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Influence of growth media and hormone concentrations on sprouting and rooting ability of terminal cutting of Ber (*Ziziphus mauritiana* Lamk) cv. Apple Ber

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Abstract
A resilient fruit tree with the chromosomal number 2n = 48, the ber (*Zizyphus mauritiana* Lamk.), is a member of the Rhamnaceae family. It may be produced successfully in dry and semi-arid regions of Indian states, including Haryana, Rajasthan, Madhya Pradesh, and Gujarat. Since there is no standardized protocol for the propagation of apple ber in Andhra Pradesh region, the current experiment was conducted with objectives of finding out the best type of rooting media, IBA concentrations and methods of application for successful propagation. The terminal cuttings were planted in three types of media *viz.*, coco peat, vermiculite and saw dust, which were treated with three different concentrations of IBA *i.e.*, 1000 ppm, 1500 ppm and 2000 ppm. For application, two methods were followed *i.e.*, cuttings treated with mixture of IBA and talcum powder and cuttings treated with IBA solution for 5 minutes. The result of this experiment showed that the maximum chlorophyll content (46.07 SPAD unit), absolute growth rate (0.35 cm /day) and early achievement of plantable size of propagules (97.66 days) was observed when the terminal cuttings were treated with 1000 ppm of IBA for 5 minutes and propagated in coco peat.

Keywords: Apple Ber, IBA, cuttings, peat, vermiculite and saw dust & chlorophyll

Introduction
India has a wealth of underutilized native and exotic fruit trees, including ber fruit, which may have a high agro-industrial potential and serve as a significant source of income for the local communities. Because its fruits are readily available at low production costs, a good source of vitamin C, protein, and minerals, it is referred to as the "poor man's apple" (Pareek and Yahia, 2013) [3]. It has large range of varieties of fruits in its basket and accounts for 10 % of world's total fruit production with an area of 6480 thousand hectares and production of 92846 MT (Anonymous, 2017) [1]. In Indian arid regions having high temperature, low and variable precipitations are spread over about 31.7 million hectares limiting the scope for high horticultural productivity. However, these conditions greatly favor development of high quality in number of fruits. The two most important auxins *i.e*, IBA and NAA have been used widely either singly or in combination for inducing rooting in cuttings of various crop species (Thimann, 1935) [9]. Hence, to find out the survival percentage of apple ber cuttings, the cuttings will be treated with different IBA combinations and will be planted in different rooting media mixtures.
Budding is the easiest method of vegetative propagation used for improved cultivars. Different types of budding techniques have been utilized with ring-budding and shield-budding being the most successful. Wild varieties of ber *Z. mauritiana* are usually used as the rootstock, the most common being *Z. rotundifolia* in India and *Z. spina-christi* in Africa. Stock and scion compatibility studies were conducted in ber cultivars with three rootstocks *viz*., Jhar ber (*Z. nummularia*) and two ecotypes of ber (Marks and Simpson, 2000) [23]. The rootstock Jhar ber showed incompatibility with inverted bottleneck symptoms budded with Gola ecotype. All other combinations were compatible with perfect union. The objective of this work was to evaluate the vegetative propagation method i.e., cutting.

Material and Methods
A slant cut was given at the basal end of the terminal cuttings to expose maximum absorbing surface area for induction of effective rooting.

The basal parts (1-2 cm depth) of the terminal cuttings were dipped in IBA solutions (at 1000, 1500 and 2000 ppm) for 5 minutes as per treatments, subsequently the cuttings were air dried for 5 minutes. For another treatment of IBA, the basal part of the terminal cuttings was dipped in the IBA powder formulations (at 1000, 1500 and 2000 ppm) and then just taped the cuttings to remove excess powder on the cuttings. The treated cuttings were planted in portrays containing respective rooting media *i.e*. coco peat, vermiculite and saw dust up to one node depth. The rooting media was pressed gently around the base of the cutting to hold the cutting in right place, to eliminate air pockets and to make sure that the base of the cutting was in good contact with the moist rooting media.

The terminal open ends of cuttings were smeared with blitox paste to avoid fungal infections. The portrays were kept in mist chamber and maintained in the congenial atmosphere for rooting of cuttings. The experiment was conducted in Factorial Completely Randomized Design with the above three factors at unequal levels and replicated thrice.

Results and Discussion

Chlorophyll content (SPAD unit)

Effect of rooting media, IBA concentration and method of application and their interactions were recorded significant for chlorophyll content and represented in Table 1 and Fig. 1.

Non-significant difference was observed among the media and concentration with respect to chlorophyll content at 60 days after planting. Among the methods of application, cuttings treated with IBA solution for 5 min (A_2) recorded maximum chlorophyll content (21.41 SPAD units) and cuttings treated with mixture of IBA and talcum powder (A_1) recorded minimum chlorophyll content (17.88 SPAD units).In first order interactions, among the interaction effects between media and concentrations (M×C) maximum chlorophyll content (23.81 SPAD unit) was recorded in sawdust media and IBA @ 1500 ppm (M_3C_2) and the minimum chlorophyll content (16.60 SPAD unit) was recorded in the coco peat media and IBA @ 1500 ppm concentration (M_1C_2). Similar results were observed in the experiments of Eccher and Noe (1989) [11].

Among the interactions between media and methods of application (M×A), highest chlorophyll content (23.00 SPAD unit) was recorded in vermiculite media and cuttings treated with IBA solution for 5 min (M_2A_2) which was followed by (M_3A_2) (20.78 SPAD units) and the interactions between concentration and methods of application (C×A), maximum chlorophyll content (22.50 SPAD units) was recorded in IBA @ 1000 ppm and cuttings treated with IBA solution for 5 min (C_1A_2).In second order interactions, among the interaction effects of media, IBA concentration and methods of application (M×C×A), maximum chlorophyll content (27.46 SPAD unit) was recorded in the treatment combination of sawdust media, IBA @ 1500 ppm concentration and cuttings treated with IBA solution for 5 min ($M_3C_2A_2$) (Table 1) which was similar to the findings of Evans *et al.,* 2003 [12].

At 90 days after planting, the highest chlorophyll content (28.85 SPAD unit) was recorded in vermiculite media (M_2) which was on par with sawdust media (M_3) (28.76 SPAD unit). Among the different IBA concentrations, highest chlorophyll content (29.41SPAD unit) was recorded in IBA @ 2000 ppm (C_3). In the method of application (A_2) cuttings treated with IBA solution for 5 min (30.53 SPAD units) recorded highest chlorophyll content. These findings were agrees with the results of Farouk *et al.,* 2008 [13] and Ludwig *et al.,* 2005 [22].

In first order interactions, among the interaction effects between media and concentrations (M×C), maximum chlorophyll content (30.53 SPAD unit) was recorded in vermiculite media and IBA @ 2000 ppm (M_2C_3). Among the interactions between media and methods of application (M×A) highest chlorophyll content (32.55 SPAD unit) was recorded in vermiculite media and cuttings treated with IBA solution for 5 min (M_2A_2) and the interaction between concentration and methods of application (C×A), highest chlorophyll content (32.08 SPAD unit) was recorded in IBA @ 1500 ppm and cuttings treated with IBA solution for 5 min (C_2A_2). In second order interactions, the interaction effects between media, concentration and method of application (M×C×A), maximum chlorophyll content (34.50 SPAD unit) was recorded in the treatment combination of vermiculite media, IBA @ 1500 ppm concentration with cuttings treated with IBA solution for 5 min ($M_2C_2A_2$). Similarly, the findings of Husen and Pal (2007) [14] agree with the current results.

At 120 days after planting, the highest chlorophyll content (35.93 SPAD unit) was recorded in sawdust media (M_3) which was followed by coco peat media (M_1) (33.77 SPAD unit). Among the IBA concentrations, highest chlorophyll content (34.69 SPAD unit) was recorded in IBA @ 1500 ppm (C_2) which was on par with IBA @ 1000 ppm (C_1) (33.62 SPAD unit) and lowest chlorophyll content (32.27 SPAD unit) was recorded in IBA @ 2000 ppm (C_3) and the methods of application cuttings treated with IBA solution for 5 min (A_2) recorded maximum highest chlorophyll content (36.20 SPAD unit) and cuttings treated with mixture of IBA and talcum powder (A_1) recorded minimum chlorophyll content (30.85 SPAD unit).In first order interactions, the interaction effects between media and concentration (M×C), maximum chlorophyll content (40.11 SPAD unit) was recorded in sawdust media and IBA @ 1500 ppm (M_3C_2) and minimum chlorophyll content (30.55 SPAD units) was recorded in the combination of saw dust media and IBA @ 1000 ppm (M_2C_1), at 120 DAP. The interaction effect of media and methods of application was found non-significant for the SPAD units at 120 DAP (Table 1). These results were agreeing with the findings of Imin *et al.,* 2005 [15].

The effect of interaction between media, IBA concentration and method of application (M×C×A), maximum chlorophyll content (46.07 SPAD unit) was recorded in the treatment combination of coco peat media, IBA @ 1000 ppm concentration and cuttings treated with IBA solution for 5 min ($M_1C_1A_2$) while minimum chlorophyll content (27.71 SPAD unit) was recorded in the treatment combination of coco peat media and IBA @ 1000 ppm concentration and cuttings treated with mixture of IBA and talcum powder ($M_1C_1A_1$) at 120 days after planting. The terminal cuttings dipping with IBA solution for 5 min @ 1000 ppm recorded maximum total leaf chlorophyll content than others. The increased leaf area with increased concentrations of auxins might have activated the process of photosynthesis resulting in more chlorophyll content of leaves per cutting. Ratnakumari (2014) [7] observed that cuttings with a greater number of leaves enhanced nutrients uptake thereby increased the photosynthates production and provided sufficient food contents for the metabolic activities of the plants by means of mounting the levels of light harvesting pigments especially chlorophyll.

Absolute growth rate (cm / day)

Among different concentrations, highest absolute growth rate (0.29 cm / day) was recorded in IBA @ 1000 ppm (C_1) and lowest absolute growth rate (0.27 cm /day) was recorded in IBA @ 1500 ppm (C_2). Cuttings treated with IBA solution for 5 min (A_2) recorded maximum absolute growth rate (0.29 cm /day) and minimum absolute growth rate (0.28 cm / day) was recorded in cuttings treated with mixture of IBA and talcum powder (A_1) as per the methods of application (Table 2). The findings of Khalafalla *et al.,* 2007 [17] agrees with current findings.

In first order interactions, among the interaction effect between media and concentration (M×C), maximum absolute growth rate (0.32 cm /day) was recorded in coco peat media and IBA @ 1000 ppm (M_1C_1), whereas the minimum absolute growth rate (0.26 cm /day) was recorded in the vermiculite media and IBA @ 1500 ppm (M_2C_2). Among the interactions between media and method of application (M×A), highest absolute growth rate (0.30 cm day) was observed in coco peat media and cutting treated with IBA solution for 5 min (M_1A_2) while the lowest absolute growth rate (0.27 cm /day) was recorded in sawdust media and cutting treated with mixture of IBA and talcum powder (M_3A_1). Similarly, the findings of Khalafalla *et al.,* 2009 [18] agrees with the current results.

Further, among interactions between concentration and methods of application (C×A) the maximum absolute growth rate (0.30 cm /day) was recorded in IBA @ 1000 ppm and cuttings treated with IBA solution for 5 min (C_1A_2) and the minimum absolute growth rate (0.26 cm / day) was recorded in IBA @ 1500 ppm cuttings treated with mixture of IBA and talcum powder (C_2A_1) (Fig. 2). These results were agreeing with the findings of Imin *et al.,* 2005 [15].

In second order interaction, among the interaction effects between media, IBA concentration and method of application, maximum absolute growth rate (0.35 cm /day) was recorded in the treatment combination of coco peat media, IBA @ 1000 ppm concentration and cutting treated with IBA solution for 5 min ($M_1C_1A_2$), while minimum absolute growth rate (0.24cm /day) was recorded in the treatment combination of vermiculite media and IBA @ 1500 ppm and cuttings treated with mixture of IBA and talcum powder ($M_2C_2A_1$) (Table 2). Terminal cutting in shade net condition showed maximum absolute growth rate due to favorable microclimate. IBA @ 1000 ppm showed maximum absolute growth rate compared to other treatments which might be due to the reason that it exhibited more plant height, also it may be due to the auxin increased the plasticity of cell wall which in turn increases permeability of cell wall for entry of water into the cell resulted in cell enlargement and causing more growth (Heyn, 1931) [4]. These results are in line with Harsha *et al*., 2012 in jackfruit.

15 Days taken for plantable size (days)

Early achievement of plantable size of the plant is the important parameter for the early yield and better plant growth. Minimum days taken for plantable size (108.22 day) was observed in coco peat media (M_1) and maximum days taken for plantable size (113.05 days) was recorded in vermiculite media (M_2). The IBA concentrations, minimum days taken for plantable size (109.33 day) was recorded in IBA @ 1500 ppm (C_2) while the maximum days taken (111.88 day) were recorded in IBA @ 2000 ppm (C_3). Among the methods of application, cuttings treated with IBA solution for 5 min (A_2) minimum days for plantable size (107.40 days) and maximum days for plantable size (112.92 days) were taken by cuttings dipped in mixture of IBA and talcum powder (A_1) (Table 3). Similar results were observed when Jamia *et al.,* 2003 [16] and Marks and Simpson, 2000 [23] conducted their experiment on pumello.

In first order interactions, among the interaction effects between media and concentration (M×C), minimum days taken for plantable size (105.50 days) was recorded in the coco peat media and IBA @ 1000 ppm (M_1C_1). The interactions between media and method of application (M×A) minimum days taken for plantable size (103.66 days) was recorded in coco peat media and cutting treated with IBA solution for 5 min (M_1A_2).These results are in line with Kuroha, and Satoh, 2007 in jackfruit.

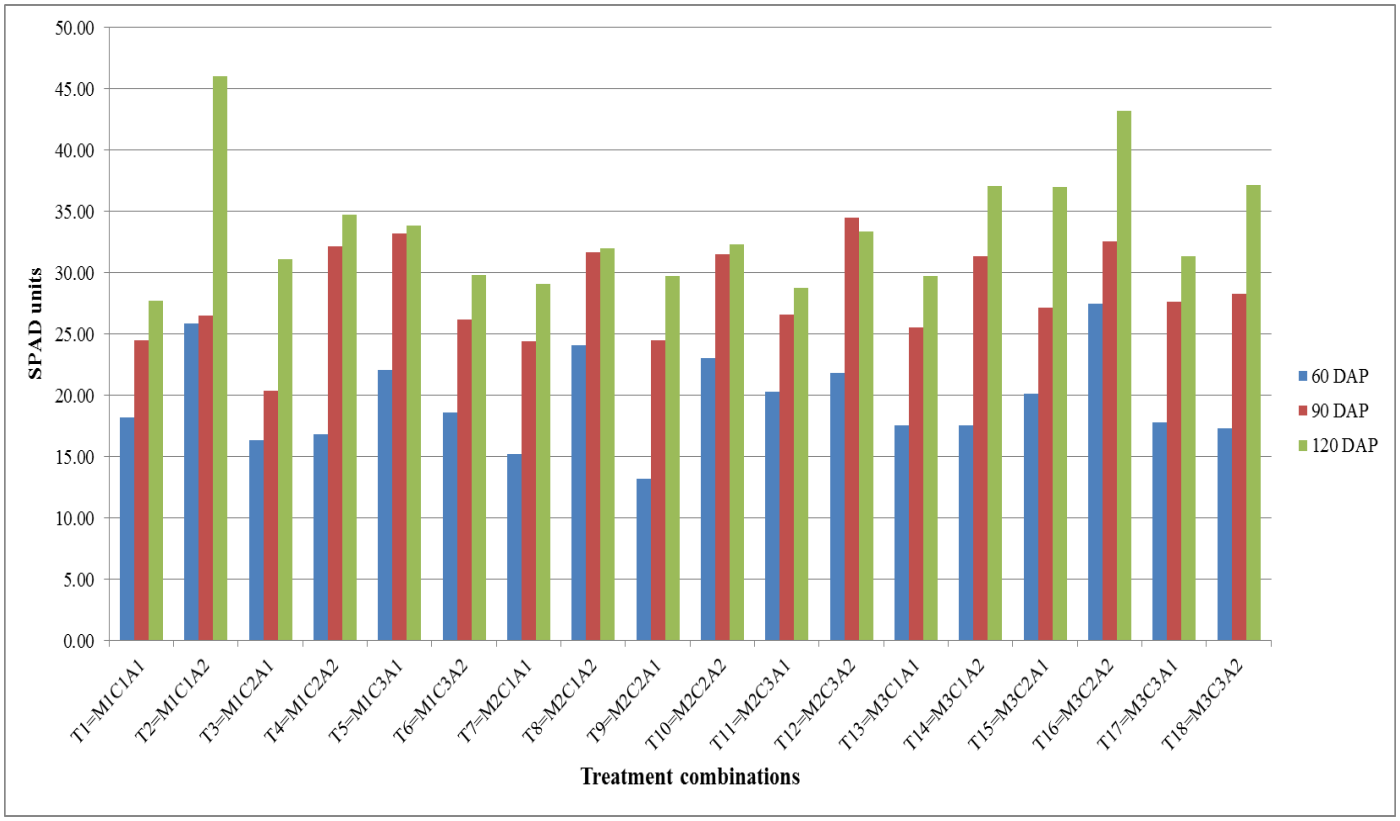
The interactions between concentration and methods of application (C×A), the minimum days taken plantable size (105.55 days) were recorded in cuttings treated with IBA solution for 5 min (C_2A_2). In second order interactions, the interaction effects between media, IBA concentration and methods of application (M×C×A), minimum days taken for plantable size (97.66 days) was recorded in the treatment combination of coco peat media, IBA @ 1000 ppm and cuttings treated with IBA solution for 5 min ($M_1C_1A_2$) and maximum days taken for plantable size (116.00 days) was recorded in the treatment combination of vermiculite media, IBA concentration with cuttings treated with mixture of IBA and talcum powder ($M_2C_2A_1$) (Fig. 3). Similarly, the findings of Leonardi *et al*., 2001 [20] agrees with the current results.

The variation in days taken for plantable size between the environmental conditions could be attributed to the growing conditions. IBA @ 1000 ppm led to best aerial growth. The emergence of longest shoot on cuttings might be attributed to the well-developed root system in such cuttings which might have tended to promote shoot growth by ensuring adequate mobilization of water and nutrients from the soil or substrate to the growing shoot and thereby reduced the days taken for plantable size (Heyn, 1931; Liu and Sanford, 1988) [4, 21]. The plant height in shade net conditions was maximum due to favorable conditions. Among the growth regulator treatments, IBA @ 1000 ppm exhibited more plant height, which might be since IBA led to best aerial growth and the emergence of longest shoot might be attributed to the well-developed root system in such cuttings, which have tended to promoting shoot growth by ensuring adequate mobilization of water and nutrients from the soil or substrate to the growing shoot. Also, IBA @ 1000 ppm increased the plasticity of cell wall which in turn increases permeability of cell wall for entry of water into the cell resulted in cell enlargement and causing more growth of shoot, consequently there was a faster growth rate of the newly emerged shoots (Heyn, 1931) [4]. The results are in conformity with research findings of Upadhyay and Badyal (2007) [10] in pomegranate, Pratima and Rana (2014) in kiwi, Singh *et al*. (2014) [8] in mulberry and Jan *et al*. (2015) [5] in olive.

Table 1: Effect of rooting media and IBA concentrations on the total leaf chlorophyll content (SPAD unit) in terminal cuttings of ber cv. Apple ber

		60 DAP			90 DAP			120 DAP		
		A1	**A2**	**Mean MxC**	**A1**	**A2**	**Mean MxC**	**A1**	**A2**	**Mean MxC**
M1	C1	18.21	25.85	22.03	24.46	26.53	25.50	27.71	46.07	36.89
	C2	16.34	16.86	16.60	20.36	32.16	26.26	31.11	34.72	32.92
	C3	22.06	18.62	20.34	33.23	26.23	29.73	33.18	29.81	31.50
Mean MxA		18.87	20.44	19.66	26.02	28.31	27.16	30.67	36.87	33.77
M2	C1	15.26	24.08	19.67	24.40	31.66	28.03	29.10	32.01	30.55
	C2	13.22	23.06	18.14	24.46	31.50	27.98	29.78	32.32	31.05
	C3	20.26	21.86	21.06	26.56	34.50	30.53	28.75	33.36	31.05
Mean MxA		16.24	23.00	19.62	25.14	32.55	28.85	29.21	32.56	30.88
M3	C1	17.60	17.57	17.58	25.53	31.33	28.43	29.75	37.08	33.42
	C2	20.16	27.46	23.81	27.13	32.60	29.86	36.98	43.2	40.11
	C3	17.83	17.30	17.56	27.66	28.30	27.98	31.33	37.20	34.27
Mean MxA		18.53	20.78	19.65	26.77	30.74	28.76	32.69	39.18	35.93
Mean CxA			Mean C		Mean C			Mean C		
	C1	17.02	22.50	19.76	24.80	29.84	27.32	28.85	38.39	33.62
	C2	16.57	22.46	19.52	23.98	32.08	28.03	32.62	36.76	34.69
	C3	20.05	19.26	19.65	29.15	29.67	29.41	31.09	33.46	32.27
Mean A		17.88	21.41	19.64	25.98	30.53	28.25	30.85	36.20	33.53
Comparing Mean		S Em (+)		CD at 5%	S Em (+)		CD at 5%	S Em (+)		CD at 5%
M		0.203		NS	0.422		1.211	0.634		1.819
C		0.203		NS	0.422		1.211	0.634		1.819
A		0.166		0.476	0.345		0.989	0.518		1.485
MxC		0.352		1.010	0.731		2.098	1.098		3.150
MxA		0.288		0.825	0.597		1.713	0.897		NS
CxA		0.288		0.825	0.597		1.713	0.897		2.572
MxCxA		0.498		1.429	1.034		2.967	1.553		4.455

M_1 = Coco peat C_1 = 1000 ppm A_1 = Cuttings treated with mixture of IBA and talcum powder
M_2 = Vermiculite C_2 = 1500 ppm A_2 = Cuttings treated with IBA solution for 5 minutes
M_3 = Sawdust C_3 = 2000 ppm DAP = Days after planting NS = Non significant



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M3 = Sawdust C3 = 2000 ppm DAP = Days after planting

Fig 1: Effect of rooting media and IBA concentration on the total leaf chlorophyll content (SPAD unit) in terminal cuttings of ber cv. Apple ber

Table 2: Effect of rooting media and IBA concentration on absolute growth rate (cm/day) in terminal cutting of ber cv. Apple ber

		A_1	A_2	Mean M×C	
M_1	C_1	0.29	0.35	0.32	
	C_2	0.28	0.26	0.27	
	C_3	0.28	0.30	0.29	
	Mean MxA	0.29	0.30	0.29	
M_2	C_1	0.29	0.28	0.29	
	C_2	0.24	0.28	0.26	
	C_3	0.30	0.28	0.29	
	Mean MxA	0.28	0.28	0.28	
M_3	C_1	0.27	0.26	0.27	
	C_2	0.25	0.29	0.27	
	C_3	0.30	0.28	0.29	
	Mean MxA	0.27	0.28	0.28	
	Mean CxA			Mean C	
	C_1	0.29	0.30	0.29	
	C_2	0.26	0.28	0.27	
	C_3	0.29	0.29	0.29	
	Mean A	0.28	0.29	0.28	
Comparing Mean			SEm(±)	CD at 5%	
M			0.001	0.003	
C			0.001	0.003	
A			0.001	0.002	
MxC			0.002	0.005	
MxA			0.001	0.004	
CxA			0.001	0.004	
MxCxA			0.003	0.007	

M_1 = Coco peat C_1 = 1000 ppm A_1 = Cuttings treated with mixture of IBA and talcum powder
M_2 = Vermiculite C_2 = 1500 ppm A_2 = Cuttings treated with IBA solution for 5 minutes
M_3 = Sawdust C_3 = 2000 ppm DAP = Days after planting

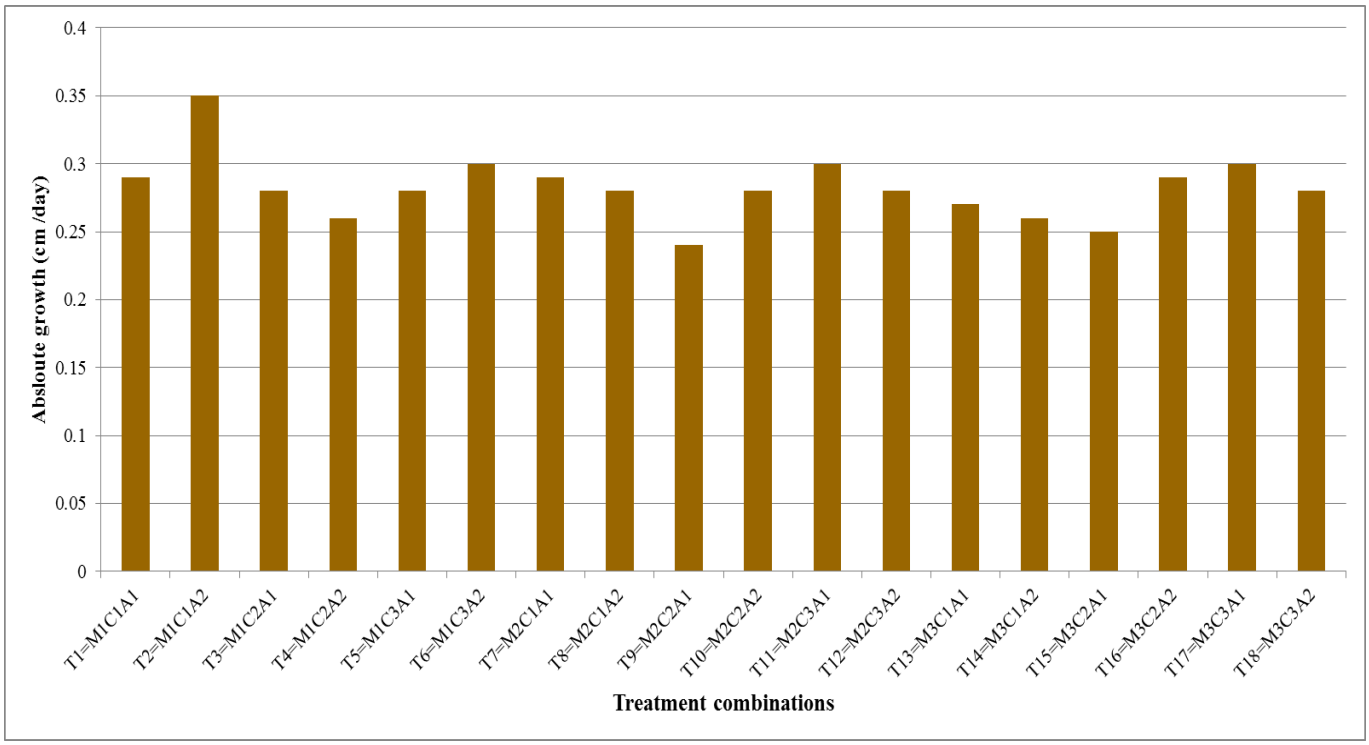
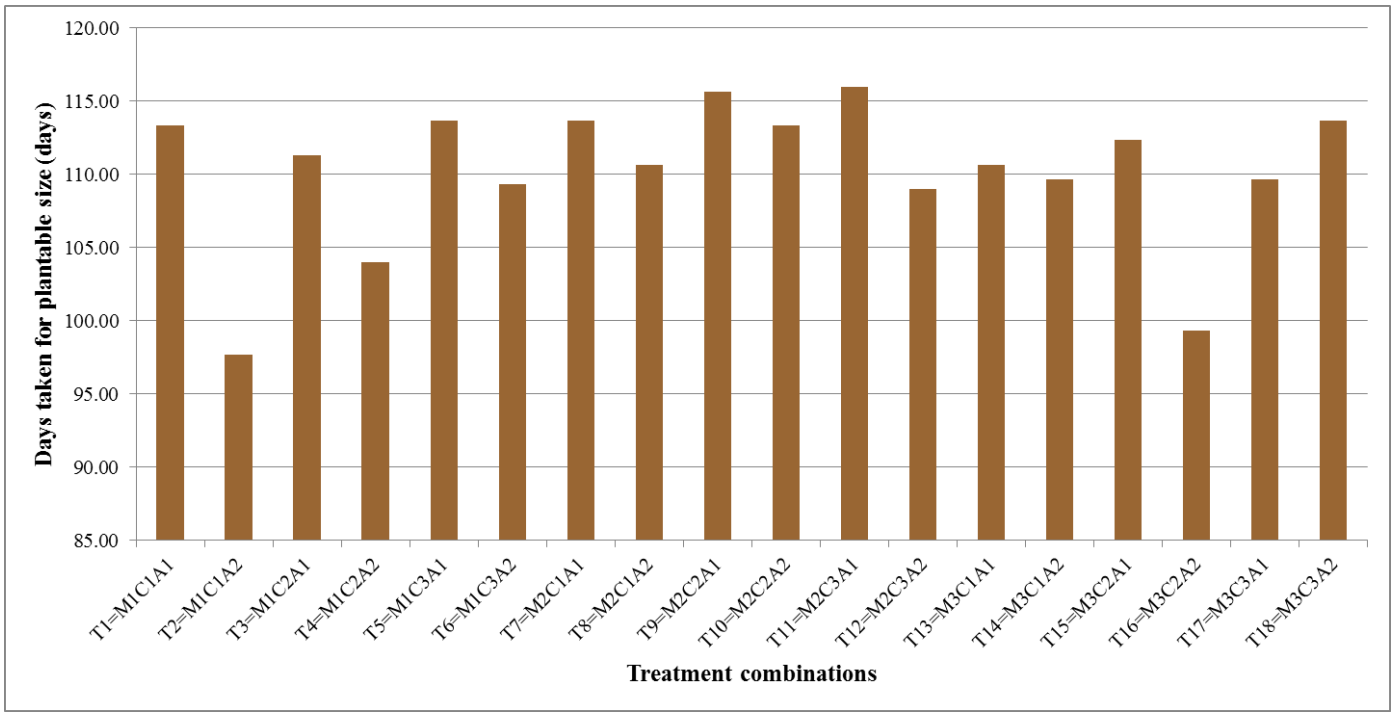


Fig 2: Effect of rooting media and IBA concentration on absolute growth rate (cm/day) in terminal cutting of ber cv. Apple ber

Table 3: Effect of rooting media and IBA concentration on days taken for palntable size (days) in terminal cutting of ber cv. Apple ber

		A_1	**A_2**	**Mean MxC**
M_1	C_1	113.33	97.66	105.50
	C_2	111.33	104.00	107.66
	C_3	113.66	109.33	111.50
	Mean MxA	112.77	103.66	108.22
M_2	C_1	113.66	110.66	112.16
	C_2	115.66	113.33	114.50
	C_3	116.00	109.00	112.50
	Mean MxA	115.11	111.00	113.05
M_3	C_1	110.66	109.66	110.16
	C_2	112.33	99.33	105.83
	C_3	109.66	113.66	111.66
	Mean MxA	110.88	107.55	109.22
	Mean CxA			MeanC
	C_1	112.55	106.00	109.27
	C_2	113.11	105.55	109.33
	C_3	113.11	110.66	111.88
	Mean A	112.92	107.40	110.16
Comparing Mean			S Em(±)	CD at 5%
M			0.694	1.990
C			0.694	1.990
A			0.567	1.625
MxC			1.202	3.447
MxA			0.981	2.815
CxA			0.981	2.815
MxCxA			1.700	4.875

M_1= Coco peat C_1 = 1000 ppm A_1= Cuttings treated with mixture of IBA and talcum powder
M_2 =Vermiculite C_2 = 1500 ppm A_2 = Cuttings treated with IBA solution for 5 minutes
M3 = Sawdust C3 = 2000 ppm DAP = Days after Planting



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M_3 = Sawdust C_3 = 2000 ppm DAP = Days after planting

Fig 3: Effect of rooting media and IBA concentration on days taken for palntable size (days) in terminal cutting of ber cv. Apple ber

Summary and Conclusion

The effect of interaction between media, IBA concentration and method of application (M×C×A), maximum chlorophyll content (46.07 SPAD unit) was recorded in the treatment combination of coco peat, IBA @ 1000 ppm and cuttings treated with IBA solution for 5 min ($M_1C_1A_2$). In second order interaction, among the interaction effects between media, IBA concentration and method of application, maximum absolute growth rate (0.35 cm /day) was recorded in the treatment combination of coco peat media, IBA @ 1000

ppm concentration and cutting treated with IBA solution for 5 min ($M_1C_1A_2$). In second order interactions, the interaction effects between media, IBA concentration and methods of application (M×C×A), minimum days taken for plantable size (97.66 days) was recorded in the treatment combination of coco peat media, IBA @ 1000 ppm and cuttings treated with IBA solution for 5 min ($M_1C_1A_2$).

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