Himachal Journal of Agricultural Research 45(1&2) : 31-38 (2019)

Standardization of different IBA concentrations on stenting method of propagation in plum cultivars under polyhouse conditions

N.D. Negi* and S.K. Upadhyay
Department of Horticulture and Agroforestry
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India.
*Corresponding author: nanak_negi@yahoo.co.in

Manuscript Received: 12.09.2019; Accepted: 22.11.2019

Abstract

This study was conducted during 2015-16 with the objective to reduce nursery production duration of plum under polyhouse conditions in mid-hill areas of HP. Non-rooted cuttings of Pixy plum having graftable thickness were collected from the mother stools in December and then kept in moist sand for two months in a pit. In the last week of January 2016, scion woods of three cultivars of plum namely; Santa Rosa, Mariposa and Frontier were grafted on to the non-rootedcuttings of Pixy plum. The grafts were then treated with different concentrations of IBA and planted in a well prepared nursery beds under ordinary tunnel type polyhouse. Stentlings (grafted stocks) started sprouting after 12 to 15 days of grafting and grafting success was recorded as high as 90.19 % with the production of saleable plants up to 94.19 % in cultivar Mariposa treated with IBA at 5000 ppm. The other growth parameters such as stentling height, number of laterals per stentlings, root number and root length were observed higher in cultivar Frontier. This method of plant propagation reduced the nursery production duration of plum from two years to one year with satisfactory results with respect to bud take success and production of percent saleable plants in polyhouse under mid-hill conditions of HP.

Key words: Stenting, Pixy plum, direct rooting and grafting, polyhouse conditions.

Stenting (cutting, grafting and rooting performed simultaneously) is the method for quick propagation in roses (Korin 2015) and hibiscus (Izadi and Hossein 2014). This technique involves direct grafting of scion onto non-rooted piece of rootstock, and development of graft union and adventitious roots simultaneously. Recent studiesin sweet cherry and pear (Negi and Upadhyay, 2016),and peach (Gill *et al.* 2014) indicated that this technique could be an innovative method to shorten the duration of plants multiplication in those fruit plants which were having easy to root clonal rootstocks.

At present, almost all of the temperate fruit plants are being multiplied conventionally through grafting and budding onto either seedling or clonal rootstocks. This technique of plant propagation takes almost two years to attain saleable size i.e. from seed sowing to raise seedling and further grafting or budding onto them. The state-of-the- art propagation technologies such as tissue culture, have proven to reduce the nursery raising duration of selected fruits but this method is highly precise and requires trained persons. Moreover, the protocol for *in-vitro* multiplication of most of the fruit plants has not been standardized yet. The other advance methods for quick multiplication of fruit plants are micro-grafting/shoot tip grafting and epicotyl grafting etc. but the commercial use of such technologies in fruit crops is limited. Hence, there is a need to device new techniques of plant propagation other than tissue culture to reduce the duration of nursery plant production to ensure regular supply of quality planting materials to the growers. The present study was therefore conducted with the primary objective of reducing the duration of nursery production in plum by grafting non-rooted cuttings of Pixy with commercial plum cultivars under polyhouse conditions in the mid-hill area of Himachal Pradesh.

Materials and Methods

The experiment was carried out in the Departmental Nursery of C.S.K.H.P.K.V., Palampur, Himachal Pradesh, India (73°23′ N and 33°26′ E) in the year 2015-16. For this study, non-rooted cuttings of Pixy (clonal rootstock of plum) were grafted with scions of plum cultivars; Santa Rosa, Mariposa and Frontier and grafts were treated with IBA in different concentrations and immediately planted in well prepared nursery beds under ordinary tunnel type polyhouse equipped with exhaust fans and mist system and oriented in W-E direction.

Collection of cuttings and stenting procedure

Well matured one-year old cuttings of Pixy plum of 30 to 45 cm length having graftable thickness were collected from the mother stool in the month of December, 2015 and were kept moist in pits lined with sand under shade conditions. The healthy scionwoods of Santa Rosa, Frontier and Meriposa plums having 3 to 4 nodes were grafted onto non-rooted cuttings in the last week of January, 2016 (Figure 1). The basal end of each grafts were then treated with different concentrations of IBA made in absolute alcohol (T_1: 5000 ppm, T_2: 2500 ppm, T_3: 1250 ppm and T_4: control) for 15seconds. The IBA treated grafts were then planted under polyhouse conditions in well prepared nursery beds.The average temperature of the polyhouse was $25\text{-}30^0 \pm 5\ ^0$C with RH 70-80 %. The soil was clay loam with pH 6.0,and at the time of bed preparation,well decomposed farm yard manure @ 5-6 kg m^{-2},120 kg of N, 50 kg of P and 80kg of K per hectare were mixed well. After planting, the grafts were given light irrigation without disturbing them. To check weeds, light hoeing was done 60 days after grafting with utmost care without disturbing the callusing at basal end of the grafts. The treatments were replicated thrice with 10 grafts per replication and experiment was laid in Randomized Block Design (RBD).

Figure 1.Grafting (tongue) of different plum cultivars onto non-rooted cuttings of Pixy and basal treatment with IBA (quick dip method)

The graft success/ bud take (%) was observed 60 days after grafting by observing the leaf emergence and growth of new shoots in scion and calculated in percentage by using standard formula.

Stentling height was measured by taking distance between the graft unions to the tip of the stentling at the end of growing season. The diameter of the stentlings was measured (5 cm below the graft union i.e. shank and 5 cm above the union) with digital Vernier caliper and average value was expressed in cm. The lateral shoots which emerged after the healing of graft union were kept as such without removing, were also counted and expressed as number of lateral shoots per stentlings. Similarly, root number, length and foliar characteristics were recorded as per the standard procedures. At the end of experiment in the month of December, number of saleable stentlings were selected as per the nursery standards of temperate fruit plants (minimum height of plants with well developed root system and proper wound healing or graft union formation) and percent saleable plants were calculated.

The data were analyzed by using ANOVA table with the help of DOS based software ASSEX. The effects of various treatments, cultivars and their interaction were compared for significance differences at 5 per cent rejection level by factorizing them.

Results and Discussion

Number of days to sprout, grafting success and number of saleable plants

In this study, the number of days taken by the grafts for sprouting weresignificantly variable among the cultivars irrespective of treatments, however, treatments and their interaction with cultivar had no significant effect on this aspect (Table 1). Higher concentration of IBA treatments resulted early bud sprout, registering an average of 11.78 (Mariposa) to 15.33 (Frontier) days for sprouting (Figure 2) with IBA at 5000ppm. In earlier studies (Negi and Upadhyay (2016) on stenting, sweet cherry took an average of 13.00 days to sprout buds when grafted onto non-rooted cuttings of clonal rootstock Colt under similar conditions. Whereas, in a studies on simultaneous graft union and adventitious root formation in rhododendron, the vascular wound in cambium was first recognizable on 21 days, and the cambium bridge (a continued strand of wound vascular cambium in callus tissue) between rootstock and scion on day 30 after grafting (Megre *et al*. 2007). In this study, none of the IBA treatments, cultivars as well as their interaction exerted any significant effect on grafting success (Table 1). However, percentage of bud take success was recorded as high as 90.19 % when grafts were treated with IBA at 5000 ppm in Mariposa (Figure 3). It is also clear in Figure 4 that all buds present on scion woods of successful grafts sprouted well with callusing at graft unions, 60 days after grafting (Figure 4). This finding is in agreement with Oguz *et al*. (2009), that the progressive callus formations were visible after 30 days of grafting and the callus started to spread into gaps between graft components, in apple. Further, Hartmann (2009)

Table 1. Analysis of variance table for number of days taken for bud break, graft success and per cent saleable plants (P=0.05)

Source	*df*	No. of days taken for bud break		Graft success (%)		Salable plants (%)	
		f values	*p* values	*f* values	*p* values	*f* values	*p* values
Treatments	3	0.20	0.8963*	2.35	0.1107*	5.16	0.0110
Varieties	2	4.17	0.0349	0.59	0.5636*	2.64	0.1020*
TxV	6	2.00	0.1256*	1.10	0.4031*	0.29	0.9343*

*Non significant

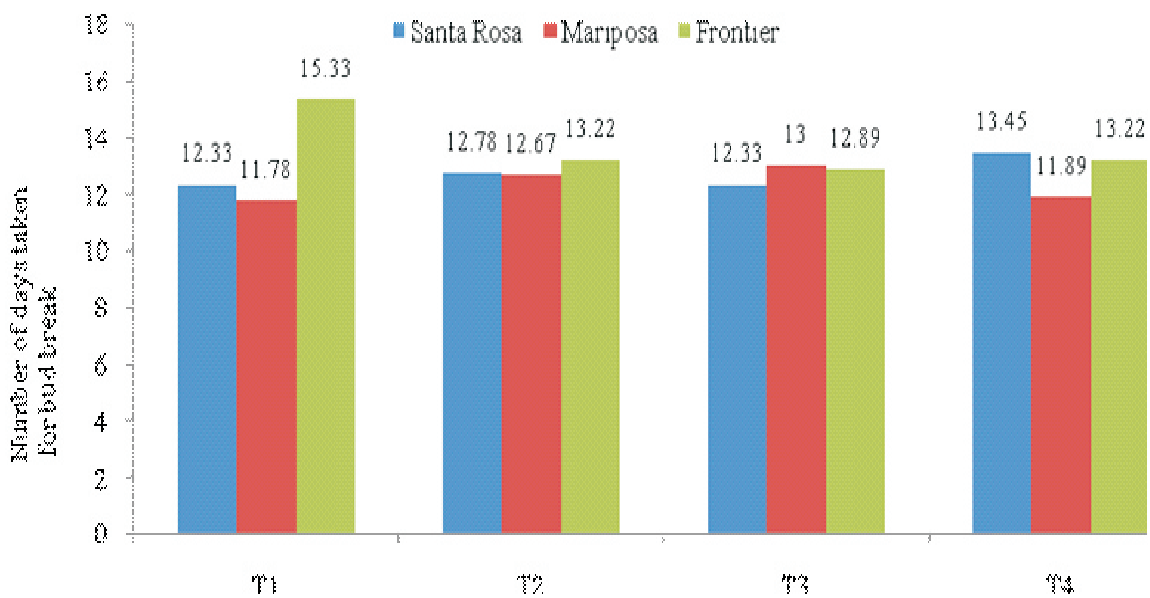

Figure 2.Effect of different IBA concentrations on number of days taken for sprouting in plum cvs. Sant Rosa, Mariposa and Frontier grafted onto non-rooted cuttings of Pixy plum under polyhouse conditions

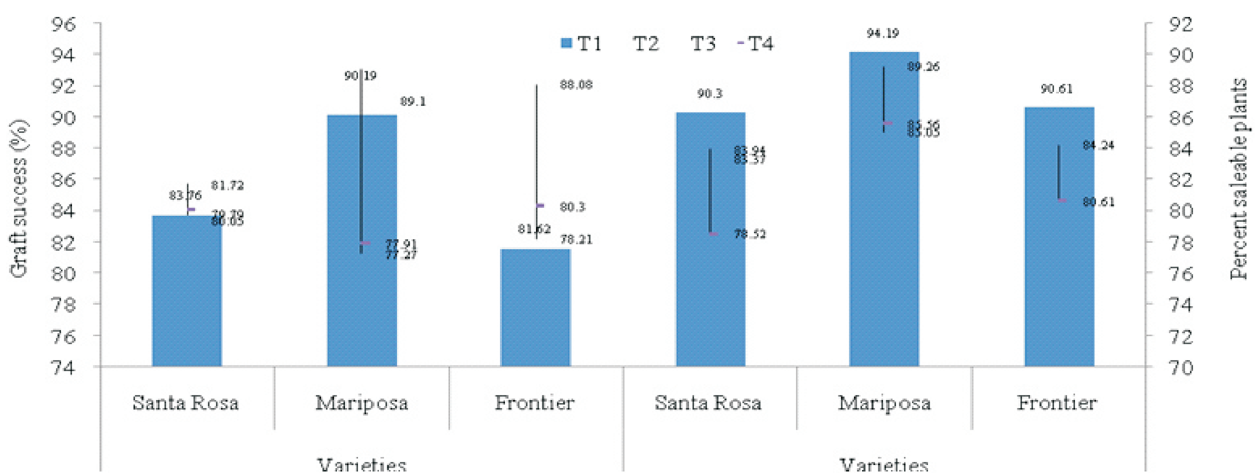

Figure 3.Effect of different IBA concentrations on graft success and production of salable plants in plum cvs. Santa Rosa, Mariposa and Frontier grafted onto non-rooted cuttings of Pixy plum under polyhouse conditions

reported that the graft union was initially formed by rapidly dividing callus cells, originating from the scion and rootstocks, which later differentiated to form the vascular cambium and the associated vascular system if scion is grafted onto rooted stocks. However**,** in this study, the histological investigations were not conducted but the grafted components were uprooted 60 days after grafting and callus formation was observed visually. Those combinations which were having leaf emergence had well developed mass of callus at the both portions i.e. at the basal end of stock and at graft union (Figure 4).

It is clear from the Figure 3 that, cultivar Mariposa produced maximum number of saleable stentlings (94.19 %) when treated with IBA at 5000 ppm, followed by Frontier and Santa Rosa in decreasing orders. This finding is in conformity with the earlier findings of Negi and Upadhyay (2016), that higher rates of IBA enhanced theroot formation and thereby increased the vegetative growthin pear and cherry on non-rooted cuttings of quince and Colt, respectively. The interaction effect of different IBA concentrations and varieties was observed non-significant however, IBA at 5000ppm concentration given to Mariposa produced more number of saleable plants as compared to other treatment combinations (Figure 3). From these findings, it can be inferred that the higher concentrations IBA might have enhanced the root growth after callusing and healing of graft union, and consequentlypromoted the further growth of the stentlings. Likewise, Karimi and Nowrozy (2017) observed that when the scions of pomegranate were grafted onto non-rooted cuttings of cvs. Gorj-e-Dadashi, Gorj-e-Shahvar and GorjSafid-e-Ashk-e-Zar in treatments with IBA at 500 ppm, resulted the production of more number of stentlings with increased height. The grafting success of woody plants depends on number of factors such as compatibility of scion and stock, proper physiological stage of the components (scion and rootstock), grafting time and various sequences of structural events during the healing of the graft union. In this study however, no stionic incompatibility was observed with all varieties grafted onto non-rooted cuttings of 'Pixy' plum but the stentlingvigour and other morphological characters differed significantly, which might be due the genetic makeup of the different genotypes and IBA treatments.

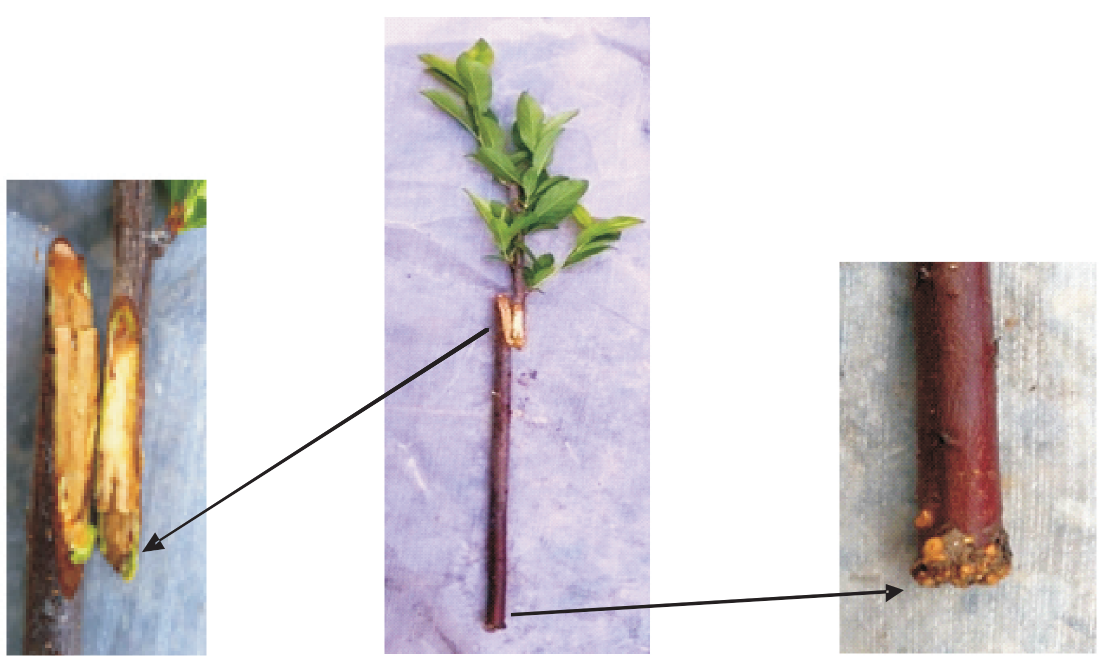

Figure 4. Callusing at the basal end of non-rooted cuttings of pixy plum, formation of callus at graft union and leaf sprouting (60 days after grafting)

Growth parameters

Various growth parameters of stentlings viz., height, internode length and number of lateral shoots per stentlings were significantly affected by different IBA treatments and cultivars (Table 2). Cultivar Frontier, produced vigorous growth,attaining an average height of grafts up to 125.0 cm, followed by Mariposa and Santa Rosa in decreasing orders. Among different IBA concentrations, the mean height at the end of growing season was observed maximum with IBA at 5000 ppm and minimum in untreated control. Similarly, the other growth parameters such as intermodal length and number of shoots per stentling were also found significantly higher with IBA treatment when applied at 5000 ppm. Among cultivars, 'Frontier' had maximum values of these two parameters and 'Santa Rosa' recorded minimum number of laterals as well as intermodal length.

It is clear from the data (Table 2) that the application of IBA at higher concentrations (2500 & 5000 ppm) resulted the production of significantly higher number of lateral shoots (8.11 per stentling) than control. Among different cultivars, 'Frontier' produced significantly higher number of lateral shoots (9.00/stentling) as compared to other cultivars.The variable growth of stentlings, in this study might be due to the cumulative effects of scion cultivars as well as the different IBA concentrations used for rooting.

Foliar characteristics

The bud break and leaf emergence was observed just 11.78 to 15.33 days after the grafting. Initially, growth of emerging leaves and shoots was slow but there was rapid growth after 45 to 60 days of grafting (data on growth rate not presented). It is clear from the plates (Figure 4) that callus formation occurred simultaneously at both ends of the grafted components. The callusing was observed visually by uprooting the grafted components however; data on callus formation and histological studies on wound healing process was not recorded.

In this study, a proportional increase in the production of leaves with stentling height was observed (Figure 5). Cultivar Frontier produced significantly larger leaves in to Mariposa and Santa

Table 2. Effect of different IBA concentrations on various growth parameters of plum cvs. Santa Rosa, Mariposa and Frontier grafted onto non-rooted cuttings of Pixy plum under polyhouse conditions

Cultivars/	Stentling height (cm),				Intermodal length (cm)				Shoots/lateral braches (number per stentling)			
Treatments	Santa Rosa	Mariposa	Frontier	Mean	Santa Rosa	Mariposa	Frontier	Mean	Santa Rosa	Mariposa	Frontier	Mean
T_1	96.61	122.2	136.1	118.3	1.51	1.82	1.99	1.77	3.67	9.00	11.67	8.11
T_2	87.50	114.1	124.5	108.7	1.40	1.78	1.86	1.68	5.00	7.00	10.00	7.33
T_3	77.91	118.4	121.0	105.8	1.36	1.62	1.80	1.59	3.67	5.67	8.33	5.88
T_4	63.02	109.2	118.4	96.89	1.31	1.43	1.67	1.47	3.00	4.33	6.00	4.44
Mean	81.26	116.0	125.0		1.40	1.66	1.83		3.83	6.50	9.00	
CD (P=0.05)												
T :		2.82				0.03				1.24		
V :		2.47				0.02				1.07		
TxV :		5.42				0.09				2.57		

Table 3. Analysis of variance table for leaf area and number/stentlings (P=0.05)

Source	*df*	Leaf area		Number of leaves	
		f values	*p* values	*f* values	*p* values
Treatments	3	3.92	0.0283	21.50	0.0000
Varieties	2	1081.04	0.0000	65.50	0.0000
TxV	6	2.00	0.1258*	1.84	0.1544*

*Non significant

Rosa. It is evident (Table 3) that the different IBA treatments and cultivars had significant effects on the production of leaves. Following 5000 ppm IBA treatment to 'Frontier', the production of number of leaves was recorded maximum, whereas, it was observed minimum in cultivar Santa Rosa (Figure 5). The morphological features of any plants is controlled by the genetic makeup of genotypes and, in this analogy during the present study, the size of leaf might be a characteristic feature of any genotype, varied according to the cultivars irrespective of different IBA concentrations.

Root growth and development

It is evident (Figure 6) that the production of roots as well as and length increased with the increase in the concentration of IBA. Frontier had maximum root length (52.68 cm) when treated with IBA at 5000ppm (T_1) and minimum average root length (15.40cm) was observed in Santa Rosa under untreated control. The root number ranged between 4.67 per stentling in Santa Rosa under untreated control to 13.76 in Frontier following the application of IBA at 5000 ppm. Among the three cultivars, the maximum root number was observed in Frontier and minimum in Santa Rosa plum. Further it was observed that stentlings having good aerial growth had more number of roots as well as length. Such an increase in root growth due to exogenous auxins have been reported earlier in the cuttings of stone fruits (Markovsky *et al*. 2015; Negi and Upadhyay 2016) on different rootstocks.

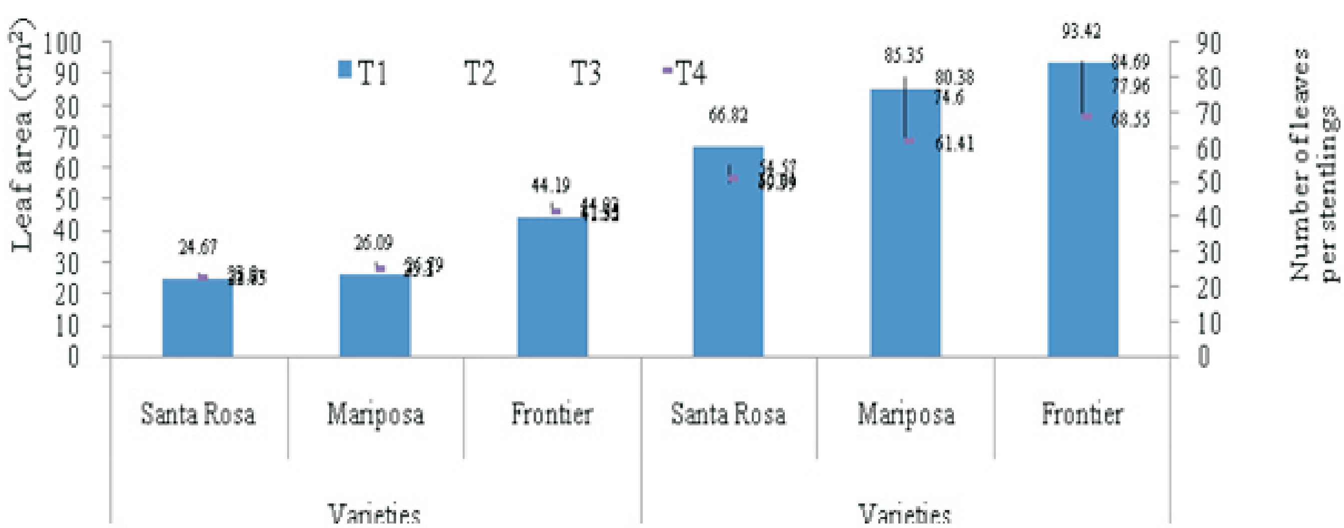

Figure 5. Effect of different IBA concentrations on leaf area and production of leaves per stentlings in plum cvs. Santa Rosa, Mariposa and Frontier grafted onto non-rooted cuttings of Pixy plum under polyhouse conditions

Table 4. Analysis of variance table for characteristics (P=0.05)

Source	Df	Root length		Number of roots		Diameter (below graft union)		Diameter (above graft union)	
		F values	*p* values	F values	*p* values	F values	*p* values	F values	*p* values
Treatments	3	34.89	0.0000	67.80	0.0000	188.13	0.0000	14.82	0.0001
Varieties	2	73.82	0.0000	97.68	0.0000	677.62	0.0000	33.31	0.0000
TxV	6	4.80	0.0056	1.32	0.3063*	13.64	0.0000	0.72	0.6374*

*Non significant

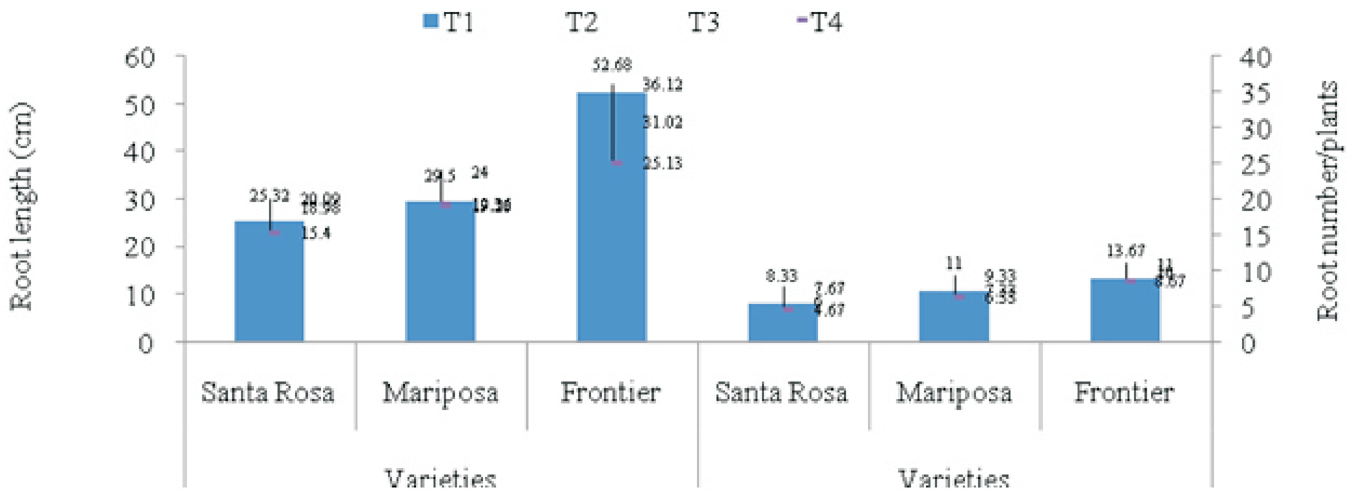

Figure 6.Effect of different IBA concentrations on average root length (cm) and production of roots in plum cvs. Santa Rosa, Mariposa and Frontier grafted onto non-rooted cuttings of Pixy plum under polyhouse conditions

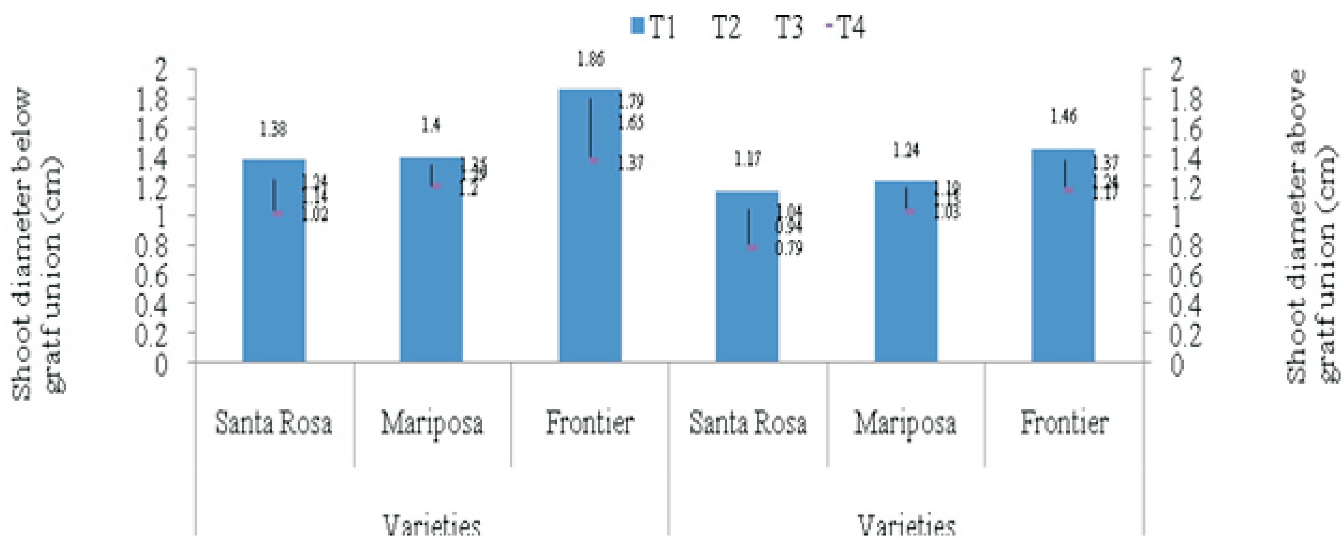

Figure 7. Effect of different IBA concentrations on diameter of stentling (above and below graft union) in plum cvs. Santa Rosa, Mariposa and Frontier grafted onto non-rooted cuttings of Pixy plum under polyhouse conditions

The radial growth of stentlings above and below graft union was also found to be significantly affected by different IBA treatments, cultivars (Table 4; Figure 7). While performing grafting on stocks of non-rooted cuttings of Pixy, it was ensured that the thickness of all stocks used were of same thickness. The average diameter of stentlings was significantly more in 'Frontier' than 'Mariposa' and 'Santa Rosa' plum. Higher concentrations of IBA resulted more radial growth when compared with untreated control (Figure 7). In this study, direct grafting the scions of plum cultivars onto non-rooted cuttings of Pixy have shown great promise in success of propagation by stenting, however, application of IBA further augmented the rooting and subsequent growth of the stentilngs.

Conclusion

From the present study, it can be inferred that simultaneous grafting and rooting in plum under polyhouse conditions can be a viable method of nursery production in plum. By this technique, nursery plants of plums can be multiplied quickly and could be made available to the growers within a year. Further, the application of IBA resulted in increased root formation which supplemented the stentling growth and development. Thus this grafting technique in plum is economical and viable option for quick multiplication for stakeholders under polyhouse conditions.

References

Gill JaspreetKaur, Harminder Singh, A Thakur and Jawandha SK. 2014. Studies on simultaneous grafting and rooting of peach Flordaguard rootstock. HortFlora Research Spectrum **3** (3): 259-262.

Hartmann Hhudson T, Kester Dale E, Davies Fred T (Jr.) and Geneve Robert L. 2009.Plant propagation principles and practices.7th Edn. PHI Learning Pvt. Ltd. New Delhi-110001.pp 411-448.

Izadi Zeinab and Hossein Zarei. 2014. Evaluation of propagation of Chinese hibiscus (*Hibiscus rosa-sinensis*) through stenting method in response to different IBA concentrations and rootstocks. American Journal of Plant Sciences **5**: 1836-1841. http://www.scirp.org/journal/ajps http://dx.doi.org/10.4236/ ajps. 2014.513197.

Karimi HR and Nowrozy M. 2017. Effect of rootstock and scion on graft success and vegetative parameters of pomegranate. ScientiaHorticulturae **214**: 280-287.

Kroin Joel. 2015. Propagation of roses: stenting-simultaneously cutting and grafting. Hortus USA file:///C:/Users/horti/Downloads/x-Kroin_Propagation_of_Roses_by_Stenting_2015.pdf.

Markovski Aleksandar, Melpomena Popovska and Viktor Gjamovski. 2015. Investigation of the possibility for production of some stone fruit rootstocks by rooting cuttings. Acta Agriculturae Serbica **39** : 75-831.

Megre Dace, Uldis Kondratovičs and Kristine Dokane. 2007. Simultaneous graft union and adventitious root formation during vegetative propagation in elepidote rhododendrons. ActaUniversitatisLatviensis Biology **723**: 155-162.

Negi ND and Upadhyay SK. 2016. Stenting-a new method of propagation for cherry and pear under polyhouse conditions. Indian Journal of Horticulture **73** (2): 296-299.

Oguz Dolgun, Adnan Yildirim, Mehmet Polat, Fatma Yildirim and Atilla Akin. 2009. Apple graft formation in relation to growth rate features of rootstocks. African Journal of Agricultural Research **4** (5): 530-534.