Hondred, D., Seltzer, J. and Pierce, D. (2000). Agrobacterium mediated sorghum transformation. Plant Molecular Biology 44: 789-798.