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# The Effect of Some Plant Growth Regulators on Callus Culture of Different Pistachio Varieties

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## HIGHLIGHTS

- Callus culture is an important method used for *in vitro* secondary metabolite production.
- Pistachio is a plant of high commercial importance that should be evaluated separately in terms of both micropropagation and secondary metabolites.
- It is critical to determine appropriate plant growth regulators in Callus Culture and micropropagation studies.

**Abstract**

Pistachio (*Pistacia vera* L) is one of the oldest cultivated plants in the world. Its fruits are rich in protein, minerals, carbohydrates, fiber, and vitamins. In addition, the demand for these plants is increasing due to the fact that they are very tasty and nutritious. On the other hand, pistachio cultivation is quite difficult. In addition, many problems are encountered in germination with seeds or reproduction with cuttings. These situations necessitate the development of different *in vitro* tissue culture protocols. In this study, callus culture optimization protocol was developed by using seeds of three different pistachio cultivars. Murashige and Skoog (MS) medium was supplemented with different concentrations of NAA, IAA, 2,4 D and BAP. When callus size (1,776 cm), callus weight (0.908 g) and embryogenic callus regenerations (27.94%) were considered, it was found that the best variety was Tekin. Again, in the evaluation made according to these factors, it was determined that the best improvement was in the MS medium containing 3 mg/L BAP and 1 mg/L 2,4D. The contamination rate detected throughout the studies ranged from 7.65% to 12.91%.

**Keywords:** Pistachioi; Callus culture; *Pistacia vera*; Plant Growth Regulators (PGRs); Secondary metabolites

## 1. Introduction

Known as *Pistacia vera* L., pistachio is an important member of the Anacardiacea family and is included in the genus Pistacia. There are 11 species belonging to the genus Pistacia and the only edible species is pistachio (Ferguson et al. 2005). It has also been reported that the genus Pistacia includes 20 species, including evergreen or deciduous species, resin-bearing shrubs, and xerophytic trees growing to a height of 5 to 15 m (Rauf et al. 2017; Bozorgi et al. 2013).

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The outer shell, which has a hard and whitish color, constitutes 50% of its weight. The seed has a thin rind and light green flesh color with a distinctive flavor. It has been reported that together with almonds, pistachios have a very important place in human consumption and even like fig and pomegranate fruits, which are known as objects of faith, pistachios are also mentioned in the Bible (Holland et al. 1992; Ergun and Bozkurt 2020; Mandalari et al. 2021; Bozkurt and Ergun 2021; Dreher 2012).

*P. vera* L. is resistant to harsh environmental conditions and is resistant to both heat and cold. Due to this feature, it grows both in semi-arid deserts and on dry slopes of low mountains and hills (Mir-Makhamad et al. 2022). It has been reported to be native to Northeast Iran, Southern Turkmenistan, and Afghanistan, and it is mentioned that there are still wild pistachio forests there. In addition, it has been reported that the commercial production of pistachios has spread far from its Eurasian origin to southwestern USA and southwestern Australia (Khezri et al. 2020). According to the data of FAO (2022), when the production amounts in 2020 are taken into account, the USA constitutes approximately 42% of the production in the whole world. This is followed by Turkey and Iran, respectively (Figure 1).

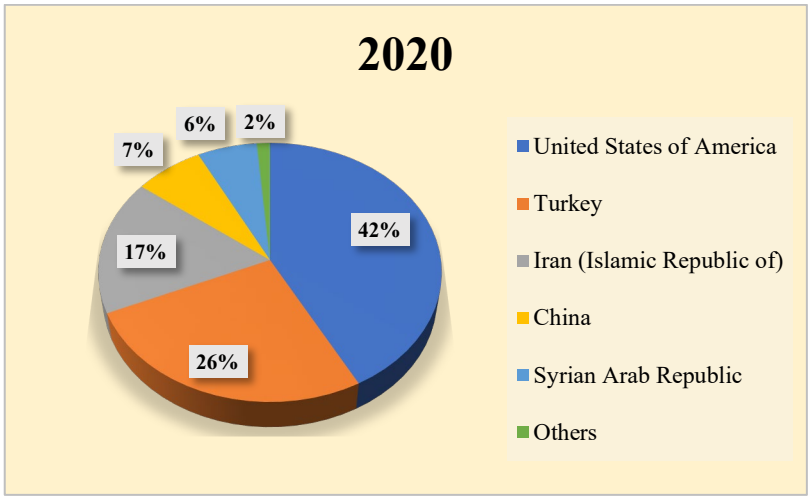


**Figure 1.** Distribution of pistachios production amount (%) in 2020

When the production of pistachios is evaluated, it is reported that it ranks fifth after cashew, walnut, almond and chestnut (Sheikhi et al. 2019). Although *Pistacia vera* L. is the most demanded species and commercially grown species in the Pistacia genus, other species are used as rootstocks for *P. vera* L. In addition, it has been reported that these species are used in the production of snacks or coffee-like beverages, and they are used as food coloring due to their anthocyanin content. It has been reported that the secondary metabolites of different parts of the pistachio plant are used for liver, heart, kidney, and respiratory system disorders (Longo et al. 2007) (Bozorgi 2013).

The production of pistachios is classically carried out by using the seeds by germinating or by using the cuttings by rooting. Propagation by seeds is not preferred since it will reveal genetic expansions as in other plants. On the other hand, rooting of cuttings is a very difficult and time-consuming process (Almehdi 2002; Benmahioul 2017). However, these methods are insufficient to produce the required number of seedlings for pistachio cultivation in the world. With *in vitro* tissue culture methods, it is possible to micropropagate disease-free pistachio rootstocks, thus enabling the production of economically important varieties in desired quantities.

The most important step in the production of plant species such as pistachios, which are very difficult to produce, by tissue culture will be the use of healthy protocols. In the selection of these protocols, many more parameters from the explant source used, the sterilization conditions applied, and the prepared nutrient media should be well evaluated. This study has been designed with the awareness that all kinds of experimental studies of tissue culture studies of pistachio are very important for sustainable production studies of this plant. The aim of this study is to determine the developmental responses of pistachio seed cells to different plant

growth regulators used *in vitro*. In this study, it was observed how the pistachio seed explant gave results to which plant growth regulator in terms of cellular growth rate and callus structure. It is aimed to be a scientific resource in terms of plant growth regulators and sterilization method for *in vitro* scientific studies to be carried out with pistachio from now on. In particular, it was predicted that this study would be beneficial for callus culture studies to be established for secondary metabolite production from pistachio.

## 2. Materials and Methods

*Plant Material*

Seeds of Tekin, Siirt and Pistachio varieties obtained from Pistachio Research Institute/Gaziantep were used as plant material.

*Surface Sterilization*

First of all, pistachio seeds were washed under a running fountain for 5 minutes. The washed seeds were taken into the laminar cabinet and the surface sterilization stage was started. At this stage, the seeds were first kept in 70% alcohol for 1 minute and then washed 4 times in sterile water. Then, it was kept in 20% (v/v) sodium hypochlorite and then cleaned from sodium hypochlorite with sterile distilled water 4 times.

*Cultural Medium and Conditions*

The content of the culture media is planned to form the most efficient callus for the pistachio varieties and their seeds to be used in this study, and the basic composition is MS (Murashige and Skoog 1962) medium. This medium was used as the basic nutrient medium and was supplemented with PGRs at different concentrations, and each was given a code (such as M1, M2, M3). The media combinations of the different PGRs clearly stated in Table 1 were determined by our own preliminary studies and research.

**Table 1.** Different PGRs and their concentrations in the media used in the study.

| | NAA (mg/L) | IAA (mg/L) | 2,4D (mg/L) | BAP (mg/L) |
|---|---|---|---|---|
| M1 | 1 | 1 | 1 | 1 |
| M2 | | | 3 | 1 |
| M3 | | | 1 | 1 |

The pH of the prepared media was adjusted to 5.8 using 1N NaOH and 1N HCl, and agar (8 g/L) was used as the solidifier. The prepared media were autoclaved at 15 psi at 121°C for 20 minutes and distributed in sterile plastic petri dishes. Endosperms were taken as explants (approximately 0.5-0.7 cm) from seeds whose surface sterilization was completed and placed in the solidified media in Petri dishes. Then, the petri dishes were cultured in a culture room with a temperature of 24°C, under 16 hours light - 8 hours dark conditions. Measurements were made every 2 weeks and the results of the observation of the developing calli were noted (Figure 2).

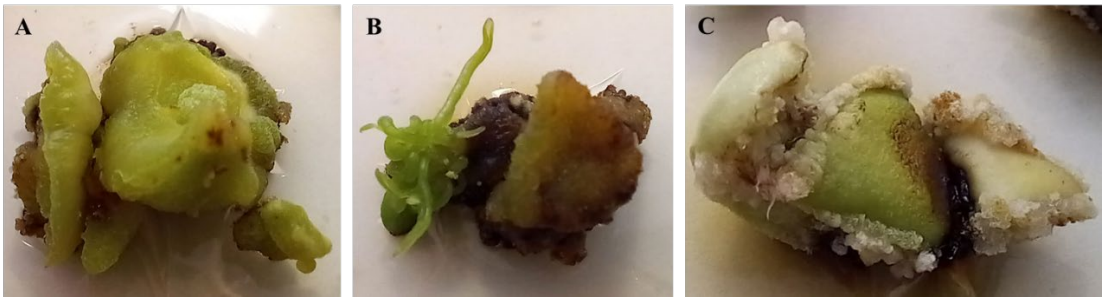


**Figure 2.** Calluses of pistachio varieties grown in different mediums. A: Antep variety growing in M1 medium, B: Tekin variety growing in M1 medium, C: Siirt variety growing in M2 medium.

*Statistical Analysis*

Experimental studies were set up in a randomized plot design with three recurrences. Calluses developed in different environments were evaluated in terms of weight, size, embryogenic status and contaminations developed in the environments. All data calculated as percentages were subjected to arcsine-transformed. The obtained data were analyzed using the SAS-JMP statistical program and the differences between them were compared with the LSD (least significant difference) multiple comparison test.

## 3. Results and Discussion

Considering the callus weights, Tekin (0.908 g) had the highest value, followed by Siirt (0.770 g) and Antep (0.755 g) varieties. There was no statistically significant difference between the varieties of Siirt and Antep. Considering the Plant Nutrient Media, M2 (0.952 g) and M1 (0.868 g) were found to be the highest and M3 (0.613 g) to be the lowest. When the medium and varieties are evaluated together; M2*Tekin (1.136 g) and M1*Tekin (1.104 g) were found to be the highest M1*Siirt (0.502 g) and M3*Tekin (0.484 g) the lowest (Table 2).

Considering the callus size, no statistical difference could be detected between the cultivars, and it was determined that Tekin (1,776 cm), Siirt (1,583 cm) and Antep (1,530 cm) cultivars were ranked from largest to smallest, respectively. When the difference between Plant Nutrient Environments is examined; M2 (1,783 cm) and M1 (1,739 cm) were the highest, while M3 (1,368 cm) was the lowest. When the medium and cultivars are evaluated together, Tekin*M1 (2,050 cm) has the highest value, Siirt*M1 (1,337 cm), Tekin*M3 (1,310 cm) and Antep*M3 (1,225 cm) has the lowest value (Table 2).

**Table 2.** Experimental results of pistachio cultivars (Tekin, Antep, Siirt)

| Varieties | Medium | Weight (gr) | Size (cm) | Embryogenic callus Regeneration rate (%) | Contamination (%) |
|---|---|---|---|---|---|
| Tekin | M1 | 1,104 a | 2,050 a | 20 b (26,335) | 2 b (8,087) |
| | M2 | 1,136 a | 1,970 ab | 40 a (39,147) | 2 b (7,947) |
| | M3 | 0,484 d | 1,310 d | 10 c (18,344) | 2 b (7,655) |
| Antep | M1 | 0,999 ab | 1,830 abc | 5 cde (12,837) | 4 a (11,477) |
| | M2 | 0,755 c | 1,537 cd | 10 c (18,303) | 4 a (11,522) |
| | M3 | 0,512 d | 1,225 d | 3 de (9,915) | 4 a (11,399) |
| Siirt | M1 | 0,502 d | 1,337 d | 3 de (9,752) | 5 a (12,910) |
| | M2 | 0,966 abc | 1,843 abc | 7 cd (15,180) | 5 a (12,879) |
| | M3 | 0,843 bc | 1,570 bcd | 2 e (7,655) | 5 a (12,824) |

P<0.05*, P<0.01**, P<0.001***, LSD value was calculated according to the angle conversion value. Different letters (a–e) indicate significant differences according to the LSD test ($p \leq 0.05$). $LSD_{Weight}$: 0.218, $LSD_{Size}$: 0.425, $LSD_{Embryogenic\ callus\ regeneration\ rate}$: 5.904, $LSD_{Contamination}$: 3.180

After evaluating the embryogenic callus regenerations as %, angle transformation was performed and shown in Table 2 in parentheses. Accordingly, when the varieties are evaluated; In terms of percentage, the best regeneration was detected in Tekin variety (27.94%), followed by Antep (13.68%) and Siirt (10.86%). When the Plant Nutrient Environments are evaluated; While M2 had the highest value (24.21%), it was followed by M1 (16.30%) and M3 (11.97%), respectively. When the varieties and mediums are evaluated together, Tekin*M2 (39.14%), Tekin*M1 (26.33%) and Tekin*M3 (18.34%) were the highest, Antep*M3 (9.91%), Siirt*M1 (9.75%) and Siirt*M3 (7.65%) were found to have the lowest rates (Table 2).

In addition to all these studies, observations have been made about contamination, one of the most important issues for *in vitro* tissue culture studies. The angle of the data obtained as a result of these observations was made as angle trasformations and sorted from the highest to the lower according to the varieties (12.87%), Antep (11.46%) and Tekin (7, 89%). When the environments were evaluated, it was found that M1 (10.82%), M2 (10.78%) and M3 (10.62%) respectively. The highest Siirt*M1 (12.91%) and Siirt*M2 (12.87%) were found when evaluated together and the environments were found together, while the lowest Tekin*M2 (7.94%) and Tekin*M1 (7.65%) were found (Table 2).

Although many studies have been carried out in this area, it is striking that each of them gives different results. When some of them are examined;

For standardization of *in vitro* propagation techniques for an endangered palm species Areca concinna (Arecaceae), Veluru et al. (2022), conducted studies. Of the three explants, only somatic embryos were obtained from mature embryos, with the best response from M72 medium containing 25 mg/L 2,4D. In our study, on the other hand, when 2,4D was used with BAP, it gave more successful results. It is clear that it would be wrong to evaluate only on the medium, and the effect of plant varieties on callus formation is also important. Another factor to consider is the diversity of essential nutrient environments. In a study in this context, Nadalizadeh Ghannad et al. (2022) MS and Driver and Kuniyuki Walnut (DKW) investigated the effects of different concentrations of PGRs added to their media on callus induction and regeneration. The highest callus formation (96%) was observed in DKW medium containing 0.5 mg/L NAA and 1 mg/L Kin. On the other hand, the shoot regeneration rate gave the best results (41%-20%) in the same medium containing 1 mg/L BAP + 2 mg/L NAA, although it differed according to the varieties. In addition, significant differences were observed between explants in terms of callus induction and shoot induction.

Khande et al. (2017) investigated different media combinations for callus formation from leaf and shoot explants of Santalum album L. Maximum callus formation was observed in MS + 0.5 mg/L 2,4-D + 0.5 mg/L NAA + 0.5 mg/L BAP medium for both leaf (67%) and shoot (46.67%) explants. Callus weights were determined as 467.67 mg and 159.67 mg in the same environment, respectively. Although the plants and explants are different, callus formation was promoted by the optimization of the environment in our study like this study. Embryogenic callus regeneration, callus weight and size were considered rather than percent callus formation. In such studies, it would be useful to measure the callus size. In support of this idea, researchers who cultured callus on different explants of potato (Haque et al., 2009) included callus weight and callus length in their measurements.

Callus culture studies mostly focus on secondary metabolites and the studies should be supported by instrumental analysis. For example, Aghaei et al. (2013) investigated the effects of different concentrations of PGRs on callus formations of wild pistachio seedling stem explants. In addition, they compared essential oil analyzes in calli. While high callus formation was detected in the medium containing 1 mg/L 6-BAP (85%), the lowest callus formation was detected in the medium containing 2 mg/L BA+1 mg/L NAA. Although the main components of callus were Bornyl acetate (9.18%), Spathulenol (5.89%) and Ledol (5.37%), a total of 8 components were reported. In this study, both explant and PGRs were designed differently. In addition, no analysis of secondary metabolites was performed. Data that will enable the development of healthy calli for secondary metabolite studies were presented at the variety level.

Differently, Ceniza et al. (1992) who studied the *in vitro* callus culture of coconut endosperm examined the development of callus and fatty acids. Callus was developed by supplementing with modified Y-3 medium (Sugimura and Salvana 1989; Branton and Blake 1983), 20 ppm 2.4 D, 1 ppm BA, 1 ppm 2 ip, 0.25% activated carbon, and 0.2% gelrite. It has been determined that while approximately 82% of the total fatty acids are short chain fatty acids, long chain fatty acids are reduced to 16%.

It has been known and investigated for many years that one of the biggest problems encountered in plant tissue culture studies is contamination (Cassells et al. 2000; Leifert et al. 1994). Cobrado and Fernandez (2016) found that they encountered two fungal species as a source of contamination in their study and that they caused the death of the culture material. There have been researchers who mentioned that there are 3-15% losses in tissue culture laboratories because of contamination, and they mentioned the importance of the sterilization protocol (Tiwari et al. 2012; Leifert et al. 1989). In this study, contaminations were observed and calculated as % and it was found to vary between 7.65%-12.91% 2-5%. Predominantly bacterial contaminations were encountered, and no typing was done at the strain level. Observations were shared to provide preliminary information for future studies.

## 4. Conclusion

Pistachio, also known as green gold, is a plant grown in the world and mostly consumed as dried nuts. Pistachio has a very important place in human nutrition thanks to its carbohydrates, proteins, minerals, and vitamins. The reproduction of the pistachio plant, which is widely produced in the world, is mostly done with

seeds and cuttings. Production with these methods is very difficult and it is not possible to create production areas of the required size in a short time. Therefore, *in vitro* tissue culture techniques are preferred as alternative production methods. It is necessary to determine the environment and method according to the rootstock plant to be produced. In addition, it is possible to produce valuable secondary metabolites in plants with rich nutrient content such as pistachios with callus culture, which is one of the *in vitro* tissue culture methods. For this purpose, callus culture study was started for 3 different pistachio cultivars in this study and the effectiveness of MS medium supplemented with different PGRs was examined. It was determined that Tekin cultivar gave better results than Siirt and Antep cultivars in terms of embryogenic callus regeneration, callus weight and callus size. MS medium containing 3 mg/L BAP and 1 mg/L 2,4D was found to be more successful than other medium combinations. Observations were also made for contaminations that cause significant problems and product losses in tissue culture, and the contamination rate was determined as 7.65%-12.91%.

**Author Contributions:** The authors have an equal contribution. All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

Aghaei P, Bahramnejad B, Mozafari AA (2013). Effect of different plant growth regulators on callus induction of stem explants in'*Pistacia atlantica*'subsp. *kurdica*. *Plant Knowledge Journal* 2: 108-112.

Almehdi AA, Parfitt DE, Chan H (2002). Propagation of pistachio rootstock by rooted stem cuttings. *Scientia Horticulturae* 96: 359-363.

Benmahioul B (2017). Factors affecting *in vitro* micropropagation of Pistachio (*Pistacia vera* L.). *Agric For J.* 1: 56-61.

Bozkurt T, Ergun Z (2021). Fatty acid composition and antioxidant capacity of pomegranate seed oil. *GSC Biological and Pharmaceutical Sciences*, 15: 103-110.

Bozorgi M, Memariani Z, Mobli M et al (2013) Five Pistacia species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. Sci World J 2013:219815. https://doi.org/10.1155/2013/219815

Bozorgi M, Memariani Z, Mobli M, Salehi Surmaghi MH, Shams-Ardekani MR, Rahimi R (2013). Five Pistacia species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. *The Scientific World Journal* pp. 1-33.

Branton RL, Blake J (1983). Development of organized structures in callus derived from explants of *Cocos nucifera* L. *Annals of Botany* 52: 673-678.

Cassells AC (1999). Aseptic microhydroponics: a strategy to advance microplant development and improve microplant physiology. In *International Symposium on Methods and Markers for Quality Assurance in Micropropagation* 530 August pp. 187-194.

Ceniza MS, Ueda S, Sugimura Y (1992). *In vitro* culture of coconut endosperm: callus induction and its fatty acids. *Plant cell reports* 11: 546-549.

Cobrado JS, Fernandez AM (2016). Common fungi contamination affecting tissue-cultured abaca (*Musa textiles* Nee) during initial stage of micropropagation. *Asian Research Journal of Agriculture* 1: 1-7.

Dreher ML (2012). Pistachio nuts: composition and potential health benefits. *Nutrition reviews* 70: 234-240.

Ergun Z, Bozkurt, T (2020). Determination of fatty acid composition and antioxidant activity of fig seed oil. *International Journal of Agricultural and Natural Sciences* 13: 101-107.

Ferguson L, Polito V, Kallsen C (2005). The pistachio tree; botany and physiology and factors that affect yield. *Pistachio production manual, 4th ed. Davis, CA, USA, University of California Fruit & Nut Research Information Center* 31-39.

Haque AU, Samad ME, Shapla TL (2009). *In vitro* callus initiation and regeneration of potato. *Bangladesh Journal of Agricultural Research* 34: 449-456.

Holland B, Unwin ID, Buss DH (1992). *Fruit and nuts: First supplement to the fifth edition of McCance and Widdowson's The Composition of Foods* (Vol. 1). Royal Society of Chemistry.

Khande BP, Burondkar MM, Sawardekar SV, Chavan SS, Gokhale NB, Bhave SG, ... & Devmore JP (2017). Effect of media combination on *in vitro* callus induction in sandalwood (*Santalum album* L.). *Adv Agri Res Technol J* 1: 98-105.

Khezri M, Heerema R, Brar G, Ferguson L (2020). Alternate bearing in pistachio (*Pistacia vera* L.): A review. *Trees* 34: 855-868.

Leifert C, Morris CE, Waites WM (1994). Ecology of microbial saprophytes and pathogens in tissue culture and field-grown plants: reasons for contamination problems *in vitro*. *Critical reviews in plant sciences* 13: 139-183.

Leifert C, Waites WM, Nicholas JR (1989). Bacterial contaminants of micropropagated plant cultures. *Journal of Applied Bacteriology* 67: 353-361.

Longo L, Scardino A, Vasapollo G (2007). Identification and quantification of anthocyanins in the berries of *Pistacia lentiscus* L., *Phillyrea latifolia* L. and *Rubia peregrina* L. *Innovative Food Science & Emerging Technologies* 8: 360-364.

Mandalari G, Barreca D, Gervasi T, Roussell MA, Klein B, Feeney MJ, Carughi A (2021). Pistachio Nuts (*Pistacia vera* L.): Production, Nutrients, Bioactives and Novel Health Effects. *Plants* 11: 18.

Mir-Makhamad B, Bjørn R, Stark S, Spengler III RN (2022). Pistachio (*Pistachio vera*) domestication and dispersal out of central Asia. *Agronomy*, 12: 1758.

Nadalizadeh Ghannad A, Marashi H, Seifi A, Moshiri F (2022). Optimization of callus induction and shoot regeneration in leaf and stem of *Pistacia vera* L. and UCB-1 (*P. atlantica* × *P. integerrima*). *Plant Biotechnology Reports* 1-9.

Rauf A, Patel S, Uddin G, Siddiqui BS, Ahmad B, Muhammad N, ... & Hadda TB (2017). Phytochemical, ethnomedicinal uses and pharmacological profile of genus Pistacia. *Biomedicine & Pharmacotherapy* 86: 393-404.

Sheikhi A, Arab MM, Brown PJ, Ferguson L, Akbari M (2019). Pistachio (Pistacia spp.) breeding. In *Advances in plant breeding strategies: nut and beverage crops*. Springer, Cham. pp. 353-400

Sugimura Y, Otsuji K, Ueda S, Okamoto K, Salvana MJ (1987) In : Applewhite TH(ed) Proceedings of world conference on biotechnology for the fats and oils industry. Kraft Inc., Illinois, pp. 51-54

Tiwari S, Arya A, Kumar S (2012). Standardizing sterilization protocol and establishment of callus culture of sugarcane for enhanced plant regeneration *in vitro*. *Research Journal of Botany* 7: 1.

Veluru A, Neema M, Shil S, Prakash K, Kavya K, & Karun A (2022). Standardization of *in vitro* multiplication technique for Areca concinna, an endangered palm species for its conservation and utilization.