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Original Research Paper

In vitro multiplication of clonal apple rootstock MM111

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ABSTRACT

The present investigation was carried out in five stages with an aim to establish the rapid and most economically viable methodology to facilitate *in vitro* propagation of clonal rootstock MM111 of apple using shoot tip and nodal segment as explants (0.5 to 0.75 cm). For culture establishment, MS medium was used either alone or in combination with 1.0 to 3.0 mg/L BAP and 1.0 to 3.0 mg/L GA_3. Rooting medium involved half-strength MS medium with 0.5 to 2.0 mg/L NAA and 0.5 to 2.0 mg/L IBA along with 0.2% activated charcoal. The highest culture establishment (61.11 and 77.66%) in minimum days (32.20 and 25.40) was recorded with MS + 1.0 mg/L BAP + 1.0 mg/L GA_3 for shoot tip and nodal segment explants, respectively. Maximum number of shoots (2.43 and 5.43) and shoot length (3.21 and 3.47 cm) were recorded using MS + 1.0 mg/L BAP + 1.0 mg/L GA_3 with an addition of 0.2% NAA. The media composition involving half-strength MS + 1.0 mg/L NAA produced the highest rooted cultures (66.67%) in fewest days (13.00) amongst all the media compositions. Furthermore, the maximum survival rate (76.67%) of *in vitro* raised plantlets was observed with soil + sand + vermiculite + FYM (1:1:1:1, v/v/v/v).

Keywords: Apple, clonal rootstock, *in vitro*, MM111

INTRODUCTION

Apple is a widely recognized fruit appreciated for its sweet taste and rich nutritional content. In India, apples are embraced both as fresh produce and for various processed products, making them a popular choice (Lal et al., 2023a). The cultivation of apples in India is primarily concentrated in the temperate parts of Jammu and Kashmir, Himachal Pradesh, and Uttaranchal, with Jammu and Kashmir leading in apple production, yielding 355.25 million tonnes annually from 28.77-million-hectare area (Anonymous, 2024). However, India's total apple production of 2452000 metric tonne, from 314,000 hectares, lags behind advanced fruit producing countries where apple orchards employ dwarfing clonal rootstocks.

The main challenges hindering the adoption of high-density plantations (HDP) in the Himalayan states, particularly in Jammu and Kashmir, is the partial accessibility to clonally propagated rootstocks. The majority clonal rootstocks of apple are traditionally propagated using the seasonal mound layering technique (Lal et al., 2021b), which has slow multiplication rate, requires significant space and labour, and yields planting material that is not financially viable for the farmers (Kakimzhanova et al., 2023). In contrast, *in vitro* propagation techniques offer an efficient and effective means of producing high-quality planting material that is genetically consistent, requiring less time and space (Ma et al., 2024). Apple rootstock MM111 has been developed as a hybrid between Northern Spy and M 793. It is vigorous in nature and resistant against drought conditions as well as woolly apple aphid attack. Multiplication of MM111 or any other clonal apple rootstock using tissue culture techniques is more economically feasible, which gives millions of disease-free plants in a very short period of time and in a very limited space (Kermani et al., 2009; Modgil et al., 2010), as compared to the traditional methods like mound layering, where we can get a maximum of 4-5 plants from a single mother plant.

In light of the significance of rootstock MM111 under Indian conditions and its increasing demand in the Union Territory of Jammu and Kashmir, the current study aimed to develop a practical commercial method for *in vitro* multiplication of MM111 using shoot tips and nodal segments.

MATERIALS AND METHODS

Selection of explants

About 10-15 cm actively growing shoots of the clonal rootstock MM111 of apple were taken from the 2-3-year-old mother block located at Regional Horticulture Research Sub-Station, Bhaderwah, of Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, India.

Preparation and sterilization of explants

Explants were taken from the shoot tip (0.5 to 0.75 cm) and nodal segment (0.5 to 0.75 cm). The explants were treated for 20 minutes with 0.2 per cent bavistin solution, then for varied periods of time with $HgCl_2$ (0.05, 0.1, and 0.2%) and NaOCl (3.0, 8.0, and 12.0%), respectively, for 2, 4, and 6 and 10, 20 and 30 minutes. Explants were rinsed three to four times in sterile (autoclaved) distilled water after surface sterilization to get rid of any adhering traces of sterilizing solution.

Culture establishment

Basal MS medium and MS media enriched with (1.0-3.0 mg/L) BAP and (1.0-3.0 mg/L) GA_3 separately as well as in combination were employed for *in vitro* cultural establishment of explants. Strong bud break explants were selected to be transplanted to new media for additional growth and the development of multiple shoots.

Shoot proliferation

After six weeks, nodal segments with budding shoots and growing shoot tips were transferred to shoot proliferation media augmented with MS basal or MS media comprising BAP (1.0-3.0 mg/litre) and GA_3 (1.0-3.0 mg/litre) alone or in combination with NAA (0.2%).

In vitro rooting

In vitro growing shoots were transferred to rooting media with half-strength MS medium that included IBA (1.0-3.0 mg/L) and NAA (1.0-3.0 mg/L) either separately or in combination with 0.2% activated charcoal in order to establish roots. The auxin was excluded from the media of the control group.

Hardening of *in vitro* raised plantlets

Complete rooted plantlets with shoots and roots were taken out of the culture tubes, and remaining agar was washed away with tap water at the root area. The plantlets were placed in containers that contained sterilized mixture of different materials, including soil (100%), sand (100%), vermiculite (100%), FYM (100%), sand + soil + vermiculite (1:1:1), sand + soil + FYM (1:1:1), sand + soil + vermiculite + FYM (1:1:1), and sand + soil + vermiculite + FYM (1:1:1:1). The different potting mixtures were tried in this stage in order to standardize the best out of all combinations. Before being effectively established in the outside environment, the rooted plantlets underwent a three-week primary hardening process in the laboratory, followed by three weeks in the greenhouse. After six weeks, statistics on the percentage of cultures that were aseptic and survived, the rate at which cultures were established, how quickly they multiplied, how well shoots rooted, and the number of plantlets that survived were recorded.

Statistical analysis

The data generated was analyzed in completely randomized block design, to work out the significant differences among different treatments.

RESULTS AND DISCUSSION

Culture establishment

The highest levels of aseptic conditions (80.00% for shoot tips and 86.67% for nodal segments) and culture survival rates (63.67% for shoot tips and 70.33% for nodal segments) in clonal apple rootstock MM111 were accomplished with 0.1% mercuric chloride for 4 minutes. Whereas, the highest culture establishment (68.89%) was recorded under EM8 (MS + 1.0 mg/L BAP + 1.0 mg/L GA_3) which was statistically at par with 67.78% in EM2 (MS + 1.0 mg/L BAP). This could be because of MS medium supplemented with BAP and auxin led to better sprouting and establishment (Boudabous et al., 2010). According to Sharma et al. (2000), it may also be due to the endogenous level of hormones, nutrients, metabolites and interaction between various factors that results in effective culture establishment. However, minimum culture establishment (0.00%) was recorded in control because of absence of BAP or any other growth hormones in MS medium prevented any culture establishment (Lal et al., 2021c). Between the two explants, nodal segment recorded maximum culture establishment (41.94%), which was significantly higher than the shoot tip (30.83%). The interaction

Table 1 : Effect of media composition on culture establishment of MM111 rootstock

Media code	Treatment	Culture establishment (%)			Culture establishment (days)		
		Tip of shoots	Nodal segment	Mean	Tip of shoots	Nodal segment	Mean
EM1	Control: MS (Basal)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-	-	-
EM2	MS + BAP (1.0 mg/L)	60.00 (50.75)	75.56 (60.35)	67.78 (55.55)	32.27	25.47	28.87
EM3	MS + BAP (2.0 mg/L)	50.00 (44.98)	63.33 (52.72)	56.66 (48.85)	33.33	27.53	30.43
EM4	MS + BAP (3.0 mg/L)	36.66 (37.24)	46.66 (43.06)	41.66 (40.15)	36.60	30.53	33.57
EM5	MS + GA_3 (1.0 mg/L)	40.00 (39.20)	54.44 (47.53)	47.22 (43.36)	33.43	27.67	30.55
EM6	MS + GA_3 (2.0 mg/L)	30.00 (33.17)	43.33 (41.14)	36.66 (37.15)	35.20	30.20	32.70
EM7	MS + GA_3 (3.0 mg/L)	20.00 (26.50)	30.00 (33.17)	25.00 (29.83)	38.27	32.27	35.27
EM8	MS + BAP (1.0 mg/L) + GA_3 (1.0 mg/L)	61.11 (51.40)	76.66 (61.12)	68.89 (56.26)	32.20	25.40	28.80
EM9	MS + BAP (1.0 mg/L) + GA_3 (2.0 mg/L)	26.66 (31.04)	40.00 (39.21)	33.33 (35.13)	35.43	30.40	32.92
EM10	MS + BAP (1.0 mg/L) + GA_3 (3.0 mg/L)	16.66 (24.08)	27.77 (31.78)	22.22 (27.93)	38.47	32.47	35.47
EM11	MS + BAP (2.0 mg/L) + GA_3 (1.0 mg/l)	51.11 (45.61)	64.44 (53.37)	57.78 (49.49)	33.27	27.43	30.35
EM12	MS + BAP (2.0 mg/l) + GA_3 (2.0 mg/L)	23.33 (28.87)	33.33 (35.24)	28.33 (32.05)	38.57	32.53	35.55
EM13	MS + BAP (2.0 mg/L) + GA_3 (3.0 mg/L)	13.33 (21.40)	23.33 (28.87)	18.33 (25.13)	38.93	32.83	35.88
EM14	MS + BAP (3.0 mg/L) + GA_3 (1.0 mg/L)	41.11 (39.86)	55.55 (48.16)	48.33 (44.01)	33.17	27.53	30.35
EM15	MS + BAP (3.0 mg/L) + GA_3 (2.0 mg/L)	13.33 (21.40)	20.00 (26.50)	16.66 (23.95)	39.33	33.33	36.33
EM16	MS + BAP (3.0 mg/L) + GA_3 (3.0 mg/L)	10.00 (18.42)	16.66 (24.01)	13.33 (21.22)	40.27	34.13	37.20
Mean		30.83 (32.12)	41.94 (39.14)		35.92	29.98	
$CD_{(0.05)}$		Explant (E) = 0.64 Media (M) = 1.79 E × M = 2.54			Explant (E) = 0.04 Media (M) = 0.11 E × M = 0.16		

Figures in parenthesis are *arc sine* transformed values

data revealed that nodal segment explants with EM8 recorded maximum culture establishment (76.66%), while shoot tip recorded only (61.11%) with EM8 (Table 1). This could be due to the endogenous concentrations of hormones, nutrients, and metabolites, as well as the interactions between numerous components (Sharma et al., 2000). The basal medium recorded minimum culture establishment (0.00%) for both the explants.

The explants cultured in media composition EM8 took minimum number of days (28.80) for culture establishment, which was statistically at par with EM2 (28.87 days). However, maximum number of days (37.20) taken for culture establishment was recorded under EM16. Between the two explants, nodal segments took minimum number of days (29.98) for culture establishment cultured in EM8 media as compared to significantly higher number of days (35.92) for establishment when shoot tips were used as explant. According to Singh & Pandey (2004), depending upon the endogenous factors, requirement of exogenous applications varies for getting optimum response of cultured explants. The interaction effect of explants and media composition showed a significant effect on percent culture establishment and number of days for culture establishment (Table 1). Nodal segment took minimum number of days for culture establishment (25.40) under EM8 followed by EM2 (25.47 days), whereas shoot tips took 32.20 days with EM8 for culture establishment followed by EM2 (32.27 days). This may due to the quantitative interaction between several growth factors which may play a crucial part in culture organogenesis (Skoog & Miller, 1957). Maximum number of days taken for culture establishment were 34.13 and 40.27 in nodal segment and shoot tip explants, respectively recorded in EM16 media. Our results are in conformity with Lizzarraga et al. (2017) who reported highest survival and minimum days required to culture establishment with the combined application of BAP and GA_3 in apple cultivar Principe Grande.

In vitro shoot proliferation

When shoots excised from established multiple shoots cultures, were placed vertically, proliferation was seen in all the combination tested (Table 2). It was observed that media composition MM8 (MS + 1.0 mg/L

Table 2 : Effect of media composition on shoot proliferation of MM111 rootstock

Media code	Treatment	Shoot proliferation (days)			Shoots/explants (Nos.)			Shoot length (cm)		
		Tip of shoots	Nodal segment	Mean	Tip of shoots	Nodal segment	Mean	Tip of shoots	Nodal segment	Mean
MM1	Control: MS (Basal)	-	-	-	-	-	-	-	-	-
MM2	MS + BAP (1.0 mg/L)	51.44	45.00	48.22	2.33	5.33	3.83	3.11	3.30	3.21
MM3	MS + BAP (2.0 mg/L)	53.77	47.57	50.67	2.00	4.66	3.33	2.82	3.15	2.98
MM4	MS + BAP (3.0 mg/L)	55.77	49.57	52.67	1.33	3.33	2.33	2.54	2.87	2.71
MM5	MS + GA_3 (1.0 mg/L)	54.44	48.14	51.29	1.66	4.33	3.00	2.75	2.93	2.84
MM6	MS + GA_3 (2.0 mg/L)	56.44	50.20	53.32	1.26	3.00	2.13	2.44	2.77	2.60
MM7	MS + GA_3 (3.0 mg/L)	58.84	52.43	55.63	1.00	2.66	1.83	2.24	2.57	2.41
MM8	MS + BAP (1.0 mg/L) + GA_3 (1.0 mg/L)	50.84	44.70	47.77	2.43	5.43	3.93	3.21	3.47	3.34
MM9	MS + BAP (1.0 mg/L) + GA_3 (2.0 mg/L)	56.67	50.37	53.52	1.16	2.86	2.01	2.34	2.67	2.51
MM10	MS + BAP (1.0 mg/L) + GA_3 (3.0 mg/L)	59.21	52.67	55.94	0.87	2.60	1.73	2.14	2.47	2.31
MM11	MS + BAP (2.0 mg/L) + GA_3 (1.0 mg/L)	53.34	47.10	50.22	2.13	4.76	3.45	2.96	3.20	3.08
MM12	MS + BAP (2.0 mg/L) + GA_3 (2.0 mg/L)	59.37	50.57	54.97	1.13	2.73	1.93	2.18	2.51	2.35
MM13	MS + BAP (2.0 mg/L) + GA_3 (3.0 mg/L)	59.67	53.10	56.39	0.66	2.33	1.50	1.98	2.31	2.15
MM14	MS + BAP (3.0 mg/L) + GA_3 (1.0 mg/ L)	54.24	48.00	51.12	1.73	4.43	3.08	2.83	2.98	2.90
MM15	MS + BAP (3.0 mg/L) + GA_3 (2.0 mg/L)	59.80	53.67	56.74	0.53	2.26	1.40	1.92	2.25	2.09
MM16	MS + BAP (3.0 mg/L) + GA_3 (3.0 mg/L)	60.15	54.11	57.13	0.33	1.66	1.00	1.82	2.15	1.99
Mean		56.27	49.81		1.37	3.49		2.49	2.77	
$CD_{(0.05)}$		Explant (E) = 0.17 Media (M) = 0.48 E × M = 0.69						Explant (E) = 0.02 Media (M) = 0.04 E × M = 0.06		

*NAA 0.2% was added to each treatment

BAP + 1.0 mg/L GA_3) took minimum number of days (47.77) for *in vitro* shoot proliferation in clonal apple MM111 rootstock which was found to be statistically at par with MM2 (48.22 days).

According to Lizarraga et al. (2017) better shoot proliferation and growth of shoots was found with MS medium supplemented with BAP (1.0 mg/L) and GA_3 (1.0 mg/L) along with 0.2% NAA, whereas, higher concentration of BAP and GA_3 deteriorates further growth and development. However, the effectiveness of any of these cytokinin in the multiplication medium will depend largely on genotype of the cultivar or may be due to their action (Kour & Singh, 2012). The maximum number of days (57.13) taken for shoot proliferation was recorded under MM16. Between the two explants, nodal segment took minimum number of days (49.81) for shoot proliferation, which was significantly higher than the shoot tip (56.27 days). The interaction data of explants and shoot proliferation media revealed that nodal segment explants under MM8 took minimum number of days (44.70) for shoot proliferation as compared to shoot tip (50.84) in MM8. However, highest number of days taken for shoot proliferation in nodal segment and shoot tip were 54.11 and 60.15, respectively was recorded under MM16 (MS + 3.0 mg/L BAP + 3.0 mg/L GA_3). The data also revealed maximum number of shoots (3.93) were recorded in MM8 media which was statistically at par with MM2 (3.83). This could be because of the presence of BAP in the MS medium which promotes maximal proliferation rate and number of shoots because of its cell division activity (Ghanbari, 2014). The minimum number of shoots (1.00) per explant was recorded under MM16 media. Between the two explants, nodal segment recorded maximum number of shoots (3.49) per explant which was significantly

superior to the shoot tip (1.37). In the interaction effect of nodal segments and shoot tips, nodal segments recorded maximum number of shoots per explants (5.43) with MM8 followed by MM2 (5.33), while shoot tip recorded only 2.43 with MM8 followed by MM2 (2.33). This may be because of the presence of 1.0 mg/litre BAP in the nutrient medium gives a greater number of shoots in MM106 and Anna (Guadie et al., 2016). However, minimum number of shoots (0.33 and 1.66) per explant in shoot tip and nodal segment, respectively was recorded under MM16. This might be because higher BAP and GA_3 concentrations in the media hindered the apple explant culture's ability to grow and develop further (Lal et al., 2023a).

Different media composition also significantly influenced the shoot length of the explants. Media composition MM8 resulted in maximum shoot length (3.34 cm) which was significantly superior among all other media composition. According to Ahmed et al. (2014), hormone treatment starts the cell on a specific development pathway and the alternative view is that hormone responsive cells are already determined and hormones evoke the expression of the combined state. The minimum shoot length (1.99 cm) was recorded under MM16. Between the two different explants, nodal segment recorded maximum shoot length (2.77 cm) which was significantly superior to the shoot tip (2.49 cm). The interaction data revealed that the maximum shoot length (3.47 cm) was recorded with nodal segments cultured in MM8 followed by 3.30 cm in MM2, while shoot tips cultured on MM8 recorded shoot length (3.21 cm) followed by 3.11 cm in MM2. However, minimum shoot length (1.82 and 2.15 cm) in shoot tip and nodal segment, respectively was recorded under MM16. This could be because of higher concentration of BAP and GA_3 which deteriorates further growth and development (Lizarraga et al., 2017). Results are in conformity with the findings of Castillo et al. (2015) who reported that MS medium supplemented with BAP and GA_3 was found to be significant with respect to number of shoots, shoot length and number of leaves per explants of CG41 apple rootstock.

In vitro root induction

Half-strength MS containing NAA has been found to be more efficient and provided the best rooting response among the auxins (NAA and IBA) used (Table 3). The media composition RM6 (half MS + 1.0 mg/litre NAA), was statistically superior to all other media compositions, and showed the highest rooting percentage (66.67). During *in vitro* multiplication of Merton 793 apple rootstock, Soni et al. (2010) reported that the presence of NAA in the medium was found to be more efficient than IBA and IAA with respect to rooting percentage. However, with RM16 (half MS + 2.0 mg/litre IBA + 2.0 mg/litre NAA), only 13.33 rooting percentage was attained. Regarding the length of time required for rooting, it was found that the minimum number of days (13.00) required for root initiation was recorded under RM6 was statistically equivalent to 13.33 in RM8 (half MS + 0.5 mg/litre IBA + 0.5 mg/litre NAA), while the longest duration of time (21.33 days) was required in media composition RM16. The addition of activated charcoal to the rooting medium makes it easier for clonal apple rootstocks to grow and spread since it facilitates the absorption of auxins from the media (Sharma et al., 2007). Renu et al. (2018) reported that NAA supported the highest rooting with an average of 7.66 numbers and a root length of 2.2 cm in Golden Delicious apple explants. RM6 recorded the maximum numbers of roots (5.00), which was statistically equivalent to RM3 (4.67). The longest root length was obtained in media composition RM6 (3.70 cm), which was statistically comparable to root lengths of 3.63 cm in RM3 and 3.33 cm in M9, while RM16 measured the shortest shoot length of 1.69 cm. This might be due to the difference in the plant growth regulators and their concentrations used in the multiplication medium (Shabani et al., 2015). Dalal et al. (2006) noted that the *in vitro* rooting was greatly influenced by half-strength MS media supplemented with NAA and 0.2 per cent activated charcoal. It is well known that half strength MS medium also reduces the callus formation (Sharma et al., 2007).

Effect of potting mixture on survival

Different potting mixtures had a significant impact on the percentage of *in vitro* produced MM111 clonal apple rootstock plantlets that survived (Table 4). The combination of SM7 (soil + sand + vermiculite + FYM) had the highest survival rate (76.67%) and was statistically the best of all the other combinations. Mixing sand, soil, vermiculite and FYM in equal volumes might have helped in giving better grip for roots ample aeration and sufficient amount of organic

Table 3 : Effect of media composition on *in vitro* rooting of MM111 rootstock

Media code	Treatment	Cultures rooted (%)	Rooting (days)	Roots/explant (Nos.)	Root length (cm)
RM1	Control: Half MS (Basal)	0.00 (0.00)	-	-	-
RM2	Half MS + IBA (0.5 mg/L)	43.33 (37.17)	15.67	3.67	2.8
RM3	Half MS + IBA (1.0 mg/L)	53.33 (43.07)	14.33	5	3.65
RM4	Half MS + IBA (2.0 mg/L)	36.67 (33.17)	18	3	2.3
RM5	Half MS + NAA (0.5 mg/L)	46.67 (41.15)	15.33	4.67	2.86
RM6	Half MS + NAA (1.0 mg/L)	66.67 (50.74)	13	5.67	3.75
RM7	Half MS + NAA (2.0 mg/L)	40.00 (35.23)	17	3.33	2.36
RM8	Half MS + IBA (0.5 mg/L) + NAA (0.5 mg/L)	33.33 (30.99)	13.33	3	2.69
RM9	Half MS + IBA (0.5 mg/L) + NAA (1.0 mg/L)	50.00 (41.15)	14.67	4.33	3.35
RM10	Half MS + IBA (0.5 mg/L) + NAA (2.0 mg/L)	26.67 (21.30)	15.67	2.33	2.16
RM11	Half MS + IBA (1.0 mg/L) + NAA (0.5 mg/L)	30.00 (31.08)	18	2.67	2.56
RM12	Half MS + IBA (1.0 mg/L) + NAA (1.0 mg/L)	46.67 (39.21)	17.33	4	3.25
RM13	Half MS + IBA (1.0 mg/L) + NAA (2.0 mg/L)	23.33 (26.50)	18.67	2	2
RM14	Half MS + IBA (2.0 mg/L) + NAA (0.5 mg/L)	20.00 (18.42)	19	2	1.96
RM15	Half MS + IBA (2.0 mg/L) + NAA (1.0 mg/L)	23.33 (26.50)	19.67	2.67	2.2
RM16	Half MS + IBA (2.0 mg/L) + NAA (2.0 mg/L)	13.33 (14.96)	21.33	1.67	1.8
$CD_{(0.05)}$		3.34	0.34	0.34	0.13

Figures in parenthesis are *arc sine* transformed values

matter (Karwa et al., 2003). The combination of soil + sand + vermiculite (SM5), had the survival rate of 66.67% for *in vitro* grown plantlets of MM111 followed by 53.33% with SM6 (soil + sand + FYM) and SM3 (46.67), and the lowest survival rate (0.00%) was found in SM1 (100% sand). The distinct morphological and physiological traits of hardening plantlets served as a critical process to aid in their adaption to natural environmental conditions (Modgil et al., 2010). Maximum plant height (3.30 cm) was observed in SM7 which outperformed all other combinations, whereas, 2.87 cm was recorded in SM5, that was followed by SM6 (2.13 cm), SM3 (0.82 cm), and SM2 (100% soil), with 0.60 cm being the lowest (Fig. 1). However, SM7 was found to have the most leaves (10.00), making it statistically the best combination out of all the others. In the media combination, SM5 recorded the most leaves (8.00), followed by SM6 (7.00), and SM3 (6.33), while, SM2 recorded the fewest leaves (5.00).

A. *In vitro* culture establishment | B. *In vitro* shoot proliferation | C. *In vitro* rooting | D. Hardening

Fig. 1 : *In vitro* multiplication of apple rootstock MM111

Table 4 : Effect of the potting mixture on survival of plantlets of MM111 rootstock

Media Code	Treatment	Survival (%)	Plant height (cm)	Leaves (Nos.)
SM1	Sand (100%)	0.00 (0.00)	-	-
SM2	Soil (100%)	26.67 (26.50)	0.52	5.00
SM3	Vermiculite (100%)	46.67 (43.06)	0.82	6.33
SM4	FYM (100%)	33.33 (35.23)	0.60	5.67
SM5	Soil + sand + vermiculite (1:1:1 v/v/v)	66.67 (54.71)	2.73	8.00
SM6	Soil + sand + FYM (1:1:1 v/v/v)	53.33 (43.89)	1.94	7.00
SM7	Soil + sand + vermiculite + FYM (1:1:1:1 v/v/v/v)	76.67 (61.12)	3.23	10.00
$CD_{(0.05)}$		6.66	0.08	0.67

Figures in parenthesis are arc sine transformed values

CONCLUSION

In vitro methods of propagating clonal apple rootstocks would ensure bulk production of true-to-type and insect, pest, and disease-free planting material throughout the season. In present study, a protocol was standardized for fastest multiplication of MM111 clonal apple rootstock. Highest culture establishment in the shortest number of days was recorded with MS + 1.0 mg/L BAP + 1.0 mg/L GA_3 which also resulted in most shoot proliferation. *In vitro* multiplied shoots performed best when rooted in a half strength MS + 1.0 mg/L NAA + 0.2% activated charcoal solution, while best hardening results were obtained in a potting mixture containing equal parts sand, soil vermiculite, and FYM.

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