J. of Plant Production, Mansoura Univ., Vol. 15 (7): 379 – 381, 2024

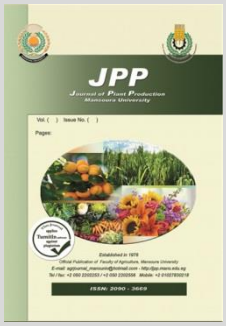
Journal of Plant Production

Journal homepage & Available online at: www.jpp.journals.ekb.eg

Shoot Apices: Promising Explants for the Regeneration and Transformation of Egyptian *Sorghum bicolor* Lines throughout the Year

Zamzam, M.[1*] and Shireen K. Assem[2]

[1] Branch of Genetics, Department of Agricultural Botany, Faculty of Agriculture at Cairo, Al-Azhar University, Egypt.
[2] Department of Plant Molecular Biology, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.



ABSTRACT

Immature embryos and immature inflorescences represent the best explants for indirect sorghum regeneration. However, obtaining these explants from field or greenhouse-grown plants requires a lengthy cultivation period. Therefore, shoot apices from seedlings offer a great advantage for readily obtaining explants to sustain the demands for gene transformation experiments throughout the year. Here we report a rapid regeneration protocol from seedling shoot apices for two Egyptian sorghum lines; LG1 and LG3. Callus induction media, CIM1 and CIM2, which differ in the concentration of the synthetic auxin 2,4-Dichlorophenoxyacetic acid (2,4-D) and Kinetin (Kin), were indifferent in their capacity to promote callus formation in the two genotypes, however, the response of the two genotypes to callus induction was significantly different. The lowest callus indication percentage and the highest callus induction percentage were 16.60% and 33.65% for LG3 on CIM1 and LG1 on CIM2, respectively. The difference in the regeneration from callus for the two genotypes was non-significant and was as low as 11.29% and as high as 20.15%. Our results show the potential of utilizing these Egyptian sorghum lines in tissue culture for transgenic and gene editing.

***Keywords*:** *Sorghum bicolor*, callus induction, regeneration, growth regulators, shoot tip explants

INTRODUCTION

Sorghum is the world's fifth most important cereal crop (Hao *et al*., 2021; Ndlovu *et al*., 2021). Typically, sorghum is grown in Africa, Asia, South America, and the United States in areas with high temperatures and low rainfall as food, fodder, fuel, beverage, and starch for industrial purposes (Hossain *et al*., 2022). In the era of genomics and genome editing, genetic improvement and functional genomics in sorghum are yet lagging behind other cereal crops (Hao *et al*., 2021; Lee *et al*., 2023), and this is majorly attributed to its recalcitrance to plant regeneration and thereby to genetic transformation (Zamzam, 2014; Hao *et al*., 2021). Therefore, improving sorghum regeneration *via* indirect somatic embryogenesis and organogenesis is essential to facilitate the genetic transformation. Sorghum regeneration has been achieved from various types of explants: immature embryos, immature inflorescence, mature embryos, anther, leaves, and shoot apices (Zhong *et al*., 1998; Maheswari *et al*., 2006; Zhao *et al*., 2010; Assem *et al*., 2014; Wu *et al*., 2024). Among these different explants, either immature embryos or inflorescences are more commonly used for transgenesis and genome editing (Liu *et al*., 2019; Chou *et al*., 2020; Che *et al*., 2022, Li *et al*., 2024), largely due to their increased regeneration frequencies. However, procuring the immature embryos and the immature inflorescences requires a lengthy cultivation period and is restricted to the season of sorghum cultivation. These difficulties in obtaining appropriate explants have constrained sorghum tissue culture and stable transformation and thereby genome editing in sorghum has fallen behind other cereals (Lee *et al*., 2023). Therefore, seedling shoot apices offer a great advantage for readily procuring explants to sustain the demands for genetic transformation experiments throughout the year. However, this necessitates continuous efforts to improve the frequency of indirect sorghum regeneration from shoot apices to increase the frequency of regenerating transformed plantlets. Accordingly, the current study investigates the capacity of callus induction and plantlet regeneration from shoot apices of two Egyptian sorghum genotypes.

MATERIALS AND METHODS

Mature grains of two Egyptian *Sorghum bicolor* local germplasms (LG): LG1 and LG3 were used. Mature grains were surface sterilized using 50% commercial Clorox (5.25% Sodium hypochlorite) supplemented with 2-3 drops of Tween 20 for 30 minutes. Following that, grains were rinsed five times with sterile double distilled water and were aseptically germinated on seed germination media (GM) in Mjenta Boxes. After 48 hours of germination, 3 mm sections of shoot apices constituting the apex and portion of the mesocotyl were carefully dissected from the germinating seedlings. A vertical slit or cut was made at the base of each dissected apex to enhance callus initiation from the wounded tissue. These explants were then cultured on Callus Induction Media (CIM) in the dark at 25 °C. Two media, CIM1 and CIM2 were tested for callus induction. Subcultures were carried out at every 13d. interval and at the time of each transfer, leaves, coleoptiles, and elongated shoots were removed from the explant to expose the shoot apices.

Calli developed on the two callus induction media were transferred to the regeneration media designated as Shooting Media (SM) after 30-35 days. Calli on regeneration media were kept at 25 °C under 16/8 h. light/darkness regime. Calli-derived shoots were transferred onto rooting media designated as

* **Corresponding author.**
E-mail address: mohamedzamzam@azhar.edu.eg
DOI: 10.21608/jpp.2024.298384.1347

Rooting Media (RM) in Majenta boxes. Subsequently, plantlets were acclimatized as described in Assem *et al.* (2014).

Table 1. The composition of culture media in the current investigation

Components	GM	CIM1	CIM2	SM	RM
MS	2.2 g/l	4.4 g/l	4.4g/l	4.4g/l	2.2 g/l
2,4-D	…….	0.5 mg/l	2 mg/l	…….	…….
Kin	…….	0.5 mg/l	0.1 mg/l	1.5 mg/l	…….
NAA	…….	…….	…….	…….	1 mg/l
Sucrose	10 g/l	30 g/l	30 g/l	30 g/l	20 g/l
PH	5.8	5.8	5.8	5.8	5.8
Phytagel	3 g/l	3 g/l	3 g/l	3 g/l	3 g/l

Statistical analysis

For callus induction, the means were derived from two independent experiments with an average number of ~50 explants (excluding the contaminants) per experiment for each genotype-media combination. For shoot regeneration, the frequency was calculated as a number of regenerated calli/number of embryogenic calli * 100. The means were derived from 4 independent experiments with an average number of ~12 embryogenic calli (excluding the contaminants) per experiment for each genotype. The statistical difference between the means was evaluated by the student t-test using GraphPad Prism Version 9.0.0.

RESULTS AND DISCUSSION

Sorghum bicolor is grouped on the basis of plant height into short (3-6 feet) and tall verities (6-12 feet) where the short verities are largely grain type and the tall verities largely silage type (Avci, 2019). In the current study, we investigated the capacity of callus induction and regeneration from shoot apices for two tall Egyptian sorghum lines; LG1 and LG3. Two callus induction media that differs in the concentration of the synthetic auxin 2,4-D and Kin were utilized. Callus initiation from both genotypes was observed typically 10-15 days after inoculation. Two types of embryogenic calli; translucent and compact white calli were observed (Figure 1 A and C). The occurrence of the translucent calli was prevalent. Importantly, both calli were capable of regeneration (Figure 1 B and D). The regenerated shoots developed roots within two weeks of transferring to the rooting medium, RM (Figure 1E). The regenerated plantlets were acclimatized as described in Assem *et al*. (2014) and the mature plants were normal (Figure 1F) and fertile.

Our results indicated that the response of LG1 and LG3 to callus induction was significantly different (Figure 1G), indicating that callus induction from shoot apices in sorghum is genotype dependent. Importantly, the frequency of callus induction from either genotype was not significantly altered on the two callus induction media (Figure 1G). The lowest callus indication percentage and the highest callus induction percentage were 16.60% and 33.65% for LG3 on CIM1 and LG1 on CIM2, respectively. The difference in the regeneration from callus for the two genotypes was non-significant and was as low as 11.29% and as high as 20.15% (Figure 1H). This suggests that differences in the overall regeneration capacity from shoot apices among different genotypes may be attributed to initial differences in the capacity of embryogenic callus induction. Therefore, highlights the requirements for optimizing the initial stage of callus induction

Sorghum is one of the most recalcitrant plant species for regeneration and transformation (Zhao *et al.*, 2010). This recalcitrance is attributed to various factors such as genotype dependence, secretion of phenolics, and low frequency and prolonged phase of somatic embryo regeneration into plantlets (Jogeswar *et al*., 2007; Raghuwanshi and Birch, 2010). Although sorghum regeneration has been rereported from various explants, immature embryos and immature inflorescences are more commonly used for genetic transformation due to their efficient regeneration (Zhao *et al*., 210; Assem *et al*., 2014; Assem *et al*., 2017a,b; Lee *et al*., 2023; Wu *et al*., 2024). However, obtaining these explants is seasonally limited and requires a lengthy cultivation period. Therefore, seedling shoot apices offer a great advantage for readily obtaining explants to sustain the demands for gene transformation experiments throughout the year. This however prerequisite for the development of efficient regeneration systems for different genotypes.

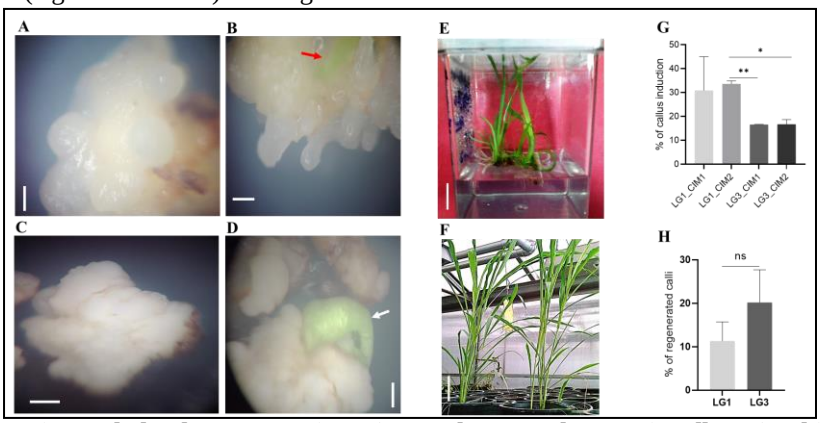


Figure 1. Callus formation and plantlet regeneration. A) Translucent embryogenic callus. B) White compact callus. C) Translucent callus on the regeneration media showing green foci developing into shoots (red arrow). D) White compact callus on regeneration media. The white arrow points at the regenerated shoot. E) Regenerated plantlets on rooting media. F) Regenerated plants grown in green house facility. G) Bar graph depicting the percentage of callus induction for the two lines; LG1 and LG3 on the two callus induction media; CIM1, CIM2. H) Bar graph showing the percentage of callus regenerated into shoots. Scale bars equal to 0.5 mm in panels A-C, 1 cm in panel E and 25 cm in panel F. One and two asterisks denote significant difference at Pvalue < 0.05 and Pvalue < 0.01, respectively.

On comparison of callus induction and shoot regeneration from immature embryos (Assem *et al*., 2014) and from shoot apices in this study, the LG3 displayed nearly twice as much increase in callus induction from immature embryos. However, the reduced callusogenesis from shoot apices may be compensated by the possibility of performing

frequent experiments throughout the off-season months. Together, our results demonstrate the regeneration capacity of two Egyptian sorghum lines from shoot apices and recommend their utilization in tissue culture for transgenic and gene editing when more competent explants, immature embryos and immature inflorescences, are unavailable.

CONCLUSION

Two Egyptian *Sorghum bicolor* lines, LG1 and LG3, produce embryogenic callus and are capable of regeneration from shoot apices. Therefore, these lines are valuable for conducting transformation experiments throughout the year and may accelerate the genome editing and the genetic improvement of the Egyptian sorghum lines.

ACKNOWLEDGEMENT

This work was funded by the Science and Technology Development Fund (STDF), EGYPT. Grant No. 983. The authors wish to thank the anonymous reviewers for their feedback to improve our manuscript.

REFERENCES

Assem, S. K., Zamzam, M. M., Hussein, B. A. & Hussein, E. H. A. Evaluation of somatic embryogenesis and plant regeneration in tissue culture of ten sorghum (*Sorghum bicolor* L.) genotypes. *African J. Biotechnol.* 13, 3672–3681 (2014).

Assem, S. K., Zamzam, M. M., Saad, M. E., Hussein, B. A. & Hussein, E. H. A. The impact of over-expression of NPK1 gene on growth and yield of sorghum under drought stress. *African J. Biotechnol.* 16, 2267–2277 (2017b).

Assem, S.K., Zamzam, M.M., Hussein, B.A. & Hussein, E.H.A. Optimization of Agrobacterium-mediated transformation in Sorghum bicolor. In Twelfth International Dryland Development Conference,' Sustainable Development of Drylands in the Post 2015 World', Alexandria, Egypt, 21-24 August 2016 (pp. 165-175). International Dryland Development Commission (IDDC). (2017a).

Avci, S. Development of an efficient regeneration system via somatic embryogenesis obtained from mature embryos in some grain and silage sorghum cultivars. *Appl. Ecol. Environ. Res.* 17, 1349–1357 (2019).

Che, P. *et al.* Wuschel2 enables highly efficient CRISPR/Cas-targeted genome editing during rapid de novo shoot regeneration in sorghum. *Commun. Biol. 2022 51* 5, 1–11 (2022).

Chou, J., Huang, J. & Huang, Y. Simple and efficient genetic transformation of sorghum using immature inflorescences. *Acta Physiol. Plant.* 42, 1–8 (2020).

Hao, H. *et al.* Sorghum breeding in the genomic era: opportunities and challenges. *Theor. Appl. Genet. 2021 1347* 134, 1899–1924 (2021).

Hossain, M. S., Islam, M. N., Rahman, M. M., Mostofa, M. G. & Khan, M. A. R. Sorghum: A prospective crop for climatic vulnerability, food and nutritional security. *J. Agric. Food Res.* 8, 100300 (2022).

Jogeswar, G., Ranadheer, D., Anjaiah, V. & Kavi Kishor, P. B. High frequency somatic embryogenesis and regeneration in different genotypes of *Sorghum bicolor* (L.) Moench from immature inflorescence explants. *Vitr. Cell. Dev. Biol. - Plant* 43, 159–166 (2007).

Lee, J. S., Bae, S. J., Kim, J. S., Kim, C. & Kang, B. C. A streamlined guide RNA screening system for genome editing in *Sorghum bicolor*. *Plant Methods* 19, 1–11 (2023).

Li, J. *et al.* A rapid and highly efficient sorghum transformation strategy using GRF4-GIF1/ternary vector system. *Plant J.* 117, 1604–1613 (2024).

Liu, G., Li, J. & Godwin, I. D. Genome editing by CRISPR/ Cas9 in sorghum through biolistic bombardment. *Methods Mol. Biol.* 1931, 169–183 (2019).

Maheswari, M. *et al.* Efficient plant regeneration from shoot apices of sorghum. *Biol. Plant.* 50, 741–744 (2006).

Ndlovu, E., van Staden, J. & Maphosa, M. Morpho-physiological effects of moisture, heat and combined stresses on *Sorghum bicolor* [Moench (L.)] and its acclimation mechanisms. *Plant Stress* 2, 100018 (2021).

Raghuwanshi, A. & Birch, R. G. Genetic transformation of sweet sorghum. *Plant Cell Rep.* 29, 997–1005 (2010).

Wu, H. *et al.* Somatic embryogenesis from mature sorghum seeds: An underutilized genome editing recipient system. *Heliyon* 10, e23638 (2024).

Zamzam M. M. (2014). Genetic Improvement of Some Sorghum Lines via Genetic Engineering Approaches. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt. 170p

Zhao L., Liu S., Song S. Optimization of callus induction and plant regeneration from germinating seeds of sweet sorghum (*Sorghum bicolor* Moench). *African Journal of Biotechnology* 9, 2367-2374 (2010).

Zhong, H., Wang, W., Sticklen, M. In vitro morphogenesis of *Sorghum bicolor* (L.) Moench: efficient plant regeneration from shoot apices. *Journal of Plant Physiology* 153, 719–726 (1998).

القمم النامية للساق: أنسجة نباتية واعدة لإنتاج نباتات كاملة من خلال زراعة الأنسجة بهدف النقل الوراثي للجينات في نباتات الذرة الرفيعة المصرية على مدار العام

محمد زمزم[1] وشيرين كمال عاصم[2]

[1] فرع الوراثة- قسم النبات الزراعي- كلية الزراعة بالقاهرة- جامعة الأزهر - مصر

[2] قسم البيولوجيا الجزيئية النباتية- معهد بحوث الهندسة الوراثية الزراعية- مركز البحوث الزراعية- الجيزة- مصر

الملخص

تعتبر الأجنة والنورات الزهرية غير الناضجة من الأجزاء النباتية (explants) الممتازة لإعادة التكشف(regeneration) الغير مباشر للذرة الرفيعة. لكن الحصول على هذه الأجزاء من النباتات المزروعة في الحقول يتطلب فترة زراعة طويلة ويمكن فقط خلال شهور موسم الزراعة الصيفي. لذلك توفر القمم النامية للساق ميزة كبيرة وذلك لإمكانية الحصول عليها بسهولة من الشتلات المستزرعة في المعمل وهذا يوفر امكانية اجراء تجارب نقل الجينات (transformation) على مدار العام. في هذه الدراسة نقدم بروتوكول لأعادة التكشف(regeneration) الغير مباشر من القمم النامية للساق لصنفين مصريين من الذرة الرفيعة هما LG1 وLG3. تم تجربة بيئتي نمو (media) لإنتاج الكلس (Callus) هما CIM1 وCIM2، تختلف بيئتي النمو في تركيز منظمات النمو- الأوكسين الاصطناعي (synthetic auxin) 2,4-D والكينيتين (kin) . استجابة الطرازين الوراثيين لاستحداث الكالس كانت مختلفة معنويا لكن لم يكن هناك اختلاف معنوي لتأثير بيئتي النمو على استحداث الكلس. ظهرت أقل نسبة استحداث وأعلى نسبة استحداث للكالس 16.60% و33.65% لـ LG3 على CIM1 وLG1 على CIM2، على التوالي. لم يظهر فرقا معنويا في أعادة تكشف النباتات من الكالس (regeneration) بين التركيبين الوراثيين محل الدراسة. تظهر نتائجنا إمكانية استخدام اصناف الذرة الرفيعة المصرية في زراعة الأنسجة لغرض النقل الجيني وتعديل الجينات.