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Effects of plant growth regulators applications on induction of lateral branching in Oregon Spur apple nursery trees

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ABSTRACT

The study presents the influence of 6-benzayladenine alone and in combination with gibberellic acid on growth and lateral branching of one-year-old 'Oregon Spur' apple nursery trees grafted on MM-106 rootstock. Different concentrations of BA and BA+GA_3 (200, 300, 400, 500, 600 and 700 ppm) were applied three times at one week interval during second vegetative growth. Investigation reveal that Oregon Spur have strong apical dominance and plant applied with growth regulators resulted in a significant increase in number of feathers (0.58 to 6.18 per tree) compared to untreated control (0.18 per tree). However, BA alone has more significant effect on number of feathers (1.45 to 6.18 per tree) than BA+GA_3 (0.58 to 4.35 per tree). Maximum number of feathers (6.18 per tree) has been obtained with 500 ppm BA treatment. This treatment also resulted in more uniform feather length (2.58 short and 2.55 medium length lateral branches per tree) and correct distribution of feathers along the trunk (20.63 cm branching zone) with appropriate average feathers crotch angle (54.74° from vertical). Furthermore, this treatment resulted in 100 per cent feathered trees compared to none in control and 2.88 trunk and mean feather diameter ratio compared to 1.54 in control. Some treatments with BA had a negative influence on the tree height. Whereas, most treatments increased trunk diameter and crotch angle compared to control.

Key words: *Malus domestica*, plant growth regulators, 6-benzyladenine, gibberellic acid.

INTRODUCTION

A high density apple orchard should be precocious and highly productive having quality fruit in an economically justified way. However, lack of early cropping is a major limitation to the development of the Indian high density apple industry. Although the use of dwarf rootstocks have certainly improved precocity for cultivars, further enhancements are likely to come from the introduction of high quality nursery tree. High quality apple nursery trees should have dominant straight central leader with sufficient feathers which are induced at desirable height and distributed along the leader at regular intervals, achieving appropriate length and crotch angle. Such type of feathered nursery trees will quickly establish, grow and fill their allotted space in orchard and consequently improve total light interception in early life of orchard. Moreover, feathers form flower buds in the first year of planting and facilitate the tree to bear fruit during second year of planting and will reach to full production potential after few years (Sadowski *et al*., 10) which help to cover the significant increased cost of establishment of apple high density orchard. Additionally, early cropping control vigour of apple trees and this give a more balanced tree. Furthermore, as well-feathered trees provide well-formed canopy, eventually result in easy and cost effective canopy management after planting. However, most apple varieties do not form adequate feathers in their growth cycle in the nursery. Therefore, it becomes essential to develop apple nursery management technique to encourage lateral branching so that apple nurseries in India can produce well-feathered nursery trees which is the critical component of most advanced high density apple orchard systems. Lateral branch induction in vigorously growing apple nursery trees is under the control of "apical dominance". The apical dominance is driven by inhibition of lateral bud development by auxin (produced in young leaves of apical top) while cytokinin promotes growth of axillary buds, and these hormones influence each other (Leyser, 5). Thus the relative amounts of auxin and cytokinin, and consequently the relative degree of inhibition or stimulation of lateral bud development, can be altered by chemical (which interact with auxin-mediated effects) as well as physical (pinching, undeveloped leaves removal, removal of peak lateral bud, notching, scoring etc.) treatments. Physical measures are time consuming and laborious task that is often not sufficiently effective. The use of PGRs has been seen as a more consistent and less

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expensive method to induce laterals branching (Sazo and Robinson, 11). Among the many PGRs tested 6-BA and BA+GAs are the most commonly used PGRs, but the recommendations are contradictory and vary from region to region (Cowgill *et al*., 1; Doric *et al*., 2; Radivojevic *et al*., 7; Robinson and Sazo, 8) which might be due to available growing period and interaction of application of PGRs with environmental variable; furthermore studies are limited to few regions and on few cultivars and many researchers found that feathering response with the application of PGRs vary with cultivar to cultivar (Doric *et al*., 2; Radivojevic *et al*., 7; Robinson and Sazo, 8). Oregon Spur is one of the most important apple cultivar in India and is highly suitable for intensive production. Therefore, the aim of this study was assessment of effectiveness of different concentration of 6-BA and BA plus GA_3 in promoting lateral branching (feathering) in one year old Oregon Spur apple nursery trees grafted on MM-106 rootstock.

MATERIALS AND METHODS

Present research work on one-year-old 'Oregon Spur' apple grafted on MM-106 was conducted in 2016 and 2017 at ICAR-CITH, Srinagar (33.58' N, 74°48' E and 1644 m a.s.l.). The treatments include the application of 6-benzyladenine and 6-benzyladenine plus gibberellic acid (1:1) @ 200, 300, 400, 500, 600 and 700 ppm respectively. The soil of experimental block was clay loam in texture with pH 6.86, EC 0.38 dSm^{-1}. Treatments were applied thrice at weekly intervals to one year old grafted nursery trees planted at 90 × 60 cm spacing in the second year of the nursery cycle, when the central leader shoot tip was growing. The apical section of the central shoot was with a hand held sprayer until run-off. Control trees were unsprayed. The experimental procedure followed a randomized block design with four replications distributed down a row with each experimental unit being a section of five trees (20 trees per treatment). All the other management practices were done as required uniformly In the fall, trees were measured in the nursery for tree height, trunk diameter (10 cm above bud union), total number of lateral (any lateral shoot longer than 5 cm), total number of feathers (any lateral shoot longer than 10 cm and above 70 cm from ground level), distance from the ground level to each of the induced feathers, length, diameter and crotch angle of each feather, branching zone (distance between the highest and lowest feathers) and per cent feathered tree (tree with at least three feathers higher than 70 cm above the ground was considered as feathered tree and their number was expressed as a per cent of total number of trees per plot). Weather data during both study years were obtained from airbase meteorological station, Srinagar and presented in Fig. 1-4. The mean annual temperature during 2016 and 2017 was respectively 0.51°C and 0.26°C higher than the mean for the years 2005 to 2015. The total precipitation during 2016 and 2017 was respectively 174.33 and 49.85 mm less than average of 2005 to 2015. Daily M^M, M^m and A^v temperature on treatment application day during 2016 and 2017 are presented in Fig.1 and Fig. 2 respectively. Whereas, during 2016 and 2017 M^M, M^m and A^v temperature of one week before first application, during applications week and one week after last application are shown in Fig. 3 and Fig. 4 respectively. The data were analysed by one-way analysis of variance (ANOVA) using software SAS 9.3. Significant differences between means were evaluated using Duncan's multiple range test at the level of p=0.05.

RESULTS AND DISCUSSION

Untreated trees of Oregon Spur revealed a lack of branching potential and on the basis of pooled value of year 2016 and 2017 only 0.85 natural lateral

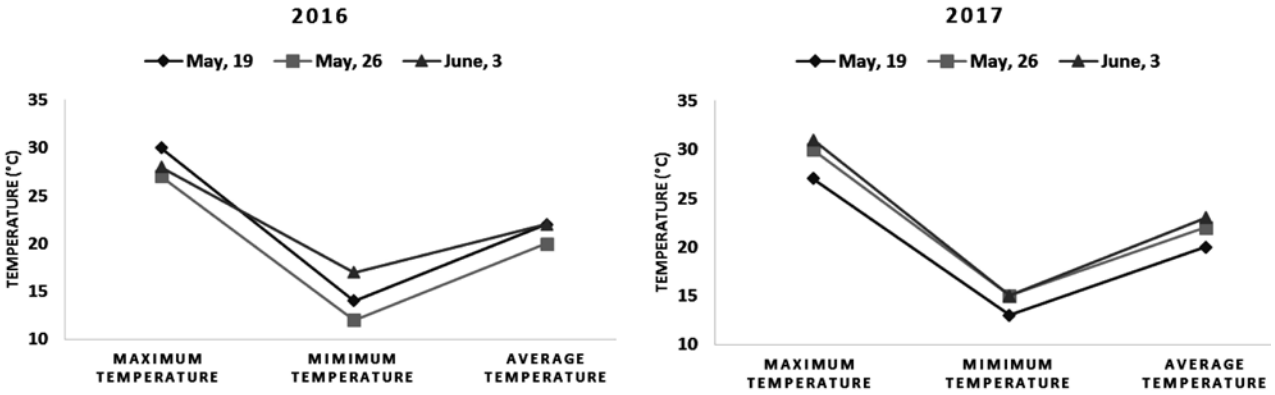

Fig. 1. and 2. Daily maximum, minimum and average temperature on treatment application days.

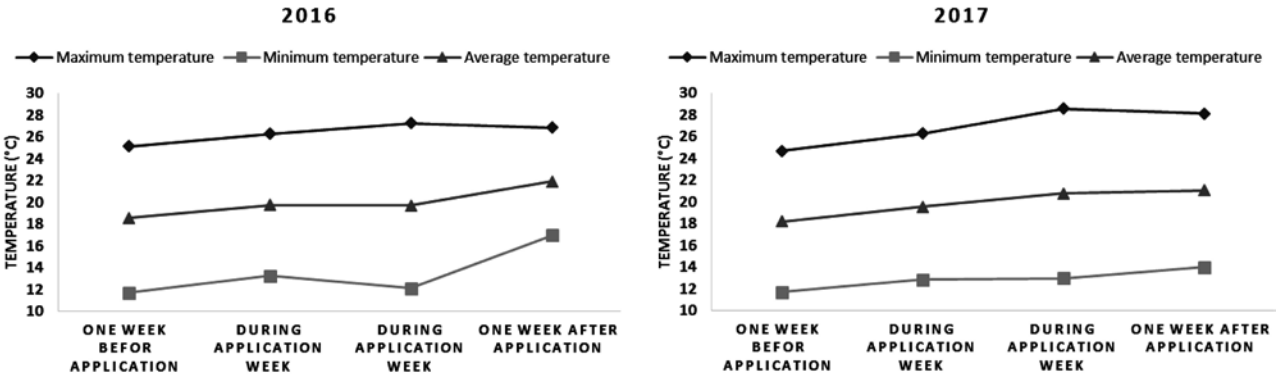

Fig. 3. and 4. Weekly maximum, minimum and average temperature one week before first application, during applications week and one week after last applications.

(more than 5 cm) were found on trees, thus cultivar revealed strong apical dominance. Whereas, the number varied from 2.10 (BA+GA_3-200 ppm) to 9.73 (BA-700 ppm) laterals per tree for the treated ones (Table 1). Moreover, control tree developed only 0.05 lateral between 5-10 cm length and at more than 70 cm tree height. Whereas, in trees other than control the number varied from 0.28 (BA+GA_3-200) to 2.33 (BA+GA_3-600 ppm) (Table 1). Untreated control trees had very few feathers (0.18) whereas, all the plant growth regulator treatments significantly enhanced the feathers in one year old apple nursery tree (*p*-<.0001). However, the lowest concentration of BA and BA+GA_3 (200 ppm) caused the low feathering of apple nursery tree, whereas increased concentration of BA and BA+GA_3 from 200 to 700 ppm increased the number of feathers more than four-fold and seven-fold, respectively (Table 1). On the basis of pooled value of two year study among all the PGR's treatments the most feathers were counted for BA-500 ppm (6.18 per tree). However, this treatment remains statistically *at par* with BA-700 ppm (6.15 per tree). Increasing concentration of BA from 500 to 700 did not positively influence the development of feathers indicating that only 500 ppm is needed for acceptable branching. Apparently, BA alone was more effective than BA+GA_3 in feather formation. It is might be due to higher concentration of benzyladenine (200-700 ppm) when applied as alone as compared to lower concentration (100-350 ppm) in combination with GA_3. That is in accordance with results of Gastol *et al*. (3) they concluded that higher doses of BA (780 mg BA+120 mg GA_3 per dm^3) promoted more branching as compared to lower (330 mg BA+570 mg GA3 per dm^3) in 'Boskoop' cultivar of apple. These results suggest that higher concentration (more than 400 ppm) would seem to provide major stimulus for feather formation in one year old Oregon Spur apple nursery tree. The application of BA alone or in combination with GA_3 significantly affected the formation of feathers might be due to application of BA affects the flow of auxins (Muller and Leyser, 6) and temporarily impedes the main shoot growth (Sazo and Robinson, 11) which helps to overcome apical dominance and create favourable environment of feather formation in one year old Oregon Spur apple nursery trees.

Feather length of nursery tree has direct relation with orchard tree productivity (Sadowski *et al*., 10) and is important in determining the quality of nursery tree. In the present investigation the length of feathers was also affected with different plant growth regulator treatments. In treated tree, short (10-20 cm), medium (21-40) and long (>40 cm) feathers and total length of feathers were significantly greater in higher doses of BA and BA+GA_3. Less than 40 cm feather length is ideally suited for high density planting (Sazo and Robinson, 12) as shorter feathers are easier to manage in the close in-row spacing. Examining the length of feathers, it is easy to see that the treatment with the higher BA concentrations (500-700) produced maximum number of short and medium feathers. On the basis of pooled value of two years investigation the highest number of short feathers (2.73) was obtained from BA-600 ppm treatment although, this treatment remained statistically *at par* with BA-500 (2.58) and BA-700 (2.6). Whereas significantly higher numbers of medium feathers (2.55) were obtained from BA-500 ppm treatment and it remained statistically *at par* with BA-400 (2.3) and BA-700 ppm (2.23) (Table 1) and length of most of the feathers on the control tree almost exceeded 40 cm. These finding thus also suggest that treatments containing higher concentration of BA are more effective in the formation of feathers of ideal length *i.e.* 10-40 cm. As far as mean feather length to concern generally trees with higher number of feathers recorded the lower mean length of feathers (Fig. 5). In trees treated with

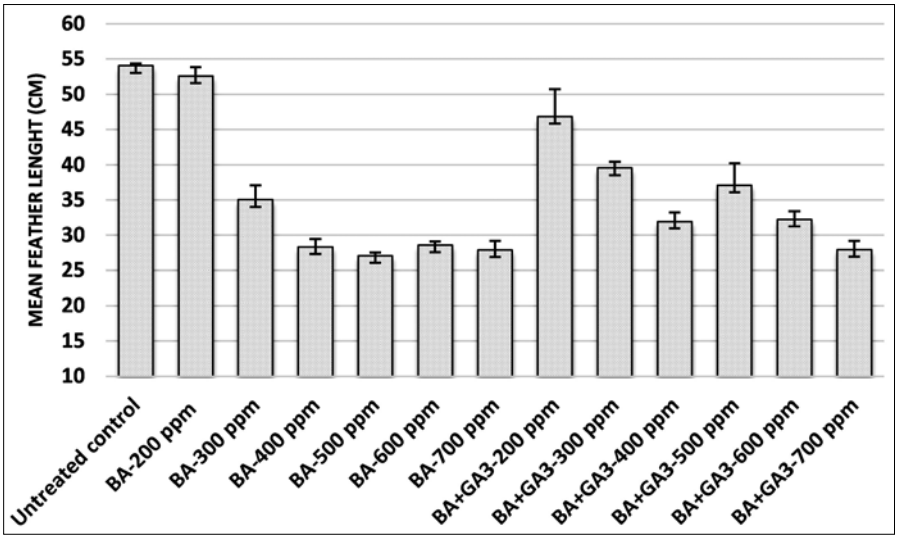

Fig. 5. Effect of various plant growth regulators treatments on mean feather length (pooled value). The bars represent ± S.E.

higher doses of BA the mean feather length value were low which might be due to higher number of feathers produced by these trees. These results are in accordance with earlier research findings, which confirmed that an increase in the number of feather permits variation in mean feather length (Doric *et al*., 2; Radivojevic *et al*., 7). Further, control, 200 ppm BA and BA+GA_3 promoted the longest feathers compared to other treated trees because most of feathers in these treatments induced in early spring thus they got more vegetative growth period, further as these treatments have few feathers therefore the water and nutrients were used for the growth of a smaller numbers of feathers. The results are in conformity with the findings of Cowgill *et al*. (1); Doric *et al*. (2) and Radivojevic *et al*. (7); Robinson and Sazo (8). The total length of feather varied from 0.71 m (untreated control and BA+GA_3-200 ppm) to 1.75 m (BA- 700 ppm). In general the trees with higher numbers of feathers produced higher total length of feathers. Application of BA alone was more effective than combine application with GA_3 in respect of total feathers this might be due to BA treatments inducing more number of feathers compared to BA+GA_3 treatments. The results are in agreement with previous reports (Doric *et al*., 2; Radivojevic *et al*., 7).

The height of first feather from ground is important determinant of quality of nursery tree. In present investigation only those laterals were considered as a feather, which were longer than 10 cm and above 70 cm from ground. Pooled value of two years investigation revealed that lateral branching below 70 cm was observed in control (0.63) as well as in all treatments (0.78-2.13) (Table 1). However, these laterals were not included in the analysis, as the main emphasis of the investigation was on feathers. According to Sazo and Robinson (12), the bottom feather of nursery tree should not be lower than 75 cm from ground level for high density planting and in all treatments except BA+GA_3-200 ppm induced feathers at desirable height *i.e.* 77.41 to 83.41 (Fig. 6). In both experimental years higher concentration of BA alone and in combination with GA_3 resulted in the expansion of the branching zone. Further, on the basis of pooled value of two experimental years branching zone value were higher when BA was applied alone (6.52-20.63 cm) than in combination with GA_3 (4.40-17.74 cm) (Table 1). However, average shoot internode length was found higher in trees treated with BA+GA_3 even though the branching zone observed was higher in BA treated tree due to higher number of induced feathers and highest branching zone (20.63 cm) was observed in treatment which induced highest number of feathers *i.e.* BA-500 ppm. The present results are also supported by Doric *et al*. (2).

The results of two years of investigation revealed that tree treated with plant growth regulators produced feathers with wider crotch angle from vertical compared to untreated control and the effect is found more prominent in trees treated with higher concentrations of PGR's (Table 1). On the basis of pooled value of two year investigation the widest crotch angle (57.42°) was achieved from BA+GA_3-500 ppm treatment followed by 56.77° and 55.10° from BA+GA_3-700 and 400 ppm treatments respectively, indicating that combine application of higher concentration of BA+GA_3 achieved a wider crotch angle compared to BA alone. Application of

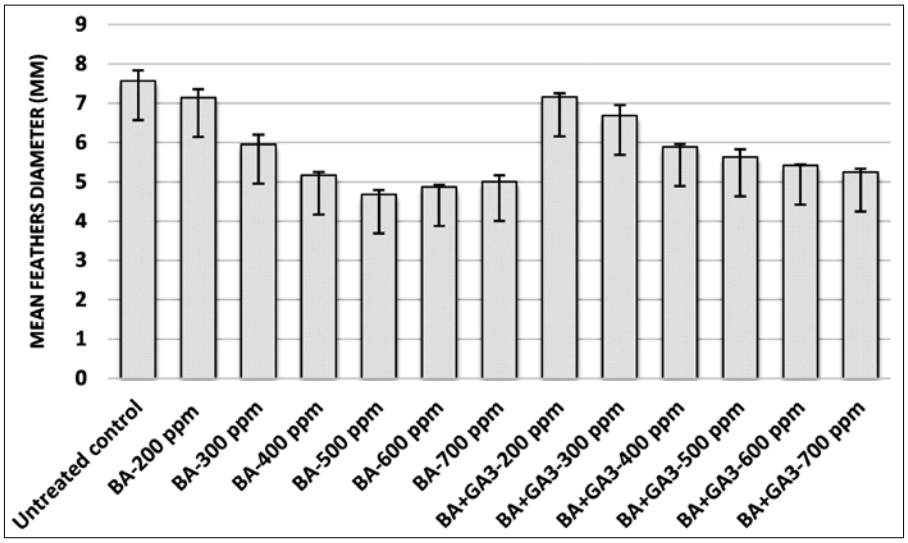

Fig. 6. Average lowest feather height from ground in response to various plant growth regulators treatments (pooled value). The bars represent ± S.E.

aggressive chemical branching agents like BA in nursery tree can result in more obtuse feather angle (Steiner *et al*., 14). From these findings we can say that higher concentration of BA acts as aggressive branching agent compared to lower rate of BA with GA_3. However, when assessing treatment effects, number of feathers (vary from 1.45 to 6.18 per tree in BA alone treatments compared to 0.58 to 4.35 per tree in BA+GA_3 treatments) should be considered as well, as it can have effect on the feather crotch angle, which tends to be wider in the lowest feathers relative to those in the higher tiers. The results are in accordance with Stanisavljevic *et al*. (13) and in contradiction with Doric *et al*. (2) and Radivojevic *et al*. (7). Wider angle produced by all the treatments compared to control would be a significant advantage for apple orchard system like the Tall Spindle since less labour would be required to tie feathers or branches down after planting.

The present research revealed that the majority of the treatments with high doses of BA (400, 500 and 700 ppm) produced significantly shorter tree (156.4-159.48 cm) compared to lower doses (165.08-166.80 cm) and untreated ones (167.1) (Table: 1) which might be because high doses of BA treatment temporarily slows the main shoot growth (Sazo and Robinson, 11), which can negatively affect the final tree height in investigated cultivar. In addition, the tree height was possibly reduced in treatments with higher doses of BA, as the more active growing laterals shoot, induced by these treatments, competing for limited nutrients and other growth factors, but still the height of the treated trees met the standard requirements. The results are in accordance with Cowgill *et al*. (1) where they reported that trees treated with higher doses of BA (1000 ppm) were significantly shorter than lower doses (500 ppm). Furthermore, control trees were significantly taller than trees treated with BA+GA_3 (156.95-162.18) which might be due to less number of feathers and apical dominance. That is in accordance with results of Rufato *et al*. (9), they reported that combine application of BA+GA_{4+7} reduced tree height compared to BA alone and untreated control in Maxi Gala cultivar of apple grafted on G.202. However, many researchers have expressed contrasting findings, stating that combine application of BA+GA_{4+7}/GA_3 significantly increase tree height compared to BA alone and untreated control in Mutsu (Gastol *et al*., 3); Golden Delicious (Cowgill *et al*., 1); Gala and Jonagold (Doric *et al*., 2) apple cultivars.

In the present study minor differences in the trunk diameter were noted among treatments in both experimental years (Table 1). However, on the basis of pooled value of both years application of BA alone (12.77-13.61 mm) and in combination with GA_3 (12.35-13.44 mm) significantly increase trunk diameter compared to untreated control (12.06 mm) whereas maximum value (13.61 mm) was recorded for BA-700 ppm treatment. The positive influence of plant growth regulator treatments observed in this study could be explained by the presence of greater number of feathers than control, which can have a considerable beneficial effect on trunk diameter (Jacyna, 4) of investigated trees. The results of present investigation are in agreement with previous research findings which confirm that trunk diameter with the application of BA and BA+GA_{4+7}

Table 1. Effect of various plant growth regulators treatments on different studied parameter of Oregon Spur apple nursery tree.

Treatment	Laterals (>5 cm)	Laterals (< 70 cm ht)	Laterals (5-10 cm and >70 cm ht)	Feathers	Short feathers (10-20 cm)	Medium feathers (21-40 cm)	Long feathers (>40 cm)	Total feather length (m)	Branching zone (cm)	Average feather crotch angle (°)	Tree height (cm)	Trunk diameter (mm)	Trunk and mean feather diameter ratio
	Pooled value of year 2016 and 2017												
Untreated control	0.85^{f}	0.63^{d}	0.05^{f}	0.18^{i}	0.00^{e}	0.03^{e}	0.15^{g}	0.71^{f}	4.58^{f}	48.75^{e}	167.10^{a}	12.06^{c}	1.54^{g}
BA-200 ppm	2.58^{e}	0.78^{cd}	0.35^{f}	1.45^{g}	0.10^{e}	0.33^{e}	1.03^{abc}	1.02^{d}	6.52^{e}	51.96^{cde}	166.80^{a}	12.77^{abc}	1.80^{f}
BA-300 ppm	5.78^{c}	1.10^{bc}	1.78^{abcd}	2.90^{e}	0.75^{d}	1.45^{bc}	0.75^{cde}	0.98^{de}	10.43^{d}	53.29^{bcd}	165.08^{ab}	13.09^{ab}	2.21^{e}
BA-400 ppm	9.38^{a}	2.13^{a}	2.13^{abc}	5.13^{c}	1.93^{b}	2.30^{a}	0.90^{bcd}	1.46^{b}	16.62^{c}	53.41^{bcd}	159.48^{bc}	13.13^{ab}	2.54^{cd}
BA-500 ppm	9.45^{a}	2.00^{a}	1.50^{cd}	6.18^{a}	2.58^{a}	2.55^{a}	1.03^{abc}	1.69^{a}	20.63^{a}	54.74^{abc}	156.40^{c}	13.43^{a}	2.88^{a}
BA-600 ppm	8.23^{b}	1.38^{b}	1.18^{de}	5.68^{b}	2.73^{a}	1.73^{b}	1.25 ^{a}	1.66^{a}	19.10^{b}	54.57^{abc}	159.58^{bc}	13.08^{ab}	2.71^{abc}
BA-700 ppm	9.73^{a}	1.95^{a}	1.63^{bcd}	6.15^{a}	2.60^{a}	2.23^{a}	1.30 ^{a}	1.75^{a}	18.45^{b}	54.82^{abc}	157.55^{c}	13.61^{a}	2.72^{ab}
BA+GA_3-200 ppm	2.10^{e}	1.20^{b}	0.28^{f}	0.58^{h}	0.03^{e}	0.10^{e}	0.45^{efg}	0.71^{f}	4.40^{f}	50.09^{ed}	162.18^{abc}	12.49^{bc}	1.75^{f}
BA+GA_3-300 ppm	2.23^{e}	1.08^{bc}	0.33^{f}	0.80^{h}	0.10^{e}	0.28^{e}	0.43^{fg}	0.75^{f}	10.06^{d}	49.46^{e}	160.66^{bc}	12.56^{bc}	1.91^{f}
BA+GA_3-400 ppm	4.18^{d}	1.10^{bc}	0.68^{ef}	2.50^{f}	0.85^{d}	1.13^{cd}	0.53^{ef}	0.80^{ef}	9.61^{d}	55.10^{abc}	156.95^{c}	12.77^{abc}	2.17^{e}
BA+GA_3-500 ppm	4.28^{d}	1.03^{bc}	0.58^{ef}	2.75^{ef}	0.65^{d}	1.00^{d}	1.13^{ab}	1.10^{cd}	10.20^{d}	57.42^{a}	159.10^{bc}	12.35^{bc}	2.20^{e}
BA+GA_3-600 ppm	7.63^{b}	1.13^{bc}	2.33^{a}	4.18^{d}	1.63^{c}	1.38^{bc}	1.18^{ab}	1.36^{b}	16.60^{c}	54.86^{abc}	159.78^{bc}	13.15^{ab}	2.43^{d}
BA+GA_3-700 ppm	7.80^{b}	1.18^{b}	2.28^{ab}	4.35^{d}	2.03^{b}	1.63^{b}	0.70^{def}	1.28^{bc}	17.74^{bc}	56.77^{abc}	157.30^{c}	13.44^{a}	2.57^{bcd}
Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0015	0.0084	<.0001
Significance	*	*	*	*	*	*	*	*	*	*	*	*	*

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

significantly increased in Jonagold (Doric *et al*., 2) whereas, slightly increased in Cadel (Radivojevic *et al*., 7) apple cultivars. Though the contradictory results are obtained too in apple cultivar Golden Delicious (Steiner *et al*., 14) Gala (Doric *et al*., 2) and Jonagold (Radivojevic *et al*., 7). As far as mean diameter of feathers to concern treatment, which obtained highest number of feathers (6.18 per tree for BA-500 ppm, Table 1) recorded the lowest mean diameter of feathers (4.68 mm, Fig. 7). Whereas, untreated control, 200 ppm BA+GA_3 and BA resulted in significantly higher diameter (7.57, 7.16 and 7.14 mm respectively, Fig. 7) compared to other treatments however, all these treatment remain statistically *at par* with each other. Generally, the mean feather diameter decreasing with increasing number of feathers on treated trees might be due to the inter-shoot competition. Trunk and mean feather diameter ratio plays a critical role in canopy management in early years of high density orchard and accordingly, to preserve a hierarchy of branch the leader should be 3 times the diameter of any of the lateral branches in the upper part of the tree. However, in this investigation the trunk diameter was taken in to consideration instead of central leader which certainly have higher diameter value than leader in upper part of trees in spite of this, in present investigation we had achieved 2.88 value in best performing treatment *i.e.* BA-500 (Table 1) indicating that almost all the feathers produced by these treatments will remain suitable after planting.

Both, BA alone and BA+GA_3 treatments results in substantial increase in numbers of per cent feathered plants. In both years of investigation, 100 per cent feathered trees were observed for BA-500, BA-700 and BA+GA_3-700 ppm, additionally during 2017 application of BA-600 ppm also observed 100 per cent feathered trees. However, it is worth mentioning that without exogenous plant growth regulators or even lower concentration of BA+GA_3 (200 ppm) no trees were feathered (Fig. 8). The findings also indicate a higher effectiveness of application of BA

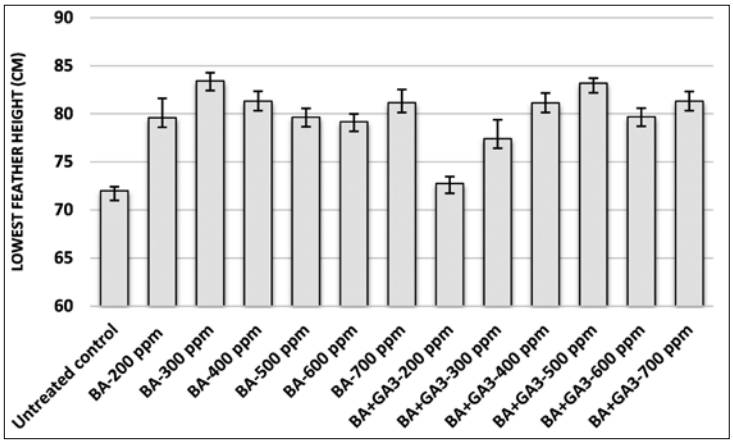

Fig. 7. Effect of various plant growth regulators treatments on average feather diameter (pooled value). The bars represent ± S.E.

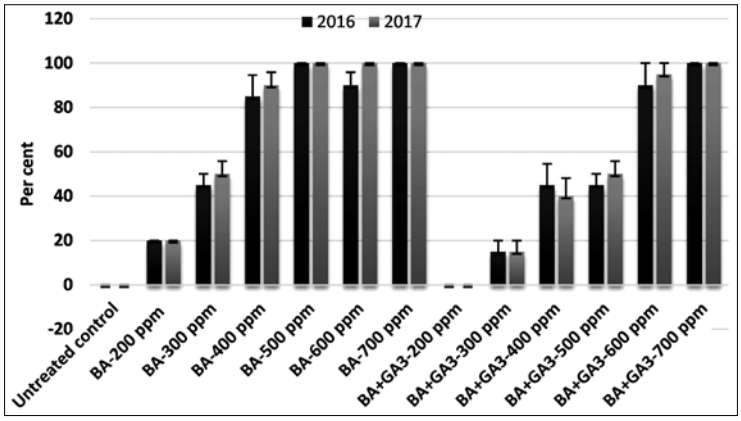

Fig. 8. Feathered trees (%) obtained in different plant growth regulators treatments during 2016 and 2017. The bars represent ± S.E.

alone compared to combine application with GA_3 for feathering in one year old Oregon Spur apple nursery trees. The results are in agreement with Gastol *et al*. (3).

In conclusion, application of BA alone had a greater positive effect compared to combine application with GA_3. Its beneficial effects were mainly reflected in the increase in total number of feathers, number of short and medium feathers, branching zone, trunk and mean feather ratio and per cent feathered trees. Results based on all the studied parameters proved that application of BA-500 performed better than rest of the treatments for feathering in one year old Oregon Spur apple nursery trees.

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