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Influence of plant growth regulators on growth and formation of sylleptic shoots in one-year-old apple cv. Gala Mast

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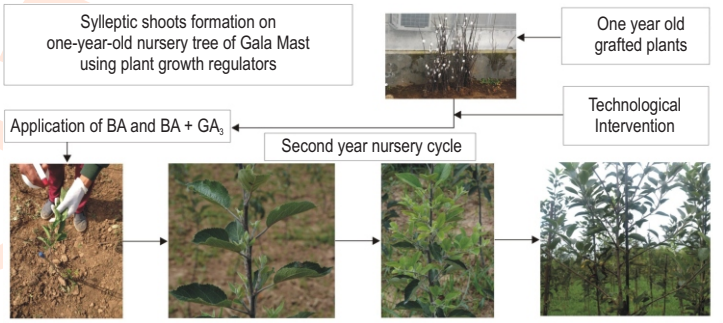
Abstract

Aim: To examine the effectiveness of 6-benzyladenine (BA) alone or in combination with gibberellic acid (GA_3) on sylleptic shoots formation on one-year-old apple cv. Gala Mast grafted on MM-106 rootstock.

Methodology: During the second vegetative period, the actively growing apical portion of the nursery tree was sprayed with varying doses of BA and BA+GA_3 (200, 300, 400, 500, 600, and 700 ppm) using a hand sprayer until run-off. Three spray treatments were performed at one week apart. The first spray was applied when the growth of the central leader reached 70-80 cm.



Application of 3 sprays of 700 ppm BA at a one-week interval during the second vegetative cycle produces most significant results in term of growth and sylleptic shoots formation. However, it is economical to apply 3 sprays of 500 ppm BA for acceptable growth and branching.

Results: All plant growth regulators treatments significantly increased the number and length of sylleptic shoots, branching zone and per cent branched trees compared to control. The unsprayed trees had an average of 1.95 useful sylleptic per tree, 1.1 m total sylleptic shoot length, 10.24 cm branching zone and 35 per cent branched trees. Application of BA (700 ppm) resulted in four-fold increase in the number of sylleptic shoots (7.8), more than two-fold increase in total length of sylleptic shoots (2.44 m), and around four-fold increase in branching zone (38.96 cm). The 100 per cent branched trees were obtained with the application of BA (500, 600, 700 ppm) and BA+GA_3 (600, 700 ppm) in both experimental years. Moreover, these treatments obtained 2.78 to 2.90 values of the trunk and mean sylleptic shoot diameter ratio.

Interpretation: Application of BA alone or in combination with GA_3 played an important role in overcoming apical dominance and therefore in the production of well-branched apple nursery tree. However, application of BA alone has a stronger positive effect on sylleptic shoot formation. More than 400 ppm BA appears to give a significant stimulation for the production of sylleptic shoots, with the highest beneficial effect reported with the application of 700 ppm BA. However, it is cost-effective to apply 3 sprays of 500 ppm BA for adequate growth and sylleptic shoot production.

Key words: 6-benzyladenine, Gibberellic acid, Nursery tree, Plant growth regulators, Sylleptic shoot

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Introduction

Planting well-branched apple nursery trees from a nursery increases total production earlier in the life of the orchard (Reig *et al.,* 2019). Therefore, farmers should plant apple nursery trees with well-formed canopies capable of starting fruit as soon as possible to get an early return on investment. Moreover, precocity regulates vegetative vigor and therefore results in optimum tree growth for flowering and fruiting. Thus improving branch development in apple nursery trees at nursery stage is going to be more and more economically important for nurserymen as well as apple growers. Apple nursery trees produce two types of lateral branches, i.e., proleptic and sylleptic. Proleptic branches arise from axillary buds after a dormant period and are made up of preformed stem units that exist in dormant buds and elongate after bud break whereas sylleptic branches arise from axillary meristems without a dormancy period while the leader is growing and are made up of neoformed stem unit by simultaneous initiation and elongation (Wilson, 2000; Costes *et al.,* 2006). Generally, vigorously growing apple nursery trees do not form any proleptic branch during first and second years of grafting. However, if terminal growth is slow, the tree may produce one to three proleptic branches depending on the genotype.

Physical intervention like heading back can interrupt the apical dominance and encourage elongation of the preformed stem unit (Elfving and Vissar, 2007). The heading back cut performed on a one-year-old apple nursery tree during the second year of nursery cycle can produce three or more proleptic branches. However, the heading cut produces vigorous, dominating, and narrow-angled proleptic branches that are limited to few buds just below the cutting area. Therefore, this prevents the well-positioning of trees (Hoying *et al.,* 2001). Furthermore, heading cut lead to a smaller trunk diameter and weakened roots (Mika *et al.,* 2003). In addition, heading discourages early production by removing potential fruiting spurs as well as wiping out some tree reserves (Ouellette *et al.,* 1996). Therefore, the induction of sylleptic shoots is the only way to obtain well-branched apple nursery trees. However, in apples, the natural ability to form sylleptic shoot is generally low (Volz *et al.,* 1994; Kumawat *et al.,* 2020). The main factor responsible for poor sylleptic shoot formation is a strong apical dominance.

Apical dominance is the control that the terminal bud exerts over the outgrowth of lateral buds (Cline, 2006) and is driven by auxin (Leyser, 2009). Thus, sylleptic shoots in the apple nursery trees can be encouraged by interrupting the apical dominance by chemical (plant growth regulators) and physical (which alter the auxin-cytokinin balance, i.e., top twisting or removal of complete or part of apical leaves, etc.) treatments (Wertheim and Webster, 2003; Elfving and Visser, 2006). But physical treatments are not found so effective and economic as bioregulators (Elfving and Visser, 2006). Several studies have been conducted on the possibilities of using plant growth regulators to stimulate sylleptic shoots in nursery trees, however, the recommendations of plant growth regulators for sylleptic shoots formation in apple nursery trees varies from region to region (Robinson and Sazo, 2014; Lordan *et al.,* 2017) because effect of plant growth regulators varies depending on the cultivar (Lordan *et al.,* 2017; Necas *et al.,* 2020), rootstock (Rufato *et al.,* 2019), and ecological condition (Lordan *et al.,* 2017). Additionally, sylleptic shoot formation may be influenced by nutrient availability (Radivojevic *et al.,* 2016), supply of irrigation water (Doric *et al.,* 2014), plant density, cultural practices, biotic and abiotic stress, etc. Gala Mast is an important apple cultivar and gaining popularity in India. Therefore, this study aimed to investigate the effect of summer spray different concentrations of cytokinin (6-benzyladenine) alone and in combination with gibberellic acid (GA_3) on the growth and sylleptic shoots formation on one-year-old 'Gala Mast'/MM-106 apple nursery tree.

Materials and Methods

A field experiment was conducted in 2016 and 2017 at ICAR-CITH, Srinagar (33.58' N, 74°48' E, and 1644 m a.s.l.) to compare the effect of summer spray of varying concentrations of 6-benzyladenine alone and in combination with gibberellic acid (1:1) on growth and sylleptic shoots development on one-year-old 'Gala Mast' apple nursery trees. The treatment include untreated control (T_1), 200 ppm BA (T_2), 300 ppm BA (T_3), 400 ppm BA (T_4), 500 ppm BA (T_5), 600 ppm BA (T_6), 700 ppm BA (T_7), 200 ppm BA+GA_3 (T_8), 300 ppm BA+GA_3 (T_9), 400 ppm BA+GA_3 (T_{10}), 500 ppm BA+GA_3 (T_{11}), 600 ppm BA+GA_3 (T_{12}) and 700 ppm BA+GA_3 (T_{13}). A silicon-based adjuvant, Wetwell (Biostadt India Ltd.) was added to each spray treatment @ 400 µl l^{-1}. The MM-106 rootstocks were planted in the nursery at 20 x 25 cm spacing in February and grafted with the 'Gala Mast' scion with 4-5 buds at 20 cm height by wedge grafting in March. Standard nursery management practices such as water, nutrient, weed, and biotic stress management, among others, were followed. The following year, these one-year-old trees with a height of 55-65 cm were lifted in February and planted at 90 x 60 cm spacing in the experimental block.

The soil of the experimental block was clay loam in texture with pH 6.86, EC 0.38 dSm^{-1}. During the second vegetative period, the actively growing apical portion of the nursery tree was sprayed using a hand sprayer until run-off. Three spray treatments were applied at one week interval. The first spray was applied when the growth of the central leader reached 70-80 cm (May, 19). The experiment was conducted using a randomized block design with four replications and five trees per replication (20 trees per treatment). The uniform cultural practices and irrigation, fertilizer, pest, and disease programme were applied in the nursery. In the fall, nursery trees were measured for height (measured by fiberglass measuring tape), trunk diameter (10 cm above bud union by digital Vernier caliper), total number of proleptic shoots (shoot produced by preformed stem unit), total number of sylleptic shoots (shoot produced by neoformed stem unit), distance from the ground level to each of the induced sylleptic shoot, length (measured by steel scale), diameter (measured by digital Vernier caliper), and crotch angle of each

sylleptic shoot (measured as degree divergence from the main axis at the sylleptic shoot base with the aid of a transparent protractor), branching zone (distance between the highest and lowest sylleptic shoots) and per cent branched trees (trees with at least three sylleptic shoots were considered as a branched tree and their number was expressed as a per cent of the total number of trees per plot). The total sylleptic shoot length per tree was estimated by aggregating the length of all sylleptic shoots of the tree. Weather data during both study years are presented in Fig. 1-4. The mean annual temperature during 2016 was 14.1°C and during 2017 was 13.85 °C which was respectively 0.51°C and 0.26°C higher than the mean for the years 2005 to 2015.

The total precipitation was 274.2 mm during 2017 and 398.68 mm during 2017 which was respectively 174.33 and 49.85 mm less than that of 2005 to 2015. Daily maximum, minimum, and average temperature on treatment application day during 2016 and 2017 are presented in Fig. 1 and Fig. 2, respectively. Whereas, during 2016 and 2017 the maximum, minimum, and the average temperature of one week before the first application, during applications week, and one week after the last application are shown in Fig. 3 and 4, respectively. The collected experimental data were subjected to statistical analysis based on the analysis of variance (ANOVA) using the programme SAS 9.3. Significant differences at p=0.05 level were obtained using Duncan's Multiple Range Test.

Results and Discussion

On the basis of pooled data of two-year study; the proleptic shoot formation on one-year-old apple nursery trees was not influenced by the application of plant growth regulators. However, the plant growth regulator treatments significantly affected the development of sylleptic shoots on one-year-old 'Gala Mast' apple nursery trees. In two years investigation, we recorded mean 1.95 sylleptic shoots in the control trees whereas the number varied from 3.64 (BA+GA_3-200 ppm) to 7.8 (BA-700

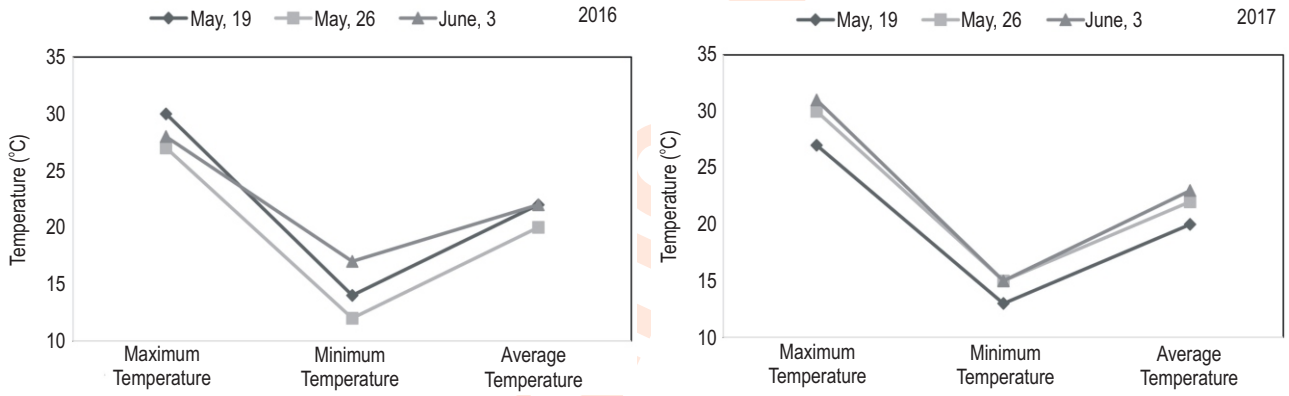

Fig. 1 and 2: Maximum, minimum, and average temperature on treatment application days.

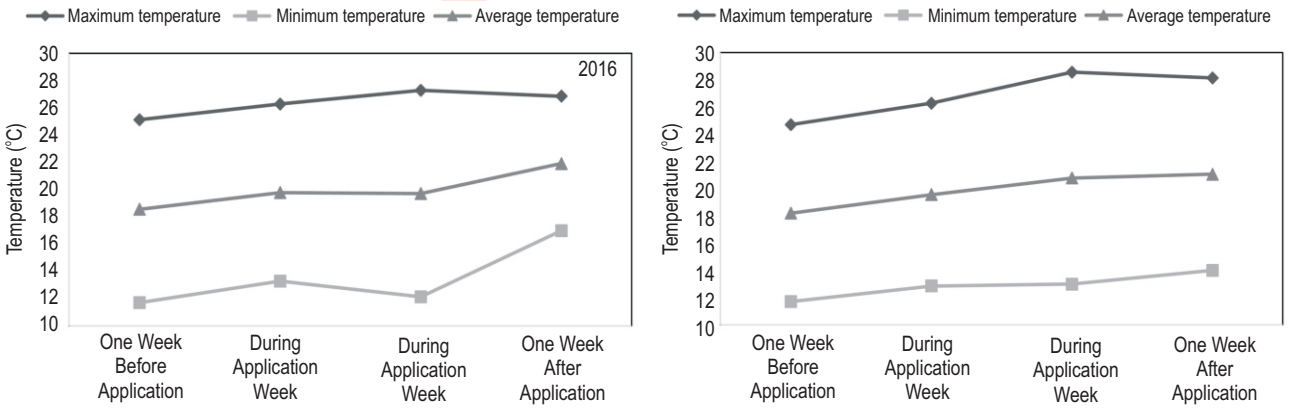

Fig. 3 and 4: Weekly maximum, minimum, and average temperature one week before the first application, during treatment applications and one week after the last application.

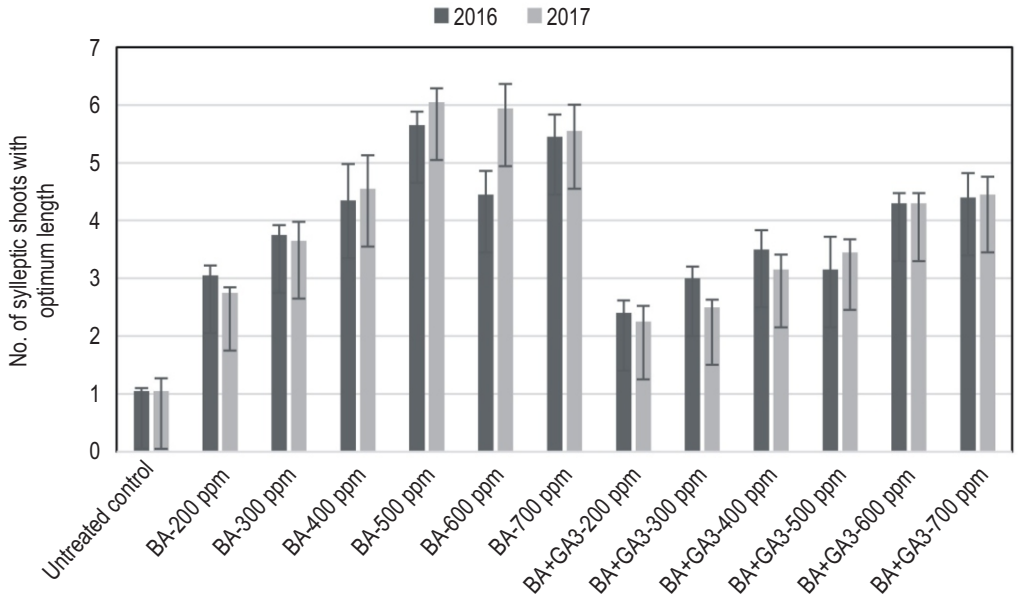

Fig. 5: Number of sylleptic shoots with optimum length (10-40 cm) produced by various plant growth regulator treatments. The bars represent ±S.E.

ppm) in treated trees. The lower concentration of BA (200, 300, and 400 ppm) and all concentrations of BA+GA_3 (200 - 700 ppm) caused low branching of apple nursery tree. In the present study, satisfactory sylleptic shoot formation was recorded in BA treatment at 500 and 600 ppm (7.53 and 7.1 sylleptic shoots/tree) whereas the higher concentration of 700 ppm produced the most positive effect (7.8 sylleptic shoots/tree) (Table 1; Fig. 8). However, less significant differences were observed between 500 and 700 ppm BA treatment indicating that only 500 ppm were needed for acceptable branching. The findings of previous studies of one-year-old maiden apple nursery trees indicates that increasing concentration of BA from 250 to 450 ppm (Doric *et al.,* 2015) and 300 to 600 ppm (Radivojevic *et al.,* 2015) led to the emergence of higher number of sylleptic shoots. Apparently, BA alone was found more effective than BA+GA_3 for inducing sylleptic shoots which might be due to higher concentration of benzyladenine (200-700 ppm) when applied alone as compared to lower concentration (100-350 ppm) in combination with GA_3.

Gastol *et al.* (2012) reported that higher doses of benzyladenine (780 mg BA+120 mg GA_3 per dm^{-3}) promoted more branching as compared to lower (330 mg BA+570 mg GA_3 per dm^{-3}) in 'Boskoop' cultivar of apple. The findings of the present investigation suggest that higher concentration (more than 400 ppm) would seem to provide a major stimulus for sylleptic shoots formation in one-year-old Gala Mast apple nursery tree. Treatment with BA and BA plus GA_3 significantly affected the formation of sylleptic shoots which might be due to BA application overcame apical dominance. Apical dominance has been attributed mainly to the export of auxins from the shoot apex to regions below. The application of benzyladenine affects the flow of auxin (Muller and Leyser, 2011) and temporarily obstructs the central leaders' growth (Sazo and Robinson, 2011), which helps in overcoming the apical dominance and creates favourable environment for sylleptic shoots formation in one-year-old nursery tree of the investigated cultivar. In the present study, the length of sylleptic shoots was affected by different plant growth regulator treatments. With some exception, in the treated tree, short (10-20 cm), medium (21-30, 31-40, and 41-50 cm), and long (>50 cm) sylleptic shoots and total sylleptic length were significantly greater than control, however, no significant difference was observed between treated and untreated control in respect of long sylleptic shoots (>50 cm) during 2016. For high-density planting, the sylleptic shoot length of the apple nursery tree should be less than 40 cm (Sazo and Robinson, 2012) to reduce branch manipulation after planting and examining the length of sylleptic shoots, it is easy to see that the treatment with higher BA concentration (400-700 ppm) produced the maximum number of short and medium sylleptic shoots (Table 2).

The maximum mean sylleptic shoots with 10-40 cm length (5.28) were recorded with the application of BA-500 ppm and this treatment remained statistically at par with BA-600 ppm (4.64) and BA 700 ppm (4.83) (Fig. 5). These findings suggest that treatments containing a higher concentration of BA are more effective in the formation of sylleptic shoots of optimum length, i.e., 10-40 cm. As far as mean sylleptic shoot length of concern trees treated with a higher concentration of BA (500, 600 and 700 ppm) and BA+GA_3 (600 and 700 ppm) resulted in lower mean sylleptic shoot length and the lowest value (31.30 mm) obtained in BA-500 ppm (Table 3) might be due to presence of a higher

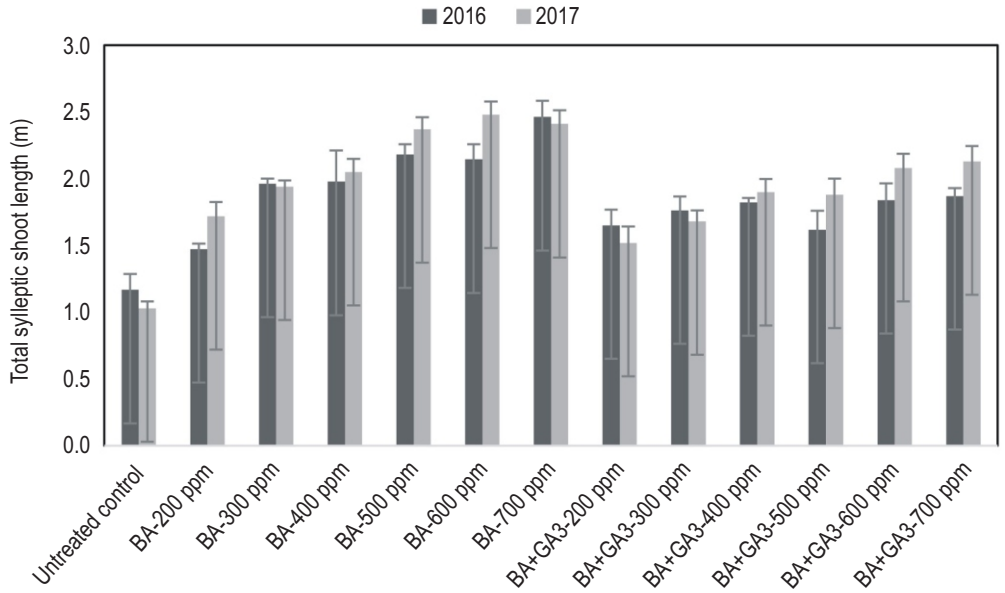

Fig. 6: Effect of various plant growth regulator treatments on total sylleptic shoots length (m). The bars represent ±S.E.

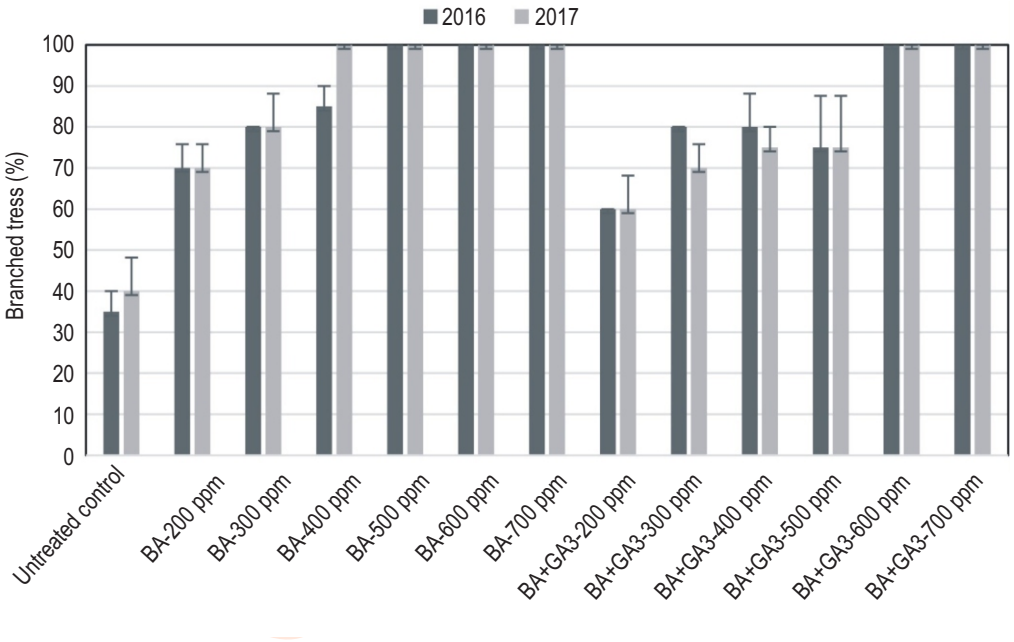

Fig. 7: Effect of BA and BA+GA_3 treatments on percentage of branched trees. The bars represent ±S.E.

number of shoots on the tree. A decrease in mean sylleptic shoot length with an increase in the number of shoots on trees is an expected pattern because the nursery tree has to divide its energy budget among a greater number of sylleptic shoots. The results are in accordance with earlier findings of Doric *et al.* (2015); Radivojevic *et al.* (2015) and Kumawat *et al.* (2020). The effects of spray treatments on total sylleptic shoot length seemed similar to those on the sylleptic number, and the total length of the sylleptic

Fig. 8: Branching performance of best treatments along with untreated control.

shoots increased as the number of sylleptic shoots increased on the nursery trees. Further, the application of BA alone was found to be more effective than a combined application with GA_3 in terms of total sylleptic shoot length. However, the application of BA+GA_3 resulted in longer sylleptic shoots, which could be attributed to gibberellic acid's positive influence on cell elongation. Still, the total length of sylleptic shoots was higher in BA application. This could be due to the fact that BA treatments induce greater number of sylleptic shoot on the nursery trees (4.30 to 7.80 per tree) than BA+GA_3 (3.64 to 5.95 per tree), resulting in higher total sylleptic shoot length (Table 1; Fig.6). The results are in agreement with previous findings (Gastol *et al.,* 2012, Doric *et al.,* 2015, Radivojevic *et al.,* 2015). The current findings revealed that the higher concentration of BA alone and in combination with GA_3 expanded the branching zone of the apple nursery tree. In spite of higher shoot internode length obtained from tree treated with BA+GA_3 branching zone values were higher in BA (31.77 to 38.96 cm) than BA+GA_3 (28.61 to 34.13 cm) treated trees. This might be due to the fact that BA treatments induced more sylleptic shoots, and the highest branching zone (38.96 cm) was observed in the treatment that induced the most sylleptic shoots, i.e., BA-700 ppm (Table 3). The results are partially supported by Doric *et al.* (2015).

In the present study, irrespective of treatments sylleptic shoots initially emerged at a very narrow-angle from vertical, which increased with the advancement of growth of nursery trees, however during 2016 final crotch angle was significantly narrow in trees treated with a higher concentration of BA (51.25° with 600 ppm and 50.79° with 700 ppm) compared to control (53.38°) and lower concentration. Likewise during 2017; a higher concentration of BA (500-700 ppm) slightly decreased the final crotch angle compared to control and lower concentration of BA but the difference was not statistically significant. Based on the mean value of both years, it was established that higher concentrations of BA (600 and 700 ppm) produce sylleptic shoots with narrow crotch angles (51.81 and 51.61°) (Table 3). This is in agreement with the work of Steiner *et al.* (2013) and Doric *et al.* (2016) in apple.

The use of aggressive chemical branching agents in apple nursery trees can result in a narrow crotch angle (Steiner *et al.,* 2013). Thus current findings suggest that a higher concentration of BA (500 - 700 ppm) acts as an aggressive branching agent compared to the lower rate of BA. Nonetheless, when assessing treatment effects, the number of the sylleptic shoot (vary from 4.30 to 5.95 per tree in lower BA concentration compared to 7.1 to 7.8 per tree in higher BA concentration) should also be taken into account, as it can affect the average sylleptic shoot crotch angle, which is wider in the lower tiers of sylleptic shoots than in the higher tiers probably due to longer available growing period. The application of higher concentration of BA or BA+GA_3 for induction of branching in a one-year-old apple nursery tree can result in a narrow crotch angle but still, it did not

Table 1: Effect of various plant growth regulator treatments on proleptic and sylleptic shoots development

Treatments	Number of proleptic shoots/tree (< 70 cm height from ground)			Number of sylleptic shoots/tree		
	2016	2017	Mean	2016	2017	Mean
Untreated control	2.10^{a}	1.55^{ab}	1.83^{a}	1.85^{h}	2.05^{f}	1.95^{l}
BA-200 ppm	1.05^{bdc}	1.25^{ab}	1.15^{ab}	4.35^{f}	4.25^{d}	4.30^{g}
BA-300 ppm	1.55^{abcd}	1.65^{ab}	1.60^{ab}	5.45^{cd}	5.40^{bc}	5.43^{d}
BA-400 ppm	1.15^{bcd}	1.35^{ab}	1.25^{ab}	5.90^{bc}	6.00^{b}	5.95^{c}
BA-500 ppm	0.85^{d}	1.20^{ab}	1.03^{b}	7.40^{a}	7.65^{a}	7.53^{ab}
BA-600 ppm	1.30^{abcd}	1.10^{ab}	1.20^{ab}	6.40^{b}	7.80^{a}	7.10^{b}
BA-700 ppm	1.15^{bdc}	1.00^{ab}	1.08^{b}	7.75^{a}	7.85^{a}	7.80^{a}
BA+GA_3-200 ppm	1.78^{abc}	1.30^{ab}	1.51^{ab}	3.63^{g}	3.65^{e}	3.64^{h}
BA+GA_3-300 ppm	1.35^{abcd}	1.40^{ab}	1.38^{ab}	4.80^{ef}	4.20^{de}	4.50^{fg}
BA+GA_3-400 ppm	1.15^{bdc}	1.20^{ab}	1.18^{ab}	5.10^{de}	4.85^{c}	4.98^{e}
BA+GA_3-500 ppm	0.95^{dc}	0.90^{b}	0.93^{b}	4.50^{ef}	5.15^{c}	4.83^{ef}
BA+GA_3-600 ppm	1.95^{ab}	1.60^{ab}	1.78^{a}	5.85^{bc}	5.80^{b}	5.83^{cd}
BA+GA_3-700 ppm	1.40^{abcd}	1.75^{a}	1.58^{a}	6.00^{bc}	5.90^{b}	5.95^{c}
Pr > F	0.0422	0.3567	0.0663	<.0001	<.0001	<.0001

Values within a column followed by same letter are not significantly different at *P*>0.05, as established by the Duncan's Multiple Range Test.

considerably affect it and produces sylleptic shoots with desired crotch angle. The height and trunk diameter of nursery trees are important parameters for determining the quality of nursery. Analysis of mean data pertaining to tree height revealed that the application of BA alone and in combination with GA_3 had a mild negative effect on tree height although, the height of the treated tree still met the standard criteria (163.58 to 170.12 cm) (Table 4). The results are in agreement with earlier research findings on one-year-old apple tree which confirmed that tree height with the application of BA as a branching agent slightly decreased in 'Cadel', and 'Granny Smith' (Radivojevic *et al.,* 2015; Doric *et al.,* 2016) apple cultivars. Likewise, tree height with the application of BA+GA_3 significantly decreased in 'Cadel' (Radivojevic *et al.,* 2015) and 'Gala' (Doric *et al.,* 2015) and slightly decreased in 'Jonagold' (Radivojevic *et al.,* 2015). The frequent formation of syllepsis resulted in a shorter tree, which could be attributed to the effect of BA, as BA application interrupts the growth of the leader, or simply to the allocation of growth resources such as carbon and nitrogen to the new syllepsis rather than the growing central leader (Lañar *et al.,* 2019; Wolf *et al.,* 2019; Kumawat *et al.,* 2020).

In the present study, significantly higher trunk diameter (14.3 to 14.63 mm) was obtained with the application of higher doses of BA (500-700 ppm), on the other hand, significantly lower trunk diameter (13.36 to 13.90 mm) was obtained with the application of lower doses of BA (200-400 ppm) compared to control (13.94 mm). The results are in accordance with Doric *et al.* (2015) who reported that higher doses of BA (450 ppm) significantly increase trunk diameter in 'Gala' and 'Jonagold' apple compared to lower (250 and 350 ppm). Further, tree treated with combined application of 600 ppm BA+GA_3 obtained significantly higher trunk diameter compared to control. The result is in conformity with the findings of Doric *et al.* (2015, 2016). However, based on research reports, the mechanism by which BA and BA plus GAs influenced the trunk diameter cannot be clearly described. Auxin, cytokinin, gibberellin, and ethylene are the four key hormonal signals that stimulate and control wood development in plants (Doric *et al.,* 2015). The flow of auxin through the cambium regulates cambial activity (Aloni, 2007). Because BA influences auxin flow (Leyser, 2005), it is highly likely that it also influences cambial activity, i.e., the thickening of the trunk and thus resulting in smaller trunk diameter with lower concentration of BA and BA+GA_3. However, the positive influence of higher concentration of BA and BA+GA_3 could be explained by the presence of a greater number of sylleptic shoots than control, as presence of higher number of sylleptic shoots on nursery tree can have a positive effect on trunk diameter (Jacyna, 2007; Wolf *et al.,* 2019).

During each year of study, fewer differences were found between the treatments for mean sylleptic shoot diameters, and based on the mean of two years study, it ranged from 4.94 to 5.85 mm in the treated trees. BA+GA_3-200 ppm treatment obtained maximum mean sylleptic shoot diameter (5.85 mm) which remains statistically at par with control. Whereas, the minimum mean sylleptic shoot diameter was recorded with the application of BA 700 ppm (4.94 mm). Examining the mean sylleptic shoot diameter it is clear that with some exceptions the treatment which produces more sylleptic shoot resulted in lower mean sylleptic shoot diameter value (Table 4), probably because of increased inter-shoot competition for resources. Trunk and mean feather diameter ratios play an important role in canopy management during the initial years of high-density planting. Cook and Strydom (2000) advised the 3: 1 pruning rule which means that the central leader should be 3 times the diameter of any of the lateral

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Table 2: Effect of various treatments of plant growth regulator on sylleptic shoot length

Treatments	Number of sylleptic shoots (10-20 cm)			Number of sylleptic shoots (21-30 cm)			Number of sylleptic shoots (31-40 cm)			Number of sylleptic shoots (41-50 cm)			Number of sylleptic shoots (> 50 cm)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Untreated control	0.50^{e}	0.40^{g}	0.45^{h}	0.20^{e}	0.40f	0.30^{f}	0.35^{c}	0.25^{d}	0.30^{e}	0.55^{ef}	0.65^{de}	0.60^{ef}	0.30	0.35^{b}	0.33^{b}
BA-200 ppm	1.25^{cd}	1.10^{ef}	1.18^{efg}	1.00^{cd}	1.10de	1.05^{e}	0.80^{bc}	0.55^{cd}	0.68^{de}	0.70^{ef}	0.75^{cde}	0.73^{de}	0.65	0.75^{ab}	0.70^{ab}
BA-300 ppm	1.35^{cd}	1.30^{de}	1.33^{de}	1.70^{b}	1.50bcd	1.60^{bcd}	0.70^{bc}	0.85^{bcd}	0.78^{cde}	0.80^{cde}	0.80^{bcde}	0.80^{cde}	0.90	0.95^{ab}	0.93^{a}
BA-400 ppm	1.25^{cd}	1.30^{de}	1.28^{def}	1.45^{bc}	1.50bcd	1.48^{bcd}	1.65^{a}	1.75^{a}	1.70^{a}	0.75^{def}	0.70^{cde}	0.73^{de}	0.75	0.75^{ab}	0.75^{ab}
BA-500 ppm	2.30^{ab}	2.00^{bc}	2.15^{ab}	2.20^{a}	2.95a	2.58^{a}	1.15^{ab}	1.10^{abc}	1.13^{bcd}	1.15^{abc}	1.05^{abc}	1.10^{ab}	0.61	0.55^{ab}	0.58^{ab}
BA-600 ppm	2.15^{ab}	2.30^{ab}	2.23^{a}	1.20^{bc}	2.14b	1.67^{bc}	1.10^{ab}	1.50^{ab}	1.30^{abc}	1.20^{ab}	1.17^{ab}	1.18^{ab}	0.65	0.70^{ab}	0.68^{ab}
BA-700 ppm	2.40^{a}	2.55^{a}	2.48^{a}	1.70^{b}	1.80bc	1.75^{b}	1.35^{ab}	1.20^{abc}	1.28^{abc}	1.30^{a}	1.25^{a}	1.28^{a}	1.05	1.05^{a}	1.05^{a}
BA+GA_3-200 ppm	0.93^{de}	0.78^{ef}	0.85^{fgh}	0.63^{de}	0.58ef	0.60^{f}	0.85^{bc}	0.90^{bcd}	0.88^{bcd}	0.38^{f}	0.50^{e}	0.44^{f}	0.85	0.90^{ab}	0.88^{a}
BA+GA_3-300 ppm	0.85^{de}	0.70^{f}	0.78^{gh}	1.55^{b}	1.15cde	1.35^{cde}	0.60^{bc}	0.65^{cd}	0.63^{de}	1.10^{abcd}	0.95^{abcd}	1.03^{abc}	0.70	0.75^{ab}	0.73^{ab}
BA+GA_3-400 ppm	1.15^{cde}	1.00^{ef}	1.08^{efg}	1.40^{bc}	1.10de	1.25^{ed}	0.95^{abc}	1.05^{abc}	1.00^{bcd}	0.90^{bcde}	1.00^{abcd}	0.95^{bcd}	0.65	0.70^{ab}	0.68^{ab}
BA+GA_3-500 ppm	1.05^{cde}	1.25^{de}	1.15^{efg}	1.35^{bc}	1.15cde	1.25^{ed}	0.75^{bc}	1.05^{abc}	0.90^{bcd}	0.65^{ef}	0.75^{cde}	0.70^{de}	0.85	0.95^{ab}	0.90^{a}
BA+GA_3-600 ppm	1.70^{bc}	1.85^{bc}	1.78^{bc}	1.40^{bc}	1.55bcd	1.48^{bcd}	1.20^{ab}	0.90^{bcd}	1.05^{bcd}	0.60^{ef}	0.65^{de}	0.63^{ef}	0.90	0.85^{ab}	0.88^{a}
BA+GA_3-700 ppm	1.65^{bc}	1.75^{cd}	1.70^{cd}	1.45^{bc}	1.30cd	1.38^{cde}	1.30^{ab}	1.40^{ab}	1.35^{ab}	0.75^{def}	0.70^{cde}	0.73^{de}	0.85	0.75^{ab}	0.80^{ab}
Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0416	0.0052	0.0005	<.0001	0.0012	<.0001	0.8704	0.7222	0.3002

Values within a column followed by same letter are not significantly different at *P*>0.05, as established by the Duncan's Multiple Range Test.

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Table 3: Effect of various treatments of plant growth regulator on mean sylleptic s hoot length, branching zone and average sylleptic crotch angle from vertical

Treatments	Mean sylleptic shoot length (cm)			Branching zone (cm)			Average sylleptic shoot crotch angle (°) from vertical		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Untreated control	37.53^{ab}	53.11^{a}	45.32^{a}	6.16^{f}	14.30^{g}	10.24^{g}	53.38^{ab}	54.12^{a}	53.75^{a}
BA-200 ppm	34.02^{ab}	40.43^{b}	37.23^{bcd}	33.36^{bc}	30.18^{de}	31.77^{de}	53.79^{ab}	53.88^{a}	53.83^{a}
BA-300 ppm	35.20^{ab}	36.08^{b}	35.64^{bcd}	32.69^{bc}	32.04^{cde}	32.37^{cd}	53.90^{ab}	53.62^{a}	53.76^{a}
BA-400 ppm	34.40^{ab}	34.27^{b}	34.34^{bcd}	34.83^{b}	36.40^{ab}	35.61^{b}	52.80^{ab}	53.72^{a}	53.26^{a}
BA-500 ppm	31.54^{b}	31.04^{b}	31.30^{d}	34.33^{b}	36.17^{ab}	35.25^{b}	53.87^{ab}	52.84^{a}	53.36^{a}
BA-600 ppm	33.70^{ab}	31.89^{b}	32.80^{cd}	27.72^{de}	36.46^{ab}	32.09^{cd}	51.25^{b}	52.37^{a}	51.81^{ab}
BA-700 ppm	32.33^{ab}	30.80^{b}	31.57^{d}	38.67^{a}	39.24^{a}	38.96^{a}	50.79^{b}	52.42^{a}	51.61^{ab}
BA+GA_3-200 ppm	38.66^{a}	41.60^{b}	40.13^{ab}	30.40^{cd}	26.80^{f}	28.61^{f}	53.10^{ab}	53.73^{a}	53.41^{a}
BA+GA_3-300 ppm	38.41^{a}	39.96^{b}	39.19^{abc}	30.43^{cd}	29.30e^{f}	29.86^{ef}	46.99^{c}	53.17^{a}	50.08^{b}
BA+GA_3-400 ppm	33.82^{ab}	39.40^{b}	36.62^{bcd}	32.16^{bc}	33.12^{bcd}	32.64^{cd}	54.90^{a}	51.80^{a}	53.35^{a}
BA+GA_3-500 ppm	38.68^{a}	36.67^{b}	37.68^{bcd}	25.86^{e}	31.18^{de}	28.52^{f}	50.33^{b}	53.26^{a}	51.79^{ab}
BA+GA_3-600 ppm	32.19^{ab}	36.06^{b}	34.13^{bcd}	33.13^{bc}	35.14^{bc}	34.13^{bc}	53.67^{ab}	52.67^{a}	53.17^{a}
BA+GA_3-700 ppm	32.25^{ab}	36.17^{b}	34.21^{bcd}	33.56^{bc}	34.61^{bc}	34.09^{bc}	55.14^{a}	52.43^{a}	53.79^{a}
Pr> F	0.0204	0.0062	0.0087	<.0001	<.0001	<.0001	0.0011	0.8483	0.0262

Values within a column followed by same letter are not significantly different at *P*>0.05, as established by the Duncan's Multiple Range Test ns

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Table 4: Effect of various treatments of plant growth regulator on tree height, trunk diameter, sylleptic shoot diameter and ratio of trunk to shoot diameter.

Treatments	Tree height (cm)			Trunk diameter (mm)			Mean sylleptic shoot diameter (mm)			Trunk and mean sylleptic shoot diameter ratio		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Untreated control	167.1^{abc}	174.09^{a}	170.60^{a}	13.91^{bcde}	13.96^{bcde}	13.94^{cde}	5.40^{ab}	6.28^{a}	5.84^{a}	2.59^{bcd}	2.24^{de}	2.42^{e}
BA-200 ppm	159.9^{d}	171.14^{ab}	165.52^{bcd}	13.29^{e}	13.42^{ef}	13.36^{f}	5.42^{ab}	5.61^{bcde}	5.52^{abcd}	2.47^{d}	2.41^{cde}	2.44^{e}
BA-300 ppm	166.2^{abcd}	171.18^{ab}	168.70^{abc}	13.99^{abcde}	13.80^{def}	13.90^{de}	5.13^{abc}	5.53^{bcdef}	5.33^{bcde}	2.73^{abcd}	2.50^{bcde}	2.62^{bcde}
BA-400 ppm	165.2^{bcd}	166.48^{bcde}	165.85^{abcd}	13.65^{de}	13.94^{bcde}	13.79^{ef}	5.24^{abc}	5.21^{def}	5.23^{bcde}	2.62^{bcd}	2.70^{abc}	2.66^{abcde}
BA-500 ppm	161.4^{cd}	165.73^{cde}	163.58^{d}	14.41^{abcd}	14.60^{ab}	14.51^{ab}	4.99^{abc}	5.18^{def}	5.09^{cde}	2.89^{ab}	2.83^{ab}	2.87^{ab}
BA-600 ppm	166.3^{abcd}	163.29^{e}	164.80^{cd}	14.74^{a}	14.51^{abc}	14.63^{a}	5.51^{a}	5.02^{ef}	5.27^{bcde}	2.69^{abcd}	2.89^{a}	2.79^{abcd}
BA-700 ppm	168.8^{ab}	165.33^{de}	167.08^{abcd}	13.80^{cde}	14.80^{a}	14.30^{abcd}	5.00^{abc}	4.88^{f}	4.94^{e}	2.77^{abcd}	3.04^{a}	2.90^{a}
BA+GA_3-200 ppm	161.5^{cd}	170.19^{abc}	165.85^{abcd}	14.70^{ab}	13.26^{f}	13.98^{cde}	5.57^{a}	6.13^{ab}	5.85^{a}	2.65^{bcd}	2.17^{e}	2.41^{e}
BA+GA_3-300 ppm	166.2^{abcd}	171.23^{ab}	168.73^{abc}	13.97^{abcde}	14.19^{abcd}	14.08^{bcde}	5.39^{ab}	5.89^{abc}	5.65^{ab}	2.59^{dc}	2.41^{cde}	2.51^{e}
BA+GA_3-400 ppm	168.7^{ab}	168.10^{bcd}	168.40^{abc}	14.52^{abc}	13.90^{cdef}	14.21^{abcde}	5.39^{ab}	5.68^{abcde}	5.54^{abc}	2.70^{abcd}	2.45^{cde}	2.58^{de}
BA+GA_3-500 ppm	168.1^{abc}	169.89^{abcd}	169.00^{abc}	14.46^{abc}	14.40^{abcd}	14.44^{abc}	5.47^{a}	5.71^{abcd}	5.59^{ab}	2.64^{bcd}	2.53^{bcd}	2.59^{cde}
BA+GA_3-600 ppm	173.2^{a}	167.08^{bcde}	170.12^{ab}	14.44^{abc}	14.62^{ab}	14.53^{ab}	4.89^{bc}	5.44^{cdef}	5.17^{bcde}	2.96^{a}	2.71^{abc}	2.84^{abc}
BA+GA_3-700 ppm	167.3^{abc}	166.74^{bcde}	167.02^{abcd}	13.38^{e}	14.55^{abc}	13.97^{cde}	4.78^{c}	5.32^{cdef}	5.05^{ed}	2.81^{abc}	2.76^{abc}	2.78^{abcd}
Pr > F	0.0031	0.0004	0.0419	0.0003	<.0001	<.0001	0.0332	0.0007	0.0014	0.0683	0.0001	0.0004

Values within a column followed by same letter are not significantly different at $P>0.05$, as established by the Duncan's Multiple Range Test ns

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branches in the upper part of the tree to preserve a hierarchy of branches. However, in this study the trunk diameter was taken into consideration instead of the central leader which obliviously has higher diameter values than a leader in the upper part of trees even though the best-performing treatments (BA-500, 600, and 700 ppm) obtained 2.69 to 2.89 (2016) and 2.83 to 3.04 (2017) value of trunk and feather diameter ratio (Table 4), indicating that almost all sylleptic shoots produced by these treatments will remain useful after planting.

As far as the percentage of branched trees are concerned (Fig. 8) in both experimental years the best results (100% branched trees) were obtained from trees treated with 500, 600, 700 ppm BA and 600, 700 ppm BA+GA_3. A lower value was noted in control (35 and 40% in 2016 and 2017, respectively). Apical dominance could be the reason for low branching in Gala Mast nursery tree. In the shoot tissues, the optimal ratio of auxin and cytokinin (decrease in auxin and increase in cytokinin concentration) is required for acceptable branching. The present findings suggest that higher concentrations of BA and BA+GA_3 are more effective in the promotion of branched trees compared to lower. The findings also indicate higher effectiveness of the application of BA alone compared to combine the application with GA_3. It might be due to a higher concentration of benzyladenine when applied alone as compared to a lower concentration in combination with GA_3. A similar effect was observed for the 'Boskoop' apple by Gastol *et al.* (2012).

From the current study, it was concluded that the use of 6-benzyladenine alone and in combination with gibberellic acid is an effective measure for promoting sylleptic shoots in a one-year-old apple nursery tree. Results based on all studied parameters it is economical to apply three sprays of 500 ppm BA during second vegetative growth for acceptable growth and sylleptic shoots formation in a one-year-old nursery tree of 'Gala Mast'.

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Add-on Information

Authors' contribution: K.L. Kumawat: Conceptualization, investigation, methodology, collection of data, statistical analysis, interpretation of data and manuscript preparation, **W.H. Raja:** Technical support for experiment, manuscript preparation and editing, **L. Chand:** Technical support for experiment, summarization and tabulation of data, **K.M. Rai:** Technical support for experiment and statistical analysis, **S. Lal:** Statistical analysis and editing.

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