

Original Research Article

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Factors Affecting Induction of Callus from Nucellus Tissue of Polyembryonic Mango Cv. Vellaikolumban and Cv. Olour

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ABSTRACT

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The protocol for high frequency induction of callus from nucellus tissue was re initiation attained in polyembryonic mango cultivars Vellaikolumban and Olour. Among different sterilization treatments followed, dipping fruits in 70 % ethanol +followed by treating them in Cetrimide 1000 ppm - 15 minutes further treating with NaOCl - 1 % +Tween 20 (one drop per 100 ml) for 15 minutes and Flame sterilisation under laminar air flow found to be the best treatment. Among the basal media, Rugini Olive (RO) medium followed by Murashige and Skoog (MS) medium was superior to Gamborg's B5 (B5) for culture establishment. Among various physiological stages of fruits used for culturing, 30-40 days old and 40-50 days old fruits found to ideal for nucellar embryogenesis in cv. Vellaikolumban and cv. Olour respectively. Embryogenic calli formed on establishment medium (RO, 6 percent sucrose, 1g activated charcoal, 2.5 g/L phytigel, 5 mg each of 2,4-D and GA3).This calli can be used for further steps in somatic embryogenesis.

Introduction

Mango, the King of fruits is generally propagated by both seeds and vegetative methods. Main problems associated with seed propagation are high heterozygosity, seasonal availability of fruits and stone weevil infestation. By employing vegetative methods of propagation, it is not possible to achieve the demand for planting material because of its

meager rate of multiplication. The alternative that can be followed for propagation and rapid multiplication is micro propagation using different explants. It is reported that Mango is a hard to deal crop for micro propagation and attempts for micro propagation using several explants such as shoot tip and nodal explants faced huge defeat due to lack of growth response from explants, high phenol exudation, complications in maintaining

axenic cultures. Somatic embryogenesis is one of the best options to get rapid multiplication and uniform planting material from mango. Nucellus tissue is reported as the suitable explant that can be used for somatic embryogenesis for clonal multiplication in Mango. Establishment of clean and microbe free cultures and callus induction from the explant is the basic step in induction of somatic embryogenesis. With this background, an experiment was set up to standardize the protocol for callus induction from nucellus tissue.

Materials and Methods

This study was conducted during 2017-2018 at Division of fruit crops, ICAR-IIHR, Bengaluru, Karnataka. Vellaikolumban and Olour are the polyembryonic rootstocks possessing horticulturally important traits such as dwarfness and abiotic stress tolerance. Microbial contamination is the main hurdle in establishment of cultures in mango. Hence standardization of surface sterilization treatments is one of the basic steps mango micro propagation. Various surface sterilization treatments reported in different studies were followed to find out the best method of sterilization to obtain maximum number of axenic cultures. Clean cultures obtained from the best treatment are utilized for culture establishment and callus induction experiments to standardize the basal medium to be used for culturing.

After subjecting to surface sterilization treatments, fruits were cut longitudinally and ovule was taken outside the fruit. Ovule was cut into two halves and discarded the embryo and ovule with intact nucellus was cultured in the establishment medium in such a way that it should face the medium. Basal medium used for the establishment of cultures is one of the important factors deciding the response obtained from the explant. Various basal

media such as half MS, half B5, half RO, full MS, full B5 and full RO supplemented with 2,4-D 5mg/L and GA₃ – 5mg/L and Sucrose 6 percent and activated charcoal 1 g/L (Thomas, 1999) were used for the study. Once the surface sterilization treatment and basal medium are standardized, fruits of less than 20 after pollination to more than 60 days after pollination were utilized for the study to find out the optimum stage for somatic embryogenesis from nucellus tissue of polyembryonic cultivars.

Results and Discussion

Effect of different sterilization treatments on survival, mortality and contamination

Among the different surface sterilization treatments followed (Table 1), T₆ recorded maximum percent survival (90.00) which is at par with T₅ (83.33) and no cultures survived in control. T₆ recorded minimum percentage of mortality (3.33) of explants which is at par with T₅ (6.67) and followed by T₃ (10.00). Percent contamination was minimum (3.33) in T₆ which differed significantly from T₅ (13.33) and followed by T₄ (33.33).

Sodium hypochlorite is reported to be a better disinfectant than hydrogen peroxide due to bleaching effects of the later and hence has been widely used for sterilization in Mango. Wei *et al.*, (2013) used sodium hypochlorite 20 % (v/v) for 30 min supplemented with 3 drops of Tween 20 as a surface - disinfectant in Mango fruits, then washed with sterile water to obtain microbe free explants. Ara *et al.*, (1999) surface sterilized mango fruits with 0.1 % (v/v) sodium hypochlorite, 2 or 3 drops of Tween -20 for 20 minutes before culturing the explant in the medium.

Flame sterilisation of mango fruits after treating them with disinfectants was reported to be most beneficial in controlling microbial

contamination (Thomas, 1999, Mishra *et al.*, 2010)

Effect of different basal media on callus induction from nucellus tissue of cv. Vellaikolumban and cv. Olour

Among different basal media used for the study (Table 2), Rugini Olive (RO) medium responded better both in cv. Vellaikolumban and cv. Olour in terms of percent response and

callus index. Full RO medium recorded maximum percent response (83.33 and 86.66) followed by full MS medium (70.00 and 76.66) in cv. Vellaikolumban and cv. Olour. Maximum callus index (83.33 and 86.66) was recorded in full RO medium followed by full MS medium (70.00 and 76.66) in cv. Vellaikolumban and cv. Olour respectively. RO is a previously unfamiliar medium for mango and it found promising in the initial stages of embryogenesis (Thomas, 1999).

Table.1 Different surface sterilization treatments followed:

T ₁	Control
T ₂	Dipping in 70 % ethanol for 5 minutes + Cetrimide 1000 ppm - 15 minutes
T ₃	Dipping in 70 % ethanol for 5 minutes + Cetrimide 1000 ppm - 15 minutes + (NaOCl - 1 % +Tween 20 (one drop per 100 ml)) - 15 minutes
T ₄	Dipping in 70 ethanol + Cetrimide 1000 ppm - 15 minutes + H ₂ O ₂ - 1% - 10 minutes
T ₅	Dipping in 70 ethanol + Cetrimide 1000 ppm - 15 minutes + H ₂ O ₂ - 1 % - 10 minutes + Flame sterilisation
T ₆	Dipping in 70 % ethanol + Cetrimide 1000 ppm - 15 minutes +(NaOCl - 1 % +Tween 20 (one drop per 100 ml)) - 15 minutes + Flame sterilisation

Table.2 Effect of different surface sterilization treatments on survival, mortality and contamination of explants.

Treatments	Percent survival	Percent mortality	Percent contamination
T ₁	0.00(0.00)	0.00(0.00)	100.00 (90.00)
T ₂	13.33(21.40)	0.00(0.00)	86.67(68.58)
T ₃	33.33(35.25)	10.00(18.42)	56.67(48.81)
T ₄	46.67(43.07)	20.00(26.55)	33.33(35.25)
T ₅	83.33(65.89)	6.67 (14.95)	13.33(21.40)
T ₆	90.00(71.58)	3.33(10.51)	3.33(10.51)
Mean	44.44	6.67	48.89
SEm±	0.55	0.23	0.39
CD (p=0.01)	1.66	0.71	1.22
CV	2.33	3.40	1.49

Table.3 Effect of different basal media on induction of callus in cv. Vellaikolumban and cv. Olour

Basal medium/ cultivars	Vellaikolumban (30-40 days fruits)					Olour (40-50 days fruits)				
	Total explants cultured	Total explants responded	Percent response	Growth score	Callus index (percent response X growth score)	Total explants cultured	Total explants responded	Percent response	Growth score	Callus index
Half MS	30	20	66.66 (54.82)	1.00	66.66	30	21	70.00 (56.99)	1.00	70.00
Half RO	30	15	50.00(44.98)	1.00	50.00	30	17	56.66 (48.84)	1.00	56.66
Full MS	30	21	70.00(56.93)	1.00	70.00	30	23	76.66(61.57)	1.00	76.66
Full RO	30	25	83.33(66.73)	1.00	83.33	30	26	86.66(71.42)	1.00	86.66
Half B5	30	17	56.66(48.83)	1.00	56.66	30	20	66.66(54.86)	1.00	66.66
Full B5	30	14	46.66 (43.05)	1.00	46.66	30	15	50.00(44.98)	1.00	50.00
Mean		18.67	62.21		62.21		20.33	67.73		67.73
SEm ±					CD (p = 0.05)					
Varieties	0.66		1.62		2.20	-		-		-
Basal medium	1.14		2.81		3.81	3.36		8.25		11.20
Varieties X Basal medium	1.61		3.97		5.39	-		-		-

Amount of callus (g)	Growth score
0.0-0.5	1
0.51 - 1.00	2
1.01-1.50	3
1.51-2.00	4

Table.4 Effect of different physiological stages of fruits of polyembryonic cultivars on callus induction from nucellus tissue.

Cultivars	Vellaikolumban					Olour				
	No. of explants cultured	No. of explants showed callusing	% explants showing callus induction	Days taken callus initiation	Days taken callusing	No. of explants cultured	No. of explants showed callusing	% explants showing callus induction	Days taken callus initiation	Days taken callusing
Less than 20 days	30.00	0.00	0.00(0.00)	0.00	0.00	30.00	0.00	0.00 (0.00)	0.00	0.00
20- 30	30.00	20.00	66.67 (54.87)	28.00	48.00	30.00	19.00	63.33(52.74)	27.00	50.00
30-40	30.00	25.00	83.33 (67.11)	25.00	38.00	30.00	23.00	76.67(61.22)	24.00	40.00
40-50	30.00	24.00	80.00(64.15)	26.00	41.00	30.00	26.00	86.67 (69.03)	25.00	34.00
50-60	30.00	15.00	50.00(44.98)	30.00	56.00	30.00	17.00	56.67(48.82)	32.00	54.00
More than 60 days	30.00	0.00	0.00 (0.00)	0.00	0.00	30.00	0.00	0.00 (0.00)	0.00	0.00
Mean	30.00	14.00	46.67(38.52)	18.17	30.50	30.00	14.17	47.22	18.00	29.67
		SEm±				CD (p = 0.01)				
Cultivars		0.49	1.21	0.63	1.06	-	-	-	-	-
Stages		0.85	2.10	1.09	1.84	2.51	6.17	3.21	5.41	
Cultivars X Stages		1.21	2.94	1.54	2.60	-	-	-	-	-

Effect of different physiological stages of fruits on callus induction

Among various physiological stages of fruits utilized for the experiment (Table 3), 30-40 days old fruits in cv. Vellaikolumban and 40-50 days old fruits in cv. Olour responded better with respect to percent callus induction, days taken for callus initiation and days taken for callus formation in comparison with remaining stages of fruits.

Maximum percent callus induction (83.33 and 86.66) was observed in 30 - 40 days old fruits and 40 - 50 days old fruits of cv. Vellaikolumban and cv. Olour respectively with no callus induction in fruits of less than 20 days after pollination and more than 60 days after pollination. Minimum days for callus initiation (25.00 and 25.00) was recorded in 30 – 40 days old fruits and 40 – 50 days old fruits of cv. Vellaikolumban and cv. Olour respectively with maximum days for callus initiation (30.00 and 32.00) was recorded in 50 – 60 days old fruits in cv. Vellaikolumban and cv. Olour respectively. Minimum days for callus formation (38.00 and 34.00) was observed in 30 – 40 days old fruits and 40 -50 days old fruits of cv. Vellaikolumban and cv. Olour respectively with maximum days for callus formation (56.00 and 54.00) was recorded in cv. Vellaikolumban and cv. Olour respectively. This may be attributed to the percentage coverage of nucellus tissue in different stages of fruits. In the present study it is observed that maximum percentage of nucellus tissue was observed at 30-40 days old fruits and 40-50 days old fruits in cv. Vellaikolumban and cv. Olour. Thirty to sixty-day-old fruits, harvested after pollination are suitable for induction of somatic embryogenic culture from the nucellus (Litz *et al.*, 1982; Dewald *et al.*, 1989; Pliego-Alfaro *et al.*, 1996; Ara *et al.*, 1999; Singh *et al.*, 2002; Sulekha and Rajmohan, 2004).

The percentage of explants showing embryogenesis was 10- 20% less in case of nucellus of older fruits as compared to that of younger fruits. Furthermore, the average number of developed embryos formed in younger explants was more, i.e., 20.75 than in case of nucellus of older fruits where it was only 12.5 (Chaturvedi *et al.*, 2004). The size of explant to be used for culturing is of great importance (Table 4).

The larger the explant, poor in the response. In this context the medium has a limited influence. On the other hand, small explants are more easily directed by the substances contained in the medium (Auge, 1995).

A protocol for induction of callus from nucellus which was found to be reproducible from polymebyronic mango cultivars (Vellaikolumban and Olour) at IIHR, Bengaluru.

Surface sterilization treatment involving dipping of fruits in 70 % ethanol followed by dipping in Cetrime 1000 ppm for 15 minutes and treating them with NaOCl - 1 % containing Tween 20 (one drop per 100 ml) for 15 minutes and flame sterilization of fruits under laminar air flow is found to be the best treatment which resulted in maximum percent survival with minimum mortality of explants and contamination. 30-40 days old and 40-50 days old fruits in cv. Vellaikolumban and cv. Olour respectively responded better with respect to callus induction, days taken for callus initiation and formation on RO basal medium supplemented with 2,4-D and GA₃ 5 ppm. Both cultivars failed to induce callus from less than 20 days and more than 60 days old fruits.

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