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Research Article

Influence of Basal Salts, Sucrose and Plant Growth Regulator Levels on Nucellar Embryogenesis and Plantlet Regeneration in Monoembryonic Mango Varieties

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Abstract: Current study described stage-wise protocols for in vitro propagation of commercially important varieties of mango. Induction of somatic embryos (SE) and plantlet regeneration was obtained using nucellar explants of three superior monoembryonic mango vars.'Saroli', 'Langra' and 'Chaunsa' cultivated in Khairpur, Pakistan. The immature fruits (2.5-4.0 cm long) were surface disinfected using 30 % sodium hypochlorite (NaOCl) solution. Results revealed that significantly highest direct somatic embryogenesis (93 %) was obtained in var. 'Chaunsa' under full dark on culture medium comprising of 2.0 mg L^{-1} N6 2-isopentenyl adenine (2iP), 0.5 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). Medium consisted of 2iP 4.0 mg L^{-1}, 2,4-D 1.0 mg L^{-1} induced significantly highest embryogenic callus (91 %) using nucellar explants in var.'Chaunsa'. Significantly highest germination (95 %) of SE was achieved in var. 'Chaunsa' on the medium comprising of microsalts of MS, macrosalts of B5, 2iP 0.1 mg L^{-1}, Kinetin (Kin) 0.5 mg L^{-1}. Highest shoot length (5.1 cm) and root length (4 cm) were obtained in var. 'Langra' on the medium consisted of microsalts of MS, macrosalts of B5, 30 g L^{-1} sucrose, 200 mg L^{-1} activated charcoal (AC), 0.1 mg L^{-1} naphthalene acetic acid (NAA), 0.2 mg L^{-1} benzyl adenine (BA). Stage-wise protocols established for the regeneration of plantlets can be useful to micropropagation of the other mango varieties of the world.

Keywords: Cuture media, *Mangifera indica* L., Micropropagation, Monoembryonic, Regeneration, Somatic Embryogenesis

1. INTRODUCTION

Mango is tropical, arborescent, evergreen tree with a life span ranging from 70 to 100 years [1, 2]. The mango propagation can be done through sexual (seed propagation) and asexual methods (grafting) [3]. The mango seeds can be categorized into monoembryonic (contain single zygotic embryo) and polyembryonic (contain one zygotic and 4-5 nucellar embryos) types [4]. The seedlings obtained from polyembryonic seeds are always true-to-type except the one that develops from the zygotic embryo [4, 5]. The monoembryonic seeds produce single zygotic seedling that is always different genotypically from the mother tree [3, 6, 7]. The grafting is traditional vegetative propagation method to grow true-to-type plants of the commercially important varieties. The in vitro propagation through vegetative tissues is an alternative method to grafting [8, 9]. Different reports [5, 10-12] utilized immature ovular halves comprising nucellus tissue as initial explant for in vitro propagation of elite mango varieties. Previously, the nucellar explants obtained from immature polyembryonic seeds [5, 10-17] and monoembryonic seeds [6, 7, 10, 18-20] have been used in somatic embryogenesis. In vitro propagation of mango was done before at different times [6, 10, 19, 21], but still there

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is need to improve the elongation and rooting stages [2]. Moreover, there is no report available for direct somatic embryogenesis in mango using nucellus tissue. Pakistan is one of the major mango producers and exporters, cultivating some of the leading exporting varieties like 'Saroli', 'Langra', 'Chaunsa', 'Sindhri', 'Fajri', 'Sonaro', 'Totapuri', 'Lal Badshah', 'Anwar Ratol'. Hence the mango propagation by in vitro method using nucellus tissue is a suitable method to produce true-to-type plantlets. In vitro cultures of mango facing severe browning due to phenolic compounds occur in tissues which was tackled by addition of antioxidants, AC, quick shifting of cultures to the fresh media, incubation of cultures under full darkness. Effects of Plant Growth Regulators (PGRs) levels, sucrose, salts, AC on direct and indirect somatic embryogenesis using nucellar explants, proliferation and germination, shoot elongation and rooting were focused in present study. The results obtained in this study will apply to other mango varieties worldwide.

2. MATERIALS AND METHODS

2.1. Plant Material

Fruits (2.5-4 cm long) obtained at different intervals from 20 years old trees, 30-40 days after pollination from vars. 'Saroli', 'Langra', 'Chaunsa' (early, mid, late season varieties respectively) (Figure 1a, b). Fifty fruits were collected from each variety and brought to the laboratory for further processing. Initially fruits were washed using tap water for twenty min followed by washing with commercial liquid Lemon Max (Colgate Palmolive, Pakistan) dissolved 5 ml in 500 ml of water to clean dust particles attached to surface of fruits. Final sterilization of fruits was carried out on laminar airflow cabinet using 30 % NaOCl solution with 5 ml Tween-20 (Scharlau, Spain) continuously up to 20 min followed by final washing with distilled autoclaved water. Fruits were divided into two halves and intact immature ovular halves comprising nucellus tissue were used as initial explants. The

Fig. 1. (a) Immature fruits on the tree during 15th April ready for picking, (b) size and morphology of the fruits of 'Saroli', 'Langra', 'Chaunsa' used for in vitro culture.

remaining fruit flesh (mesocarp with epicarp) were discarded. Each isolated ovule was preserved in filtered solution of citric acid (100 mg L^{-1}) for 2-3 min. Later, ovules dissected longitudinally from the middle into two halves (Figure 2a), and each half lacking zygotic embryo was used as an initial explant.

2.1.1. Effect of 2,4-D and 2iP on Proembryogenic Callus Induction, Maturation, and Direct Somatic Embryogenesis under Full Dark Conditions

Medium consisted of (1) 2,4-D 0.0, 0.5, 1.0 or 2.0 mg L^{-1} and 2iP 0.0, 0.5, 1.0 or 2.0 mg L^{-1}. (2) 2,4-D 0.0, 0.5, 1.0 or 2.0 mg L^{-1} and 2iP 1.0, 1.5, 2.0 or 4.0 mg L^{-1}, microsalts of MS (Merck, Germany), macrosalts of B5 (Merck, Germany), agar 2.2 g L^{-1} (Oxoid, United Kingdom), gelrite 1.4 g L^{-1} (Gellan Gum, Caisson Laboratories, USA), sucrose 60 g L^{-1}, glutamine 400 mg L^{-1}, 200 mg L^{-1} AC. pH of medium fixed at 5.8 prior to autoclaving. 20 ml solution poured into each culture vessel and sterilized in autoclave at 15 psi pressure at 121 °C up to 20 min. Cultures put in complete dark at 24 °C. Transfer of cultures onto the fresh media was done at 2-3 weeks intervals.

2.1.2. Effect of PGRs on SE Germination and Plantlet Formation under Light (16h Photoperiod) consisting of cool white fluorescent light (40-60 µmol m^{-2} s^{-1})

Medium comprising of 2iP 0.1 mg L^{-1}, Kin 0.5 mg L^{-1} used for germination and plantlet formation. Shoot elongation and rooting medium was comprised of NAA 0.1 mg L^{-1}, BA 0.2 mg L^{-1}. Microsalts of MS, macrosalts of B5, sucrose 30 g L^{-1}, agar 2.2 g L^{-1}, gelrite 1.4 g L^{-1}, AC 200 mg L^{-1}, glutamine 400 mg L^{-1} used in both types of media used to multiply somatic embryos induced either directly from nucellus or indirectly via callus.

2.1.3. Effect of Basal Salts and AC on SE Multiplication and Germination

Media used for multiplication and germination of embryos was consisted of (1) micro and macrosalts of MS, 200 mg L^{-1} AC (2) micro and macrosalts of MS without AC (3) micro and macrosalts of B5, AC 200 mg L^{-1}, (4) micro and macrosalts of B5 without AC.

2.1.4. Effect of Basal Salts, AC, and Sucrose on Shoot Elongation and Rooting under White Fluorescent Light (160 µmol m^{-2} s^{-1})

Medium consisting of (1) micro and macrosalts of MS, 200 mg L^{-1} AC (2) micro and macrosalts of MS without AC (3) micro and macrosalts of B5, AC 200 mg L^{-1}, (4) micro and macrosalts of B5 without AC. (5) microsalts of MS, macrosalts of B5, sucrose 30 g L^{-1}, (6) microsalts of MS, macrosalts of B5, sucrose 40 g L^{-1}, (7) microsalts of MS, macrosalts of B5, sucrose 50 g L^{-1}, (8) microsalts of MS, macrosalts of B5, sucrose 60 g L^{-1}.

2.2. Data Analysis

Three varieties were used in the study. Four different treatments were tested from induction of proembryogenic callus up to proliferation of SE (Table 1-4) and eight treamtnets were tested for shoot and root development (Table 5-6). Three replicates were selected for each treatment and each culture vessel contained single explant. Completely Randomized Design (CRD) was used. Data were recorded after every month and analyzed as two-way ANOVA and the difference between all mean values identified by LSD ($p<0.05$) by XLSTAT.

3. RESULTS AND DISCUSSION

3.1. Effect of Auxins and Cytokinins on Induction, Proliferation and Maintenance of Embryogenic Callus from Nucelli under Dark

The callus formation from nucellus tissue depends upon the type of PGRs used in media. Auxin 2,4-D mainly induce callus in primary explants was added in the media for induction of proembryogenic callus. Results of two-way ANOVA exhibited significant effect of variety (< 0.001), treatment (< 0.002) and combined effect of variety and treatment (0.004). Data in Table 1 show that highest embryogenic callus induction (Figure 2b) was obtained in vars. 'Chaunsa' (88 %), 'Langra' (80 %) and 'Saroli' (70 %) from nucellar explants (Figure 2a) on medium comprising of 2,4-D 0.5 mg L^{-1} and 2iP 4.0 mg L^{-1} within two months of initiation stage under dark. Medium comprising of 2,4-D 0.5 mg L^{-1} and 2iP 2.0 mg L^{-1} also induced callus from nucellar explants in vars. 'Chaunsa' (81 %), 'Langra'

Table 1. Induction of proembryogenic callus in initial nucellar explants of three mango varieties under full dark conditions.

PGRs (2iP+2,4-D mg L^{-1})	Embryogenic callus induction (%)		
	Saroli	**Langra**	**Chaunsa**
1 + 0.5	55 ± 1.2^{c}	65 ± 0.8^{c}	61 ± 1.6^{c}
1.5 + 0.5	61 ± 3.2^{b}	63 ± 0.5^{c}	70 ± 2.2^{ab}
2.0 + 0.5	67 ± 2.5^{a}	77 ± 2.9^{ab}	81 ± 1.4^{a}
4.0 + 0.5	70 ± 2.6^{a}	80 ± 2.2^{a}	88 ± 2.1^{a}
Variety (mean)	63.3	71.3	75.0
Source of variability			
Treatment	< 0.002		
Variety	< 0.001		
Treatment × Variety	0.004		

Mean values in columns with standard error denoted with different superscript letters show significance level at $p \leq 0.05$.

(77 %) and 'Saroli' (67 %). On the contrary, the significantly lowest induction of embryogenic callus was noted from nucellar explants in vars. 'Saroli' (55 %),'Chaunsa' (61 %) and 'Langra' (65 %) on medium comprising of 2,4-D 0.5 mg L^{-1}, 2iP 1.0 mg L^{-1}.

Embryogenic calli induced in nucellar explants was compact, proliferated rapidly under full dark (Figure 2b). Ara *et al.* [22] observed similar results regarding formation of embryogenic calli in nucellar explants of monoembryonic mango vars. 'Amrapali' and 'Chaunsa' on medium comprising of 1.0 mg L^{-1} 2,4-D. Nower [12] noted that B5 medium comprising of 2,4-D 1.0 mg L^{-1} induced compact callus in cv. Zebda. Al-Busaidi et al. [6] described the medium comprising of 2,4-D 2.0 mg L^{-1} and BAP 0.5 mg L^{-1} to induce embryogenic callus using nucellar explants. Successful *in vitro* propagation of mango was reported previously by several workers using nucellar explants [7, 10, 14, 15]. Nucellar explants have been exploited as an initial explants in the current study since plantlets obtained using nucellus tissue are usually free from viruses due to lack of a vascular link between maternal tissue and nucellus.

Krishna and Singh [23] described that formation of embryogenic calli in mango depends on morphogenic capability of nucellus. Litz *et al.* [14] reported the formation of embryogenic callus in nucellus tissue of some polyembryonic mango varieties in 1-2 months obtained from 40-60 days old fruits. Consequently, appropriate circumstances for the formation of embryogenic calli in nucellar explants observed in monoembryonic mango varieties [10]. Young fruits (3.5 to 5.0 cm) of var. Baramasi obtained after 30-40 days of pollination for *in vitro* propagation [6, 24]. Ara *et al.* [25] utilized immature fruits of vars. 'Amrapali' (2 to 3.5 cm) and 'Chaunsa' (1.5 to 2.5 cm) for *in vitro* propagation. Malabadi *et al.* [26] induced embryogenic callus in nucellar explants obtained from 3-4 cm long fruits. Similarly, in this study excellent results were obtained using nucellar explants obtained from 2-4 cm long fruits.

Embryogenic callus (Figure 2b) was maintained on initiation medium under full darkness for 1-2 months until the formation of proembryos. Abul-Soad *et al.* [7] maintained embryogenic callus cultures on callus inducing medium comprising of 2,4-D 1.0 mg L^{-1}, 2iP 4.0 mg L^{-1}, 400 mg L^{-1} glutamine, microsalts of MS, macrosalts of B5 under full dark. The combined effect of auxin, cytokinin, and full dark was the key factor to maintain the embryogenic callus and formation of proembryos into mature SE [27, 28]. The cytokinins are important to stimulate and organize the growth of the apical meristem at the maturation stage [23].

3.2. Effect of 2,4-D + 2iP on SE Induction from Proembryogenic Callus and Maturation

Early cotyledonary stage embryos induced under dark in embryogenic callus developed into mature germinating embryos under light. Two-way ANOVA exhibited significant effect of variety (<

0.001), treatment (< 0.001) and combined effect of variety and treatment (< 0.001). Comparing the four PGR treatments within three studied varieties ('Saroli', 'Langra', 'Chaunsa'), significantly highest percentage (91 %) of SE (Figure 2d) was noted in var. 'Chaunsa' on medium comprising of 2iP 4.0 mg L^{-1}, 2,4-D 1.0 mg L^{-1} followed by 85.6 % of SE induced in same variety on medium comprising of 2iP 2.0 mg L^{-1}, 2,4-D 0.5 mg L^{-1} (Table 2). Similarly, SE induced in var. 'Langra' (82 %) and in var. 'Saroli' (76 %) on medium comprising of 2iP 4.0 mg L^{-1}, 2,4-D 1.0 mg L^{-1}. Whereas, the least SE induction percentage (44.3 %) was achieved in var. 'Saroli' on medium comprising of 2iP (1.5 mg L^{-1}). Pateña *et al.* [11] devised a reliable procedure in mango for induction of SE and formation of plantlets on medium consisted of B5 macrosalts, MS microsalts, vitamins of MS, 10-20 % of coconut water, Fe-EDTA, glutamine 0.4 mg L^{-1}, sucrose 2-6 %, 2,4-D 0.5-2.0 mg L^{-1} and gelrite 2.5 g L^{-1}.

Different reports [1, 23] observed that occurrence of 2,4-D in medium for long periods retarded the formation of SE. Occurrence of 2iP and reduced level of 2,4-D observed necessary to develop SE in date palm [29]. Nevertheless, 2,4-D is utilized largely in plant tissue culture for callus induction, however, exclusion of 2,4-D from a medium at a particular stage is also important for maturation of embryos. Later, globular stage embryos were induced in the embryogenic calli, converted into heart and torpedo stage embryos and finally developed into cotyledonary stage SE (Figure 2d). Cotyledonary stage embryos were utilized to produce mature dicotyledonous embryos

Table 2. Impact of PGR concentrations on SE formation from proembryogenic callus in three mango varieties under dark.

PGRs (2iP + 2,4-D mg L^{-1})	SE induction (%)		
	Saroli	**Langra**	**Chaunsa**
1.5 + 0.0	44.3 ± 0.2^{i}	66.0 ± 1.2^{h}	73.0 ± 0.6^{efg}
2.0 + 0.0	71.6 ± 0.4^{g}	74.6 ± 1.4^{efg}	76.3 ± 1.3^{de}
2.0 + 0.5	72.6 ± 0.2^{fg}	78.3 ± 0.5^{d}	85.6 ± 1.2^{b}
4.0 + 1.0	76.0 ± 1.2^{def}	82.0 ± 0.2^{c}	91.0 ± 1.1^{a}
Variety (mean)	66.1 ± 0.6^{c}	75.2 ± 0.3^{b}	81.5 ± 0.5^{a}
Source of variability			
Treatment	< 0.001		
Variety	< 0.001		
Treatment × Variety	< 0.001		

Mean values in columns with standard error denoted with different superscript letters show significance level at $p \leq 0.05$.

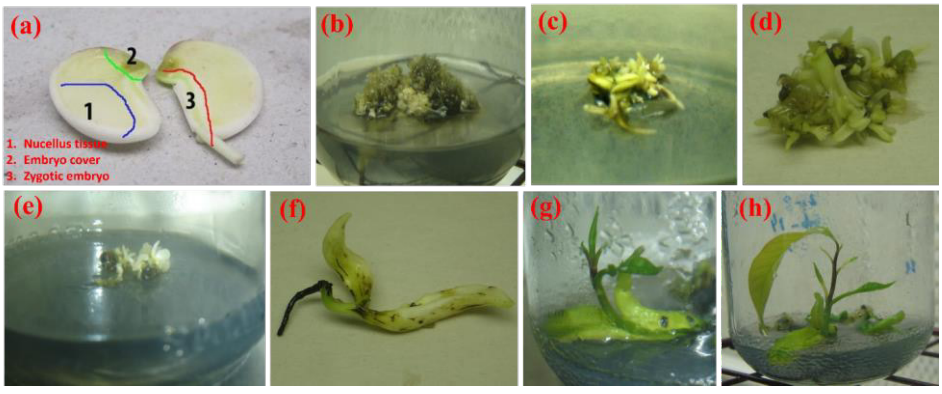

Fig. 2. (a) Immature ovular halves of variety 'Chaunsa' containing nucellus tissue, (b) embryogenic callus, (c) formation of SE from embryogenic callus, (d) maturation of SE, (e) direct somatic embryogenesis, (f) root formation in SE, (g) shoot formation in SE, (h) shoot elongation and rooting.

followed by formation of quick plantlets under light. In this way, further development of SE (ready to produce true leaves) took place under light via subsequent subcultures (Figure 2f-g).

3.3. Effect of 2,4-D + 2iP on Direct Somatic Embryogenesis from Nucellus Tissue under Full Dark

Results of two-way ANOVA revealed the significant effect of variety (< 0.003), treatment (< 0.001) while combined effect of variety and treatment (< 0.07) remained nonsignificant. The data presented in Table 3 indicate that the initiation media comprising of several treatments of 2,4-D and 2iP induced direct SE in nucellar explants of 2-4 cm fruits under full dark within two weeks, which were shifted quickly under light for further proliferation and germination (Figure 2e). Significantly highest induction of direct induction of SE recorded in nucellar explants obtained from var. 'Chaunsa' (86 %) followed by var. Langra (75 %) and Saroli (71 %) on medium comprising of 2iP 2.0 mg L^{-1}, 2,4-D 0.5 mg L^{-1}, 200 mg L^{-1} AC, ascorbic acid 100 mg L^{-1}, glutamine 400 mg L^{-1}, microsalts of MS, macrosalts of B5 (Table 3). On the contrary, significantly lowest percentage of direct somatic embryogenesis (51 %) was obtained in var. Saroli followed by var. Langra (55 %) from nucellar explants obtained from 2.5 cm fruits on medium comprising of 1.5 mg L^{-1} 2iP. In var. 'Saroli' direct SE (71 %) also induced in nucellar explants on medium comprising of 2,4-D 0.5 mg L^{-1}, 2iP 2.0 mg L^{-1}, 200 mg L^{-1} AC, 100 mg L^{-1} ascorbic acid, 400 mg L^{-1} glutamine, microsalts of MS, macrosalts of B5. SE are also induced directly in var. 'Chaunsa' (70 %) from nucellar explants of 3.5 cm long fruits on medium comprising of 2 mg L^{-1} 2iP. Furthermore, medium comprising of 2,4-D and 2iP induced significantly highest direct somatic embryogenesis compared to the media containing only 2iP, the embryos were observed to be multiplying rapidly while transferred under light. Several studies [15, 23, 30, 31] utilized the medium comprising of B5 macrosalts, MS microsalts, 4.52 to 9.04 µM 2,4-D, for somatic embryogenesis in mango using nucellus tissue. SE induced directly on nucellar explants (Figure 2e) shifted quickly from dark to light conditions for further proliferation and maturation (Figure 2d). The germination (Figure 2f-g), plantlet formation and rooting (Figure 2h) were also obtained under light.

3.4. Effect of Basal salts of MS and B5, AC on Proliferation of SE under Light (16h Photoperiod with cool white fluorescent light (40-60 µmol m^{-2} s^{-1})

Two-way ANOVA show significant effect of variety (< 0.001), treatment (< 0.001) and combined effect of variety and treatment (< 0.001) on multiplication of SE onto the media consisting of different basal salts with and without AC, 2iP (0.1 mg L^{-1}), Kin (0.5 mg L^{-1}) (Table 4). Data in Table 4 show that highest proliferation of SE obtained in var. 'Chaunsa' (95 %) followed by var. 'Langra' (88 %) and var. 'Saroli' (77 %) on medium comprising of micro and macrosalts of MS, AC 200 mg L^{-1} (Figure 3a). Medium consisted of micro and macrosalts of MS without AC exhibited significant reduction in

Table 3. Effect of different treatments of PGRs on direct induction of SE from nucellus tissue in three mango varieties under full dark.

PGRs	**Direct somatic embryogenesis (%)**		
(2iP + 2,4-D mg L^{-1})	**Saroli**	**Langra**	**Chaunsa**
1.5 + 0.0	51±0.57^{c}	55±0.43^{c}	62±0.27^{c}
4.2 + 1.0	69±2.88^{ab}	71±3.15^{ab}	80±2.66^{b}
2.2 + 0.5	71±2.15^{a}	75±1.23^{a}	86±2.11^{a}
2.2 + 0.0	55±1.73^{c}	64±2.74^{c}	70±1.22^{c}
Variety (mean)	61.5	66.3	74.5
Source of variability			
Variety	< 0.003		
Treatment	< 0.001		
Variety × Treatment	< 0.07		

Mean values in columns with standard error denoted with different superscript letters show significance level at $p \leq 0.05$.

Table 4. Impact of basal salts and AC on proliferation of SE in three mango varieties under light.

	Proliferation of SE (%)		
Treatment	**Saroli**	**Langra**	**Chaunsa**
Micro and macrosalts of MS, AC 200 mg L^{-1}	77.0 ± 1.9^{c}	88.0 ± 1.4^{b}	95.0 ± 0.3
Micro and macrosalts of MS	45.0 ± 1.5^{h}	51.0 ± 1.2^{fg}	49.0 ± 0.6^{gh}
Micro and macrosalts of B5, AC 200 mg L^{-1}	70.0 ± 0.6^{d}	92.0 ± 1.5^{ab}	94.0 ± 0.2^{a}
Micro and macrosalts of B5	58.0 ± 1.3^{e}	51.0 ± 0.4^{fg}	55.0 ± 1.9^{ef}
Variety (mean)	62.5	70.5	73.3
Source of variability			
Variety	< 0.001		
Treatment	< 0.001		
Variety × Treatment	< 0.001		

Mean values in columns with standard error denoted with different superscript letters show significance level at $p \leq 0.05$.

proliferation of embryos in vars. 'Saroli' (45 %), 'Langra' (51 %) and 'Chaunsa' (49 %) (Figure 3b). Medium comprising of micro and macrosalts of B5, 200 mg L^{-1} AC revealed significant proliferation of SE in var. 'Chaunsa' (94 %) followed by var. 'Langra' (92 %) and var. 'Saroli' (70 %) (Figure 3c). In contrast, the significantly lowest proliferation was noted in vars. 'Saroli' (58 %), 'Langra' (51 %) and 'Chaunsa' (55 %) on medium consisted of micro and macrosalts of B5 but without AC (Figure 3d). Different reports recommended the medium solidified with gelrite and phytagel for proliferation of embryos [15, 32, 33]. Chaturvedi *et al.* [34] used liquid medium for somatic embryo

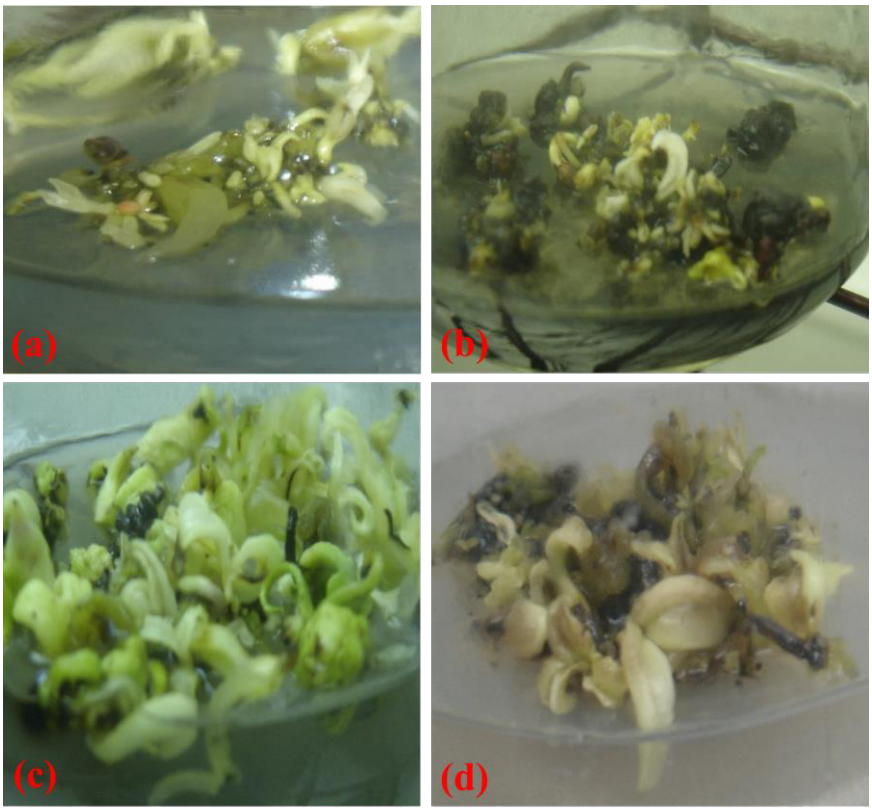

Fig. 3. (a) proliferation of SE on medium containing MS microsalts, B5 macrosalts, 0.1 mg L^{-1} 2iP, 0.5 mg L^{-1} Kin (b) proliferation of SE on medium consisted of micro and macrosalts of MS, 0.1 mg L^{-1} 2iP, 0.5 mg L^{-1} Kin (c) multiplication of SE on medium consisted of 200 mg L^{-1} AC, 2iP 0.1 mg L^{-1}, Kin 0.5 mg L^{-1} (d) multiplication of SE on medium without AC, 2iP 0.1 mg L^{-1}, Kin 0.5 mg L^{-1}.

maturation, proliferation and plantlets formation. Xiao *et al.* [35] used 23 µ M Kin in medium for proliferation and germination of SE. Laxmi *et al.* [32] suggested addition of GA_3, N6-benzyl amino purine, MS microsalts, B5 macrosalts and additives for proliferation and germination of embryos.

3.5. Effect of Basal Salts, AC and Sucrose on Shoot Elongation under Light (16h Photoperiod with cool white fluorescent light (160 µmol m^{-2} s^{-1})

Two-way-ANOVA for shoot length revealed impact of treatment as significant ($p<0.001$) while effect of variety and interaction between variety and treatment were found significant, but at lesser significant levels as $p<0.008$ and $p<0.025$, respectively (Table 5). The shoot elongation medium comprising of micro and macrosalts of MS with 200 mg L^{-1} AC revealed significantly highest shoot length in var. 'Langra' (5.1 cm) followed by vars. 'Chaunsa' (4.2 cm) and 'Saroli' (4.0 cm). Medium comprising of similar salts but without AC showed poor shoot length in var. 'Saroli' (2.0 cm), var. 'Chaunsa' (2.2 cm) and var. 'Langra' (2.3 cm). Medium comprising of micro and macrosalts of

Table 5. Impact of different treatments of MS and B5 salts, AC and sucrose on *in vitro* shoot length of three mango varieties under light.

Treatment	Saroli	Langra	Chaunsa
Micro and macrosalts of MS, AC 200 mg L^{-1}	4.0 ± 0.2^{c}	5.1 ± 0.4^{a}	4.2 ± 0.6^{bc}
Micro and macrosalts of MS	2.0 ± 0.5^{efgh}	2.3 ± 0.3^{de}	2.2 ± 0.4^{def}
Micro and macrosalts of B5, AC 200 mg L^{-1}	4.7 ± 1.2^{ab}	4.3 ± 0.7^{bc}	4.6 ± 0.3^{ab}
Micro and macrosalts of B5	1.5 ± 0.5^{hi}	2.1 ± 1.6^{efg}	1.3 ± 0.2^{i}
Microsalts-MS, macrosalts-B5, Sucrose 30 g L^{-1}	4.3 ± 1.3^{bc}	4.9 ± 0.8^{a}	4.3 ± 0.2^{bc}
Microsalts-MS, macrosalts-B5, Sucrose 40 g L^{-1}	2.7 ± 0.6^{d}	2.3 ± 0.4^{de}	2.2 ± 0.2^{def}
Microsalts-MS, macrosalts-B5, Sucrose 50 g L^{-1}	1.7 ± 0.6^{fghi}	1.6 ± 0.2^{ghi}	1.7 ± 0.3^{fghi}
Microsalts-MS, macrosalts-B5, Sucrose 60 g L^{-1}	1.5 ± 0.2^{hi}	1.7 ± 0.2^{fghi}	1.3 ± 0.9^{i}
Variety (mean)	3.0 ± 0.0^{a}	2.8 ± 0.1^{b}	2.7 ± 0.1^{b}
Source of variability			
Variety	0.008		
Treatment	< 0.001		
Variety × Treatment	0.025		

Mean values in columns with standard error denoted with different superscript letters show significance level at $p \leq 0.05$.

Fig. 4. Impact sucrose treatments, (a) 30 g L^{-1}, (b) 40 g L^{-1}, (c) 50 g L^{-1}, and (d) 60 g L^{-1} on *in vitro* shoot elongation and rooting in vars. 'Saroli', 'Langra', 'Chaunsa' under light.

B5, 200 mg L^{-1} AC enhanced shoot length in vars. 'Saroli' (4.7 cm), 'Chaunsa' (4.6 cm) and 'Langra' (4.3 cm), whereas the plantlets cultured on the medium with similar salts but lacking AC showed poor growth of plantlets. Medium comprising of 30 g L^{-1} sucrose, NAA (0.1 mg L^{-1}), BA (0.2 mg L^{-1}) induced significantly highest shoot length in vars. 'Langra' (4.9 cm), 'Saroli' (4.3 cm) and 'Chaunsa' (4.3 cm) (Figure 4a) than rest of the sucrose treatments (40, 50, 60 g L^{-1}) (Figure 4b-d). Al-Busaidi *et al*. [6] utilized B5 macrosalts and MS microsalts throughout all *in vitro* growth stages of mango including shoot regeneration. Litz [27] and Laxmi *et al.* [32] observed that 20 g L^{-1} sugar was important for plantlet formation in mango. Hemphill *et al.* [36] observed elongation of cotton (*G. hirsutism*) shoots cultured on medium comprising of 3 g L^{-1} AC. Current protocols also described positive impact of 200 mg L^{-1} AC on mango shoot-root development under light.

3.6. Effect of Basal Salts, AC and Sucrose on Root Elongation under Light (16h Photoperiod)

Treatment effect (p<0.001) significantly influenced the root elongation, and effect of variety found significant but on the least level (p<0.046), whereas the interaction effect of both failed to express any significance in root elongation (Table 6). Data in Table 6 show that highest root length was obtained on medium comprising of micro and macrosalts of MS, 200 mg L^{-1} AC in var. 'Langra' (4.0 cm) followed by 'Chaunsa' (3.7 cm) and 'Saroli' (3.2 cm). On the contrary, the root length was decreased significantly on medium comprising of micro and macrosalts of MS but without AC. Medium consisted of micro and macrosalts of B5 with 200 mg L^{-1} AC improved root length in vars. 'Saroli' (3.2 cm), 'Chaunsa' (3.1 cm), 'Langra' (3 cm), whereas the medium with similar salts but without AC caused significant reduction in root length. Simultaneously, the significantly highest root length was obtained on the medium comprising of 30 g L^{-1} sucrose, microsalts of MS, macrosalts of B5. Out of four concentrations of sucrose (30, 40, 50 and 60 g L^{-1}), with microsalts of MS and macrosalts of B5, only 30 g L^{-1} sucrose produced highest root length in var. 'Langra' (3.8 cm) (Figure 4a), var. 'Saroli' (3.6 cm) and var.'Chaunsa' (3 cm). Laxmi *et al.* [32] recommended lower sucrose quantity in addition to B5 macrosalts, MS microsalts for germination SE of mango, led to the plantlet formation. In the current study noted that 30 g L^{-1} sucrose was effective in better growth of roots. Obtained results are in agreement with Abul-Soad *et al.* [7] regarding vigorous growth of roots in mango shoots on medium comprising of 30 g L^{-1} sucrose in addition to 200 mg L^{-1} AC, B5 macrosalts, MS microsalts. Ara *et al.* [37] suggested a procedure for rooting in mango plantlets obtained from nucellar SE and described that IBA was most responsive in rooting. NAA (0.1 mg L^{-1}) is also

Table 6. Impact of different treatments of MS and B5 salts, AC and sucrose on *in vitro* root length of three mango varieties under light.

Treatment	Saroli	Langra	Chaunsa
Micro and macrosalts of MS, AC 200 mg L^{-1}	3.2 ± 0.7^{bcd}	4.0 ± 0.6^{a}	3.7 ± 0.7^{ab}
Micro and macrosalts of MS	1.6 ± 0.4^{efgh}	1.5 ± 0.8^{efghi}	1.8 ± 0.4^{ef}
Micro and macrosalts of B5, AC 200 mg L^{-1}	3.2 ± 0.5^{bcd}	3.0 ± 1.6^{d}	3.1 ± 0.5^{cd}
Micro and macrosalts of B5	1.4 ± 0.7^{fghi}	1.7 ± 1.2^{efg}	1.0 ± 0.3^{i}
Microsalts-MS, macrosalts-B5, Sucrose 30 g L^{-1}	3.6 ± 1.3^{abc}	3.8 ± 0.2^{a}	3.0 ± 0.2^{d}
Microsalts-MS, macrosalts-B5, Sucrose 40 g L^{-1}	1.6 ± 0.6^{efgh}	2.0 ± 0.3^{e}	1.5 ± 0.7^{efghi}
Microsalts-MS, macrosalts-B5, Sucrose 50 g L^{-1}	1.3 ± 0.3^{fghi}	1.2 ± 0.8^{ghi}	1.0 ± 0.3^{i}
Microsalts-MS, macrosalts-B5, Sucrose 60 g L^{-1}	1.1 ± 0.3^{hi}	1.0 ± 0.6^{i}	1.0 ± 0.4^{i}
Variety (mean)	2.1	2.3	2.0
Source of variability			
Variety	0.046		
Treatment	< 0.001		
Variety × Treatment	0.137		

Mean values in columns with standard error denoted with different superscript letters show significance level at $p \leq 0.05$.

a extensively utilized PGR in rooting media for growth of different plant species including date palm [38].

4. CONCLUSION

Successful somatic embryogenesis and plantlet regeneration, shoot elongation and rooting was obtained using nucellar explants obtained from immature fruits of vars.'Saroli', 'Langra' and 'Chaunsa' through direct and indirect somatic embryogenesis. Surface sterilization of immature fruits resulted in maximum survival of initial nucellar explants. PGRs were observed effective for callogenesis, direct and indirect somatic embryogenesis. Browning was reduced using ascorbic acid, AC, and culture conditions (i.e full dark). 2,4-D and 2iP combinations induced embryogenic callus or direct somatic embryogenesis. High proliferation of proembryogenic callus in dark, somatic embryogenesis and plantlet regeneration accomplished under light conditions. Different experiments were conducted at shoot elongation and rooting stages improved the shoot and root growth and elongation. Sucrose 30 g L^{-1}, microsalts of MS and macrosalts of B5 were better for healthy growth of plantlets. The current *in vitro* protocols of the superior monoembryonic mango varieties will be helpful to propagate other superior monoembryonic and polyembryonic varieties grown in the area and worldwide. Current study described the protocols induced direct somatic embryogenesis will support to obtain true-to-type plantlets of the elite varieties.

5. CONFLICT OF INTEREST

There is no competing interest among the authors.

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