

Original Research Paper

***In vitro* rooting protocol for clonal rootstocks of apple (*Malus × domestica* Borkh.)**

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ABSTRACT

Geneva (G.) apple clonal rootstocks are a novel series known for their super-dwarfing nature, cold hardiness, disease and insect-pest resistance, and resilience to replant disease. This study evaluated the *in vitro* rooting performance of two specific clonal rootstocks i.e. G.41 and G.214, using 1.5 cm shoot cultures. The microshoots were cultured on full- and half-strength Murashige and Skoog (MS) media, as well as Woody Plant Medium (WPM), each supplemented with varying concentrations (1.0–5.0 mg/L) of either NAA or IBA. Among the treatments, half-strength MS medium supplemented with 5.0 mg/L IBA induced the fastest response in the G.41 rootstock, resulting in root initiation at 6.54 days and root emergence at 10.63 days, outperforming G.214. Furthermore, G.41 achieved a perfect 100% rooting rate when grown on half-strength MS medium combined with IBA at 1.0, 3.0, or 5.0 mg/L. The interaction of G.41 with half-strength MS medium containing 5.0 mg/L IBA also yielded the highest number of roots per shoot (17.00), the maximum average root length (82.70 mm), and the longest individual root length (146.13 mm). Ultimately, this optimized protocol offers an efficient, year-round micropropagation strategy to rapidly produce true-to-type, virus-free, and elite clonal apple rootstocks.

Keywords: Apple, clonal rootstocks, *in-vitro*, rooting

INTRODUCTION

The domesticated apple (*Malus × domestica* Borkh.) is the predominant fruit crop widely grown in temperate areas of the world. Among temperate fruit crops, apple stands at first position both in area and production in the world (FAOSTAT, 2025). Over the last few years, the conventional apple orchards using standard trees with wider spacing have been replaced by high-density planting system where clonal rootstocks are used in place of seedling rootstocks (Mir et al., 2021). High density plantation is a recent concept to increase the productivity per unit area using clonal rootstocks of apple from M and MM series which imparts dwarfness, precocity and desired quality attributes to the scion (Kafle et al., 2021). Nevertheless, a malady of specific replant disease complex is manifested in high-density apple orchards of western Himalayan states of India with an incidence of 25-70 percent which has a deleterious impact on the yield and overall quality (Sharma et al., 2020). To overcome this problem, Geneva series of clonal rootstocks can be a prominent alternative. G.41 from

Geneva series is the new clonal hybrid rootstock of apple originated from a planned cross between *Malus domestica* ‘Malling 27’ × *Malus robusta* ‘Robusta 5’ in 1975 from Geneva, New York. It is an ultra-dwarfing rootstock i.e. the size of the trees raised on this rootstock are 15 to 20 per cent of the standard seedling tree, and has many attributes *viz.*, winter hardiness, resilience to powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*), and is not vulnerable to the woolly apple aphid (*Eriosoma lanigerum*) pest with non-susceptibility to the replant problem (Cummins et al., 2006). Another Geneva series rootstock G.214 is cold hardy and possesses resilience to crown and root rot diseases caused by *Phytophthora cactorum* and produces plants which are 30 to 35 per cent compared to that on seedling rootstocks. Besides, *Malus robusta* ‘Robusta 5’ contributes resistance to powdery mildew (*Podosphaera leucotricha*) and fire blight and immunity to the woolly apple aphid. Further, ‘Robusta 5’ has depicted non-vulnerability to the replant problem (Fazio et al., 2013). Therefore, Geneva series clonal apple rootstocks offering advantage over other



rootstocks should be multiplied and further tested in HDP under different agro-climatic conditions.

Apple clonal rootstocks are generally propagated through mound layering or trench layering. These methods are totally dependent on season, take longer time and require large land area for plantlet production and the resultant planting material produced may be exposed to soil borne pathogens, attacked by insect pests and viruses that lead to degradation in the quality of planting material. For the production of healthy, disease-resistant plants and with desirable traits in scions and rootstocks of apple rapid multiplication techniques have been done by many research groups employing micro-propagation under suitable *in vitro* conditions over the last two decades (Modgil & Thakur, 2017; Samanmalie et al., 2019; Verma et al., 2021) but there is a need of fast and efficient *in vitro* vegetative propagation method to pave the way for production of new tree fruit varieties/rootstocks that may be highly useful for commercial and private apple growers. Therefore, the present study focused on the development of a systematic and reliable protocol for *in-vitro* rooting for micropropagation using various growth regulators under different concentrations.

MATERIALS AND METHODS

Explants of two clonal apple rootstocks (G.41 and G.214) maintained in Tissue Culture Laboratory, Seven Star Fruits Pvt. Ltd., Maharashtra, India, were taken to study the *in-vitro* rooting propensity. Shoot explants were further multiplied by a monthly sub-culture in standard multiplication medium in which 0.5 to 1.0 cm long shoots were transferred. *In vitro* growing shoots having length of 1.0-1.5 cm were used for rooting, which were then transferred to different rooting media for induction of roots. In the beginning, explants were treated for 20 minutes with 0.2 per cent bavistin solution, succeeded by HgCl_2 (0.05, 0.1 and 0.2%) and NaOCl (3.0, 8.0 and 12.0) for 2, 4 and 6 minutes and 10, 20 and 30 minutes, respectively.

In vitro rooting

For *in vitro* rooting, full and half-strength Murashige and Skoog and Woody Plant medium were prepared, augmented with IBA and NAA ranging from 1-5 mg/L were used. *In vitro* growing shoots (1.0-1.5 cm) of G.41 and G.214 clonal apple rootstocks were transferred to rooting media supplemented with 0.5 mg/ L thiamine, 100 mg/L myo-inositol,

30 g/L sucrose, NAA (1.0-5.0 mg/L) and IBA (1.0-5.0 mg/L) separately, in which concentrations of 1.0 mg/L, 3.0 mg/L and 5.0 mg/L were standardized from the range of 1.0-5.0 mg/L for both the growth regulators (NAA and IBA) and forty-eight treatment combinations were used. Days to root initiation was recorded one week after subculture by determining callus formation and expressed in days. Root emergence was recorded one week after callus formation and was determined by adventitious root formation and expressed in days. The roots of ten randomly cultured shoots from each replication and each treatment were counted after 4-5 weeks of culturing, averaged, and expressed in numbers. Rooting percentage was calculated with the help of the given formula after 4-5 weeks of culturing and was expressed in percentage.

$$\text{Per cent rooting} = \frac{\text{Number of rooted shoots}}{\text{Total number of shoots cultured}} \times 100$$

The root length of ten randomly cultured shoots from each replication and each treatment was measured with the help of a measuring scale after 4-5 weeks of culturing, averaged and expressed in millimetres. The data was analysed by comparing means using one way ANOVA and the significance was determined by CD ($p \leq 0.05$) using R software.

RESULTS AND DISCUSSION

Significantly earlier root initiation and root emergence was recorded in G.41 (9.83 and 11.89 days, respectively) rootstock as compared to G.214 which took 13.93 and 14.78 days for root initiation and root emergence, respectively (Table 1 & 2). While, comparing different rooting media, half strength WPM medium registered minimum number of days to root initiation i.e. 10.29 days followed by half strength MS medium which took 10.54 days to root initiation and both were found statistically at par, whereas, WPM medium took maximum number of days for root initiation i.e. 13.82 days. Statistically significant results were observed among growth regulators, i.e. IBA (11.26 days) and NAA (12.35 days) for root initiation and among their concentration as 5.0 mg/L took minimum number of days to root initiation (11.61 days) which was statistically higher among other concentrations. Interaction studies of media, growth regulators, their concentrations and rootstocks also had significant effect on number of days taken to root initiation, and minimum number of days to root

Table 1 : Influence of media and growth regulators on number of days taken to root initiation of clonal rootstocks of apple

Rootstock Media		Growth regulators								
		G ₁ : NAA			Sub mean	G ₂ : IBA			Sub mean	Overall mean
		C ₁ :1 mg	C ₂ : 3 mg	C ₃ : 5 mg		C ₁ : 1 mg	C ₂ : 3 mg	C ₃ : 5 mg		
S ₁ : G.41	M ₁ : MS	12.63	13.17	13.22	13.01	8.96	8.76	8.55	8.76	10.88
	M ₂ : ½ MS	7.44	7.54	7.96	7.65	7.00	6.99	6.54	6.84	7.25
	M ₃ : WPM	13.31	13.33	13.50	13.38	13.53	12.50	10.63	12.22	12.80
	M ₄ : ½ WPM	8.08	9.33	10.30	9.24	7.99	7.65	7.06	7.57	8.40
Sub mean		10.37	10.84	11.25	10.82	9.37	8.98	8.20	8.85	9.83
S ₂ : G.214	M ₁ : MS	14.10	14.22	14.32	14.21	14.33	14.30	14.22	14.28	14.25
	M ₂ : ½ MS	13.30	13.83	13.88	13.67	14.11	13.98	13.96	14.02	13.84
	M ₃ : WPM	14.55	14.97	15.10	14.87	15.18	14.98	14.33	14.83	14.85
	M ₄ : ½ WPM	12.11	12.99	13.22	12.77	13.22	12.33	9.21	12.78	12.77
Sub mean		13.52	14.00	14.13	13.88	14.21	13.90	14.17	13.98	13.93
Overall mean		11.94	12.42	12.68	12.35	11.79	11.44	10.56	11.26	
Factor mean	Media	M ₁ : 12.56			M ₂ : 10.54		M ₃ : 13.82		M ₄ : 10.29	
Concentration		C ₁ : 11.87			C ₂ : 11.93		C ₃ : 11.61			
CD _(p ≤ 0.05)										
Rootstock (S)			: 0.22							
Media (M)			: 0.32							
Growth regulators (G)			: 0.22							
Concentration (C)			: 0.25							
S x M x G x C			: 1.04							

Table 2 : Influence of media and growth regulators on root emergence (days) of clonal rootstocks of apple

Rootstock Media		Growth regulators								
		G ₁ : NAA			Sub mean	G ₂ : IBA			Sub mean	Overall mean
		C ₁ :1 mg	C ₂ : 3 mg	C ₃ : 5 mg		C ₁ : 1 mg	C ₂ : 3 mg	C ₃ : 5 mg		
S ₁ : G.41	M ₁ : MS	18.84	0.00	0.00	6.28	16.76	16.53	16.53	16.61	11.44
	M ₂ : ½ MS	12.63	12.96	13.96	13.18	11.30	11.00	10.63	10.98	12.08
	M ₃ : WPM	0.00	0.00	0.00	0.00	18.98	18.43	17.76	18.39	9.20
	M ₄ : ½ WPM	14.63	16.53	17.73	16.30	15.10	13.06	11.96	13.37	14.84
Sub mean		11.53	7.37	7.92	8.94	15.54	14.76	14.22	14.84	11.89
S ₂ : G.214	M ₁ : MS	21.63	0.00	0.00	7.21	20.73	19.96	19.77	20.15	13.68
	M ₂ : ½ MS	18.53	20.33	20.97	19.94	19.63	19.50	19.30	19.48	19.71
	M ₃ : WPM	0.00	0.00	0.00	0.00	0.00	21.88	21.33	14.40	7.20
	M ₄ : ½ WPM	18.53	19.11	19.63	19.09	18.50	18.20	17.30	18.00	18.55
Sub mean		14.67	9.86	10.15	11.56	14.72	19.89	19.43	18.01	14.78
overall mean		13.32	8.39	9.03	10.25	15.12	17.32	16.82	16.42	
Factor mean	Media	M ₁ : 12.56			M ₂ :15.89		M ₃ : 8.20		M ₄ : 16.69	
Concentration		C ₁ : 14.22			C ₂ : 12.85		C ₃ : 12.93			
CD (p ≤ 0.05)										
Rootstock (S)			: 0.22							
Media (M)			: 0.31							
Growth regulators (G)			: 0.22							
Concentration (C)			: 0.27							

Figures with zero values show no root emergence & further rooting

Table 3 : Influence of media and growth regulators on the rooting (%) of clonal rootstocks of apple

Rootstock Media		Growth regulators								
		G ₁ : NAA			Sub mean	G ₂ : IBA			Sub mean	Overall mean
		C ₁ :1 mg	C ₂ : 3 mg	C ₃ : 5 mg		C ₁ : 1 mg	C ₂ : 3 mg	C ₃ : 5 mg		
S ₁ : G.41	M ₁ : MS	59.99 (50.80)	0.00 (0.51)	0.00 (0.51)	20.00 (17.27)	70.00 (56.82)	72.22 (58.23)	73.33 (58.96)	71.85 (58.00)	45.92 (37.64)
	M ₂ : ½ MS	84.44 (67.00)	81.11 (64.29)	81.10 (64.34)	82.22 (65.21)	100.00 (89.47)	100.00 (89.47)	100.00 (89.47)	100.00 (89.47)	91.11 (77.34)
	M ₃ : WPM	0.00 (0.51)	0.00 (0.51)	0.00 (0.51)	0.00 (0.51)	54.44 (47.57)	62.22 (52.11)	64.44 (53.50)	60.37 (51.06)	30.18 (25.79)
	M ₄ : ½ WPM	79.99 (63.51)	73.33 (58.96)	66.66 (54.78)	73.33 (59.08)	76.66 (61.18)	81.10 (64.40)	89.99 (71.76)	82.58 (65.78)	77.96 (62.43)
	Sub Mean	56.11 (45.46)	38.61 (31.07)	36.94 (30.04)	43.89 (35.52)	75.28 (63.76)	78.89 (66.05)	81.94 (68.42)	78.70 (66.08)	61.29 (50.80)
S ₂ : G.214	M ₁ : MS	31.11 (33.91)	0.00 (0.51)	0.00 (0.51)	10.37 (11.64)	39.99 (39.24)	41.11 (39.89)	42.22 (40.54)	41.11 (39.89)	25.74 (25.77)
	M ₂ : ½ MS	40.00 (39.25)	37.77 (37.93)	34.44 (35.94)	37.40 (37.71)	48.88 (44.38)	50.00 (45.02)	51.11 (45.65)	50.00 (45.02)	43.70 (41.36)
	M ₃ : WPM	0.00 (0.51)	0.00 (0.51)	0.00 (0.51)	0.00 (0.51)	0.00 (0.51)	27.77 (31.81)	33.33 (35.28)	20.37 (22.53)	10.18 (11.52)
	M ₄ : ½ WPM	61.11 (51.44)	53.33 (46.94)	45.55 (42.47)	53.33 (46.95)	61.11 (51.44)	64.44 (53.51)	68.88 (56.25)	64.81 (52.48)	57.11 (49.16)
	Sub mean	33.06 (31.28)	22.78 (21.47)	20.00 (19.86)	25.28 (24.20)	37.49 (33.89)	45.83 (42.55)	42.22 (40.49)	41.84 (39.98)	34.18 (31.95)
Overall mean		44.58 (38.36)	30.69 (26.27)	28.47 (24.94)	34.58 (29.86)	56.80 (49.08)	61.94 (54.04)	65.41 (56.42)	62.78 (53.18)	
Factor Mean	Media	M ₁ : 35.83 (31.70)		M ₂ : 67.50 (59.35)		M ₃ : 20.18 (18.65)		M ₄ : 68.41 (56.39)		
Concentration		C ₁ : 50.69 (43.72)		C ₂ : 46.31 (40.16)		C ₃ : 46.94 (40.68)				
CD (p ≤ 0.05)										
Rootstock (S)		: 0.66			Media (M) : 0.94			Growth regulators (G) : 0.66		
Concentration (C)		: 0.81			S x M x G x C : 3.07					

Figures in parenthesis are arc sine transformed values of percentage data

initiation (6.54) were recorded in G.41 clonal rootstock with ½ MS medium supplemented with IBA (5.0 mg/L), which was statistically at par with G.41 with ½ MS medium supplemented with IBA 3.0 mg/L (6.99), whereas, maximum number of days for root initiation (15.18) was recorded for G.214 with WPM medium supplemented with IBA 1.0 mg/L. Full strength WPM medium recorded minimum days for root emergence (8.20 days), while, maximum it was in half strength WPM medium (16.69 days) (Table 2).

Minimum number of days to root emergence (10.25 days) was taken by NAA whereas IBA took maximum (16.42) days for root emergence and both the growth regulators were found to be statistically significant. Root emergence was first noticed at 3.0 mg/L (12.85 days) which was statistically at par with 5.0 mg/L (12.93 days), however, maximum days for root emergence was taken at 1.0 mg/L

(14.22 days). Significant results were obtained for interaction effect of media, growth regulators, their concentrations. Rootstocks early root emergence (10.63 days) was recorded for G.41 with ½ MS medium fortified with IBA 5.0 mg/L) followed by G.41 with ½ MS medium fortified with IBA (3.0 mg/L) and IBA (1.0 mg/L) with the value of 11.00 and 11.30 and all combinations were found to be statistically at par with each other, whereas, late root emergence (21.88 days) was noticed in G.214 cultured on full strength WPM medium supplemented with IBA 3.0 mg/L. Variations among the rootstock for root initiation and root emergence might be due to clonal variation in two different rootstocks (Amiri & Elahinia, 2011) and Modgil & Thakur (2017) who also reported that different genotypes had different potential of rooting. Fallahpour et al. (2015) and Magyar et al. (2002) also reported that ½ WPM and 5.0 mg/L took minimum

days to root initiation and root emergence as optimal concentration of IBA necessary for rooting is depended on genotype. Indole-3-butyric acid (IBA) showed early results for root initiation as compared to Naphthalene acetic acid (NAA) and the successful rooting could be due to the specific effect of IBA which was found to increase the level of endogenous free IAA as also reported by Nag et al. (2001). Podwyszynska & Cieślińska (2017) and Soni et al. (2011) also observed accelerated root emergence driven by naphthalene-acetic acid (NAA). Their findings demonstrate that applying NAA alone, or combined with indole-3-acetic acid (IAA) during a 7-day induction window, significantly improves rooting success in recalcitrant cultivars.

Differential gene expression (epigenetic variation) can affect plant micro-propagation (George et al., 2008). Significantly higher rooting (61.29%) was observed in G.41 as compared to G.214 (34.18%). Among growing media, significantly maximum rooting (68.51%) was obtained with $\frac{1}{2}$ strength WPM medium, while, minimum rooting (20.18 %) was recorded in full strength WPM medium (Table 3). Higher rooting percentage recorded was with half strength of WPM in G.41 might be due to less nutrient availability in half strength WPM due to which plant shoots try to access more nutrients by inducing root formation. So, half strength WPM may serve as an additional induction of roots along with rooting hormones. Fallahpour et al. (2015) also reported similar results.

Maximum rooting (67.78%) was recorded with IBA, while minimum (34.58%) with NAA. Maximum rooting (50.69%) was obtained in 1.0 mg/L, which was statistically higher among all other concentrations, whereas, minimum rooting was observed in 3.0 mg/L (46.31%). Rooting percentage was significantly influenced with the interaction effect of rootstocks, media, growth regulators and their concentrations and cent per cent (100%) rooting was observed in G.41 with $\frac{1}{2}$ strength MS medium+IBA 1.0 mg/L, 3.0 mg/L and 5.0 mg/L which was found to be statistically higher among all other interactions, while, minimum (27.77%) was recorded for G.214 having full strength WPM medium supplemented with IBA 3.0 mg/L.

Maximum number of roots/shoot (10.13), average root length (41.95 mm) and length of longest root (77.70 mm) was recorded in G.41, whereas, G.214

recorded minimum number of roots/shoot (5.64), average root length (15.07 mm) and length of longest root (27.81mm) (Table 4, 5 & 6). Amiri & Elahinia (2011) and Modgil & Thakur (2017) reported that different rootstocks show different rooting behaviour. Half strength MS medium recorded maximum number of roots/shoot (11.37), average root length (45.85 mm) and length of longest root (84.39 mm) where only number of roots/shoot was statistically at par with $\frac{1}{2}$ strength WPM medium (11.35), whereas, minimum number of roots/shoot (3.00), average root length (8.75 mm) and length of longest root (15.47 mm) was obtained full strength WPM medium. The main reason behind is that the half strength MS medium, shows low osmotic potential which creates partial nutrient stress and plants produce more roots which get easily acclimatized during hardening and promote autotrophism in plants, which is in conformity with Fadel et al. (2010).

IBA registered maximum number of roots per shoot (10.17), average root length (38.02 mm) and length of longest root (67.59 mm) as compared to NAA (5.70, 19.00 and 37.92 mm, respectively). Maximum roots per shoot, root length and longest root length was observed in IBA as compared to NAA and under root inducing ability there is slow and continuous release of IAA from IBA (Stefancic et al., 2005; Ali et al., 2009), and release of IBA through hydrolysis of conjugates (Ana et al., 2024). Statistically highest number of roots per shoot (8.33) was registered in 1.0 mg/L among other concentrations of growth regulators, however, lowest (7.73) was counted in 3.0 mg/L. Madhavrai et al. (2014), Haddadi et al. (2010) and Diengngan & Murthy (2014) in strawberry also reported best rooting at IBA 1.0 mg/L. Average root length was registered maximum (29.06 mm) with 5.0 g/L., whereas, maximum length of longest root (55.39 mm) was registered in 1.0 mg/L. Minimum average root length (27.75 mm) and length of longest root (50.57 mm) was recorded in 3.0 mg/L. Significantly higher number of roots per shoot (17.00), average root length (82.70 mm) and length of longest root (146.13 mm) was recorded during interaction effect of G.41 rootstock with $\frac{1}{2}$ strength MS medium + IBA (5.0 mg/L), followed by G.41 rootstock with $\frac{1}{2}$ strength MS medium + IBA 3.0 mg/L with the value of 15.52, 80.86 and 145.03 mm, respectively, whereas, lower number of roots per shoot (2.70), average root length (3.37 mm) and length of longest root

Table 4 : Influence of media & growth regulators on number of roots per shoot of clonal rootstocks of apple

Rootstock Media		Growth regulators								
		G ₁ : NAA			Sub mean	G ₂ : IBA			Sub mean	Overall mean
		C ₁ :1 mg	C ₂ : 3 mg	C ₃ : 5 mg		C ₁ : 1 mg	C ₂ : 3 mg	C ₃ : 5 mg		
S ₁ : G41	M ₁ : MS	9.5	0.00	0.00	3.17	12.57	12.60	12.66	12.61	7.89
	M ₂ : ½ MS	14.53	14.43	13.26	14.07	14.91	15.52	17.00	15.81	14.94
	M ₃ : WPM	0.00	0.00	0.00	0.00	9.25	10.16	10.55	9.99	4.99
	M ₄ : ½ WPM	12.78	11.69	10.67	11.71	12.87	13.62	14.65	13.71	12.71
Sub mean		9.20	6.53	5.98	7.24	12.40	12.98	13.72	13.03	10.13
S ₂ : G214	M ₁ : MS	2.83	0.00	0.00	0.94	6.72	7.44	7.82	7.33	4.14
	M ₂ : ½ MS	8.47	7.24	4.17	6.63	8.88	8.89	9.20	8.99	7.81
	M ₃ : WPM	0.00	0.00	0.00	0.00	0.00	2.70	3.39	2.03	1.02
	M ₄ : ½ WPM	9.42	9.21	8.63	9.09	10.30	10.52	11.88	10.41	9.62
Sub mean		5.18	4.11	3.20	4.16	6.48	7.39	6.80	7.19	5.64
Overall mean		7.19	5.32	4.59	5.70	9.46	10.15	10.89	10.17	
Factor mean	Media	M ₁ : 6.01		M ₂ : 11.37		M ₃ : 3.00		M ₄ :11.35		
Concentration		C ₁ : 8.33		C ₂ : 7.73		C ₃ : 7.74				
CD _(p ≤ 0.05)										
Rootstock (S)		: 0.27								
Media (M)		: 0.38								
Growth regulators (G)		: 0.27								
Concentration (C)		: 0.33								
S x M x G x C		: 1.25								

Table 5 : Influence of media & growth regulators on the average root length (mm) of clonal rootstocks of apple

Rootstock		Growth regulators								
Media		G ₁ : NAA			Sub mean	G ₂ : IBA			Sub mean	Overall mean
		C ₁ :1 mg	C ₂ : 3 mg	C ₃ : 5 mg		C ₁ : 1 mg	C ₂ : 3 mg	C ₃ : 5 mg		
S ₁ : G41	M ₁ : MS	31.10	0.00	0.00	10.37	38.03	39.63	60.00	45.89	28.13
	M ₂ : ½ MS	68.78	66.57	61.56	65.64	74.80	80.86	82.70	79.43	72.53
	M ₃ : WPM	0.00	0.00	0.00	0.00	29.10	32.86	33.33	31.76	15.88
	M ₄ : ½ WPM	36.50	36.33	31.10	34.64	60.67	68.70	74.34	67.90	51.27
Sub mean	34.10	25.73	23.17	27.66	50.65	55.46	62.63	56.25	41.95	
S ₂ : G214	M ₁ : MS	3.66	0.00	0.00	1.22	14.54	17.71	20.13	17.46	9.34
	M ₂ : ½ MS	16.27	13.90	10.66	13.61	23.33	25.33	25.54	24.73	19.17
	M ₃ : WPM	0.00	0.00	0.00	0.00	0.00	3.37	6.40	3.26	1.63
	M ₄ : ½ WPM	31.21	25.86	22.50	26.52	31.60	33.08	36.60	33.76	30.14
Sub mean		12.79	9.94	8.29	10.34	17.37	19.87	22.17	19.80	15.07
Overall mean		23.44	17.83	15.72	19.00	34.01	37.67	42.39	38.02	
Factor mean	Media	M ₁ : 18.73			M ₂ : 45.85		M ₃ : 8.75		M ₄ : 40.70	
Concentration		C ₁ : 28.72			C ₂ : 27.75		C ₃ : 29.06			
CD _(p ≤ 0.05)										
Rootstock (S)		: 0.35								
Media (M)		: 0.50								
Growth regulators (G)		: 0.35								
Concentration (C)		: 0.43								
S x M x G x C		: 1.88								

Table 6 : Influence of media & growth regulators on length of the longest root (mm) of clonal rootstocks of apple

Rootstock		Growth regulators								
Media		G ₁ : NAA			Sub mean	G ₂ : IBA			Sub mean	Overall mean
		C ₁ :1 mg	C ₂ : 3 mg	C ₃ : 5 mg		C ₁ : 1 mg	C ₂ : 3 mg	C ₃ : 5 mg		
S ₁ : G.41	M ₁ : MS	52.71	0.00	0.00	17.57	62.21	73.96	100.76	78.98	48.27
	M ₂ : ½ MS	128.46	126.96	124.76	126.73	140.53	145.03	146.13	143.90	135.31
	M ₃ : WPM	0.00	0.00	0.00	0.00	42.38	58.82	61.08	54.09	27.05
	M ₄ : ½ WPM	102.2	67.78	57.25	75.74	113.86	127.82	132.13	124.60	100.17
Sub mean		70.84	48.69	45.50	55.01	89.75	101.41	110.03	100.39	77.70
S ₂ : G.214	M ₁ : MS	45.03	0.00	0.00	15.01	24.96	29.06	29.82	27.95	21.48
	M ₂ : ½ MS	29.27	23.70	18.61	23.86	41.66	42.50	45.03	43.06	33.46
	M ₃ : WPM	0.00	0.00	0.00	0.00	0.00	10.23	13.20	7.81	3.91
	M ₄ : ½ WPM	49.73	44.06	39.56	44.45	53.23	59.28	68.51	60.34	52.40
Sub mean		31.01	16.94	14.54	20.83	29.96	35.27	39.14	34.79	27.81
Overall mean		50.92	32.81	30.02	37.92	59.85	68.33	74.58	67.59	
Factor mean	Media	M ₁ : 34.87		M ₂ : 84.39		M ₃ : 15.47		M ₄ : 76.28		
Concentration		C ₁ : 55.39		C ₂ : 50.57		C ₃ : 52.30				
CD _(p ≤ 0.05)										
Rootstock (S)		: 0.70								
Media (M)		: 1.00								
Growth regulators (G)		: 0.70								
Concentration (C)		: 0.86								
S x M x G x C		: 3.26								

(10.23 mm) was obtained in G.214 rootstock with full strength WPM medium fortified with IBA at the concentration (3.0 mg/L).

CONCLUSION

Current research demonstrates the optimized protocol for *in vitro* rhizogenesis of clonal rootstocks of apple (G.41 and G.214) using MS and WPM media fortified with different growth regulators. Maximum rhizogenesis in G.41 and G.214 clonal rootstocks of apple was recorded in half strength MS medium fortified IBA 5.0 mg/L and half strength WPM medium added with IBA 5.0 mg/L, respectively. The standardized protocol is helpful in production of large scale, true to the type, disease free plants in short period.

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