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Callus induction through different explants in alphonso mango (*Mangifera indica* L.)

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Abstract
A Laboratory experiment was conducted during year 2022-2023 at Plant Biotechnology Centre, College of Agriculture, Dapoli, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli to callus induction by using different explant and in Alphonso Mango (*Mangifera indica* L.). The four different sources of explant *viz*., Apical buds, Auxiliary bud, Nucellar and leaf were used with MS media. This experiment imposed that the callus induction in mango is depend upon the quantity of 2,4-D and NAA present in the nutrient media. The result revealed that apical bud and axillary bud media combination AM3- (MS+ 2 mgl-1 2, 4 - D + 1 mgl-1 NAA) founded superior. In case of nucellar tissue media combination AM7- (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA) gave the best result. In respect to leaf explant highest success was observed in media combination AM7- (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA) as compare to other three explants.

Keywords: Apical bud, axillary bud, nucellar tissue, leaf, MS medium

1. Introduction
The Alphonso mango, usually referred to as the "Mango King," is a well-liked mango variety that is cherished for its flavour, which is rich, sweet, and aromatic. One of the best mango kinds in the world is thought to be this one. Alfonso de Albuquerque, a Portuguese general and military specialist who founded Portuguese colonies in India, is honoured in the name of Alphonso mangoes. A member of the Anacardiaceae family, the mango (*Mangifera indica* L., 2n = 40) is one of the most ancient and important tropical fruits in history. It is properly referred to as the "King of Fruits" due to its high nutritional value, distinctive flavour, and alluring perfume. It is additionally referred to as India's "National Fruit." It has been cultivated in India for more than 4,000 years and is said to have originated in the Indo-Burma region of South-east Asia (Mukherjee, 1953; Kostermans and Bompard, 1993) [6, 4].
A single mango can meet up to 40% of a person's daily fibre requirements, which is an effective defence against cancer, heart disease and cholesterol buildup. Additionally, this delicious fruit is a storehouse of antioxidants, beta-carotene and potassium. Mangoes are mostly farmed in India in tropical and subtropical areas between sea level and 1,500 m above sea level. Around 27 °C is the ideal temperature for mango growth. In the main production zones, state-of-the-art packhouses have been established to ensure the highest quality requirements. Internationally renowned treatment facilities, such as hot water treatment, vapour heat treatment, and irradiation facilities, have also been put up at various sites along the industrial belt in consideration of the requirements of the various countries (APEDA).
India plays an important role in major export in respect to mango. During the fiscal year 2021–2022, the nation exported 22963.76 MT of fresh mangoes to other countries for a total of Rs. 378.49 crores/48.53 USD million. United Arab Emirates, United Kingdom, United States, Qatar, and Kuwait are major export destinations (2021-22) (APEDA).
Mangoes can also be grown from seeds, though grafting and budding are the preferred techniques for ensuring particular traits in mango trees. It's crucial to remember though, that mango trees grown from seeds do not always have the same traits as the parent tree. It is necessary to remove the seed from a ripe mango, clean it and plant it in a soil mixture that drains properly. A mango tree raised from seed normally takes several years to develop fruit and the resulting fruit may differ in taste, size and quality.

It's important to note that grafting or budding is frequently used in commercial mango production to ensure uniform quality and features throughout the plantation. With the help of these techniques, gardeners may generate appealing mango types with well-known traits and ensure that the trees mature more quickly than trees that are grown from seeds.

Mango plants are propagated via the contemporary biotechnological method known as *in vitro* propagation, commonly referred to as tissue culture from tiny explants of plant tissue. Explants are placed in a sterile and nutrient-rich growing medium under carefully monitored environmental conditions. Rapid multiplication, disease-free plantlets and genetic consistency are just a few benefits of *in vitro* propagation that increase crop yields and preserve favourable features. It is a useful technique for maintaining uncommon cultivars and breeding superior mango variants. However, effective adoption necessitates specialised lab infrastructure, knowledgeable staff and consideration of contamination and genetic stability issues. *In vitro* propagation can be achieved with the right knowledge and skills.

Mango *in vitro* propagation is crucial because it has the ability to revolutionise mango production and solve major problems the industry is now facing. Rapid multiplication, disease-free plantlets, genetic consistency and the preservation of rare variations are just a few benefits of *in vitro* propagation. Researchers can create effective protocols that permit the mass production of healthy mango plants by researching and improving tissue culture techniques.

Enhancing the productivity and sustainability of mango production depends on this research. It may result in the creation of enhanced mango cultivars that have desirable qualities like better fruit quality, disease resistance and adaptation to shifting environmental factors. Additionally, by retaining genetic variation, *in vitro* propagation can aid in the conservation of uncommon and endangered mango cultivars.

By using different explants, we can check the growth percentage of different explants in different medium. Implementing *in vitro* propagation in mango is predicated on the idea that this cutting-edge method will enable quick multiplication of disease-free and fungus-free, genetically uniform mango plants. A little piece of mango tissue can yield multiple plantlets using tissue culture under regulated circumstances. This strategy makes sure that premium mango varieties are propagated and that desirable features are preserved. Micropropagation of mango could be revolution in mango production, which offers effective ways to produce superior mango plants, boost crop yields and improve food security worldwide. Through this research, in future we will able to induce the whole plantlet.

2. Materials and Methods

2.1 Genotype

The present investigation was carried out with Alphonso mango. The experimental material for present investigation was collected from Shirgaon research station, Centre for Excellence of Mango, Dapoli and mother orchard of Dept. of Agriculture Biotechnology Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri.

2.2 Preparation of explant for culture establishment

2.2.1 Leaf: Leaf explants were collected from nursery in a bottle containing DDW + Carbendazim solution and hence sterilization steps were followed. Midrib was removed from leaf explant prior to inoculation. The culture was incubated in dark up to the regeneration of callus tissue.

2.2.2 Apical meristem

Before the inoculation, Bavistin 5% with streptomycin 200 mg/l were sprayed on the mother orchid for 10days. The plant material was procured from the nursery. The explants were trimmed to the length of 4 cm. These apical meristems were collected in a bottle containing DDW + Carbendazim solution.

2.2.3 Axillary bud

The nursery was used to obtain the plant material. The explants were trimmed to 4 cm in length. These axillary buds were collected in a bottle containing DDW + Carbendazim solution.

2.2.4 Nucellar tissues

The immature nucellar embryos of Alphonso fruit from different stages were dissected as initial explant. The explant was disinfected with different sterilization agents for varied time period followed by washing with double distilled water for 3 to 4 times. 30 to 35days old fruits are selected for culture establishment. The explants were sterilized with Bavistin, streptomycin, tween 20 (absorbent), ethanol, mercuric chloride, sodium hypo chloride and before inoculation the explants are treated under flame (heat sterilization).

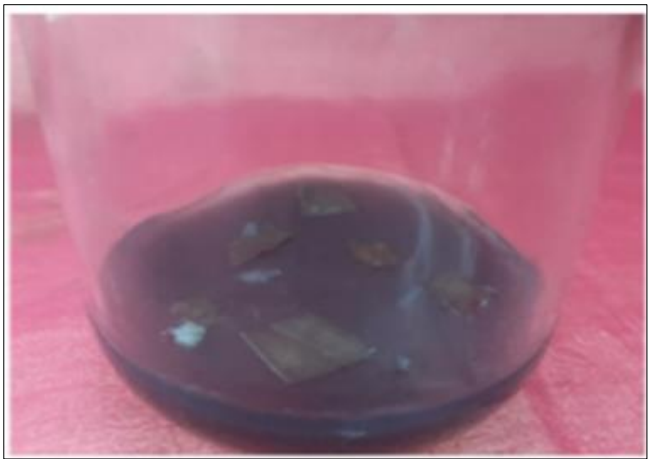
Table 1: Media combinations

Sr. No	Media combinations
AM1	MS CONTROL (MS basal salt solution)
AM2	MS+ 2 mgl^{-1} 2, 4 - D+ 0.5 mgl^{-1} NAA
AM3	MS+ 2 mgl^{-1} 2, 4 - D + 1 mgl^{-1} NAA
AM4	MS+ 2 mgl^{-1} 2, 4 - D + 1.5 mgl^{-1} NAA
AM5	MS+ 2 mgl^{-1} 2, 4 - D + 2 mgl^{-1} NAA
AM6	MS+2.5 mgl^{-1} 2, 4 – D+ 0.5 mgl^{-1} NAA
AM7	MS+2.5 mgl^{-1} 2, 4 - D + 1 mgl^{-1} NAA
AM8	MS+2.5 mgl^{-1} 2, 4 - D + 1.5 mgl^{-1} NAA
AM9	MS+ 2.5 mgl^{-1} 2, 4 - D + 2 mgl^{-1} NAA
AM10	MS+2.5 mgl^{-1} 2, 4 - D + 2.5 mgl^{-1} NAA

3. Results

3.1 Frequency of callus induction

The callus induction frequency is given in Table 2. These frequencies are calculated in terms of percentage. Highest callus induction frequency was observed in the leaf (49.74%) in media AM7 (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA). In respect to leaf explant, highest callus induction was observed in media AM7 (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA) about (49.74%) followed by AM6 medium (MS+2.5 mgl-1 2, 4 – D+ 0.5 mgl-1 NAA) was about (40.45%). In respect to nucellar tissue both media combinations AM7 (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA) and AM2 (MS+ 2 mgl-1 2, 4 - D+ 0.5 mgl-1 NAA) gave good callus intensity about (44.6%) and (44.5%) respectively. In case of apical bud and axillary bud explants, both media combination AM3 (MS+ 2 mgl-1 2, 4 - D + 1 mgl-1 NAA) and AM2 (MS+ 2 mgl-1 2, 4 - D+ 0.5 mgl-1 NAA) showed good success rate about (8.6%) and (4.75%) in AM3 media and (3.6%) and (3.4%) in AM2 medium respectively.



Callus induction in leaf

Callus induction in axillary bud

Callus induction in apical bud

Callus induction in nucellar tissue

Table 2: Frequency of callus induction in (%)

Tr. No.	Media combination	Types of Explants				Mean
		Apical bud	Axillary bud	Nucellar tissue	Leaf	
AM1	MS CONTROL(MS basal salt solution)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
AM2	MS+ 2 mgl^{-1} 2, 4 - D+ 0.5 mgl^{-1} NAA	3.60 (15.61)	3.40 (10.5)	44.5 (41.9)	0.00 (0.00)	12.9 (17)
AM3	MS+ 2 mgl^{-1} 2, 4 - D + 1 mgl^{-1} NAA	8.6 (17.00)	4.75 (12.6)	42.0 (40.41)	0.00 (0.00)	13.82 (17.5)
AM4	MS+ 2 mgl^{-1} 2, 4 - D + 1.5 mgl^{-1} NAA	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
AM5	MS+ 2 mgl^{-1} 2, 4 - D + 2 mgl^{-1} NAA	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
AM6	MS+2.5 mgl^{-1} 2, 4 – D+ 0.5 mgl^{-1} NAA	1.6 (7.17)	3.00 (9.84)	40.1 (39.3)	40.5 (39.5)	21.3 (24.1)
AM7	MS+2.5 mgl^{-1} 2, 4 - D + 1 mgl^{-1} NAA	2.90 (9.89)	3.50 (10.64)	44.6 (41.9)	49.74 (44.83)	25.2 (26.80)
AM8	MS+2.5 mgl^{-1} 2, 4 - D + 1.5 mgl^{-1} NAA	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	37.17 37.54)	9.3 (9.4)
AM9	MS+ 2.5 mgl^{-1} 2, 4 - D + 2 mgl^{-1} NAA	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
AM10	MS+2.5 mgl^{-1} 2, 4 - D + 2.5 mgl^{-1} NAA	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean		2.10 (5.02)	1.5 (4.35)	17.14 (16.34)	12.73 (12.18)	8.24 (9.5)
SE(m)		0.20 (0.3)	0.4 (0.64)	1.51 (0.9)	0.82 (0.5)	
CD at 1%		0.61 (0.80)	1.2 (1.90)	4.50 (2.6)	2.45 (1.42)	

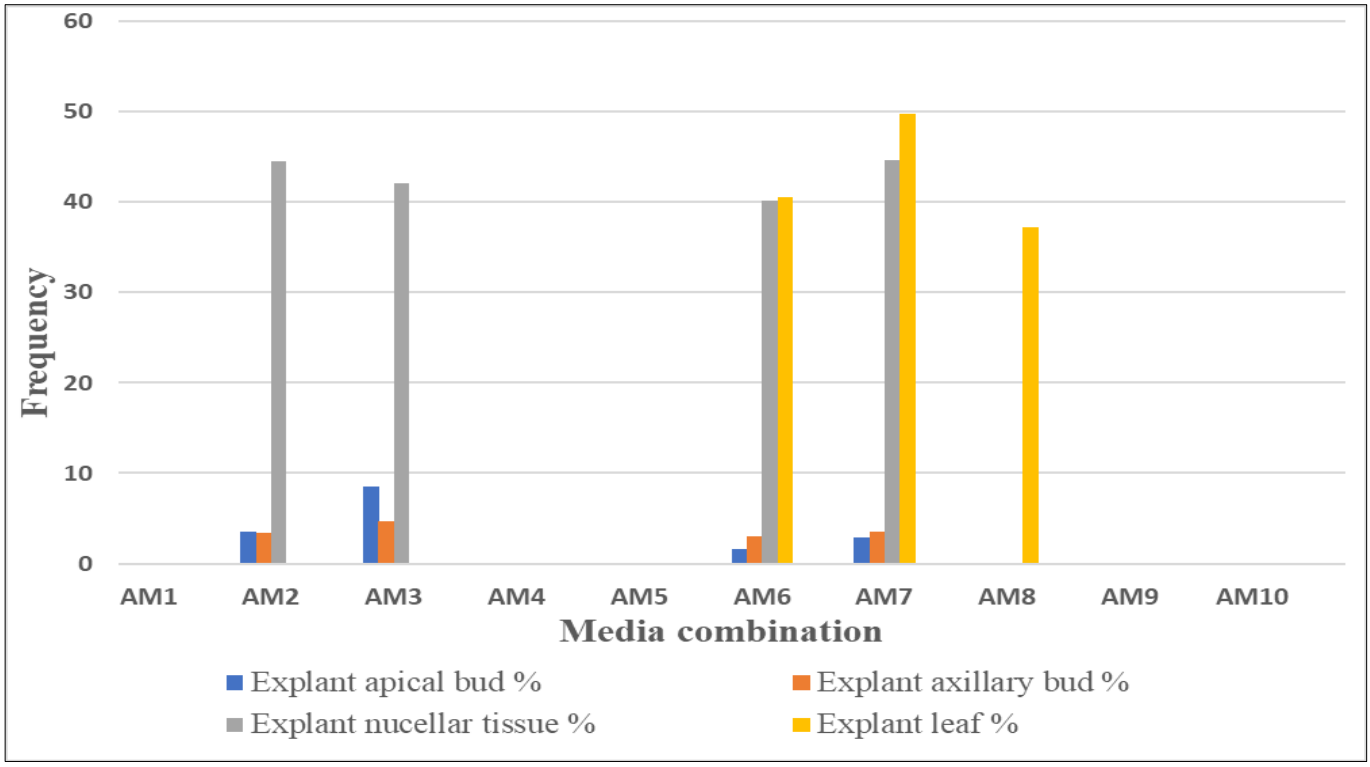


Fig 1: Frequency of callus induction observed in (MS) media combination

The callus induction success is measured in frequency of callus induction. Frequency of callus induction is dependent on several factors *viz*., nutrient medium Murashige and Skoog's (MS) (1962), Plant growth regulator *viz*., auxins, cytokinin. Different tissues from different parts have wide range of nutrient requirement. Tissue like apical bud, axillary bud is deficient in cytokinin. Frequency of callus induction vary from the quantity of nutrient and plant growth regulators are applied for that.

4. Discussion

The frequency of callus induction is highest in leaf explant in AM7 medium about (49.7%) with addition of 2,4-D and NAA followed by nucellar tissue (44.6%) in the same AM7 medium. In nucellar tissue explants MS medium with 2,4-D and NAA are used. Lad *et al*. (1997) [5] established nucellar culture by using 2,4-D and MS minor nutrient medium and also observed that there is no relation between age of nucellar explants and induction of embryogenic culture. In multiple attempts, Raghuvanshi and Shrivastava (1995) [9] used leaves to revive mango. They created several mango sprout form calluses from mature leaf explants using auxin such as NAA, in MS media. Because it contains all necessary salt and medium, MS medium was utilized in the current investigation as the base medium supplemented with plant growth regulators for callus induction. The results showed that all explants responded better to this medium.

In case of apical bud and axillary bud, callus induction is observed from cut part of the explants sometimes, the callus initiation started from primordial. Similar outcomes were obtained by Hesami *et al*. (2018) [3] when callus induction was started from the cut margins of explants that were grown in MS media.

In case of nucellar tissue, the callus growth is observed in AM7 (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA) medium about (44.6%). Al- Busaidi *et al*. (2016) [2] get callus growth in MS minor with addition of 2,4-D.

5. Conclusion

Among the nutrient media, the following frequencies were the highest for callus induction:

In the case of leaf explants, the greatest callus induction frequency, reaching 49.74%, was achieved when using medium AM7 (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA). For apical bud explants, the medium AM3 (MS+2 mgl-1 2, 4 - D + 1 mgl-1 NAA) yielded the maximum response for callus induction, at 8.56%. Similarly, axillary bud explants showed the highest response for callus induction, with a rate of 4.75%, when cultured in medium AM3 (MS+2 mgl-1 2, 4 - D + 1 mgl-1 NAA). Lastly, in the case of nucellar tissue, the most successful callus induction, at 44.5%, was observed in medium AM7 (MS+2.5 mgl-1 2, 4 - D + 1 mgl-1 NAA). These findings indicate the specific conditions and media that yielded the highest success in inducing callus for each type of explant.

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