# Effect of genotype and leader type on benzyladenine induced sylleptic branching in apple nursery trees

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**Abstract:** The planting of branched nursery trees is an essential component of most high-density orchard planting systems to induce precocity. However, in apples, most cultivars do not produce the desired numbers of sylleptic shoots naturally due to the presence of apical dominance. Benzyladenine (BA) applications alter the apical dominance and consequently encourage sylleptic shoot formation in nursery trees. However, the response to an exogenous BA application can vary with the genotype. Currently, most nurseries produce branched apple nursery trees using the renewal leader method. However, apart from the renewal leader method, branched nursery trees can also be produced using the central leader method. A comparative study of these two methods had not been conducted previously, and this investigation aimed to determine the effects of both the central and renewal leader methods, as well as the genotype, on sylleptic branching in apple nursery trees in response to repeated BA sprays. The genotype showed significant variation in the sylleptic shoot numbers (5.25-9.41), their average length (26.86–33.34 cm), and crotch angle (48.95° –54.27°) in response to the BA application. Among the genotypes, 'Shireen' produced the highest number of sylleptic shoots, whereas the opposite results were obtained in 'Top Red'. Furthermore, irrespective of the genotype, the central leader method was found to be more effective than the renewal leader for the development of high-quality branched nursery trees. The central leader method not only significantly increased the number of sylleptic shoots on the tree but also positively affected their length, final tree height, and diameter.

Keywords: central leader; cytokinin; feathering; lateral branching; renewal leader

With advancements in orchard planting systems, high-density planting is gaining momentum in apple-growing regions of the world. The economic success of high-density planting depends on the pace at which it starts production. Planting branched

apple trees can increase the profitability of highdensity plantations by enhancing the precocity and yield at an early stage. As a result, nurseries all over the world are being urged to supply branched apple nursery trees with higher trunk diameters. However,

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most apple genotypes do not produce the required number of sylleptic shoots during the nursery cycle due to the strong apical dominance (Robinson, Sazo 2014; Dorić et al. 2016). To overcome this, many research studies have proven that sylleptic shoots in apple nursery trees can be encouraged by the repeated foliar spraying of benzyladenine (BA) on the actively growing apical portion (Lordan et al. 2017; Kumawat et al. 2023). However, the response of nursery trees to BA spraying for sylleptic shoot induction is greatly influenced by the genotypes due to the different degrees of apical dominance (Robinson, Sazo 2014; Lordan et al. 2017; Nečas et al. 2020). Hence, the response of sylleptic branching to repeated summer foliar spraying of BA can be well established by studying the genotypes. By considering the importance of the genotypes/cultivars, ten commercial apple cultivars grafted on an M.9 rootstock were used in the present study to find out their sylleptic branching response to the repeated foliar spraying of BA. Furthermore, the renewal leader method is the most extensively used method for the induction of sylleptic shoots in apple nursery trees (Laňar et al. 2020; Nečas et al. 2020). It involves the heading back of a one-year-old nursery tree and the selection of one newly developed proleptic shoot as the renewal leader for the induction of sylleptic shoots with the use of phytohormones. However, the process of selecting one proleptic branch as the renewal leader from those generated after heading back, while removing the rest, is time-consuming and costly. As an alternative to the renewal leader, the unpruned, continuously growing central leader can also be used for sylleptic shoot induction. However, a question emerges: Can the central leader be as effective as the renewal leader in producing well-branched apple nursery trees? In light of this, a study was conducted using BA as a branching agent to compare the renewal and central leader methods for the production of sylleptic shoots in apple nursery trees of ten apple cultivars.

# MATERIAL AND METHODS

**Experimental site and material.** A field experiment was conducted to establish the effect of the method and genotype on the sylleptic branching and growth of one-year-old apple nursery trees in 2019 at ICAR-Central Institute of Temperate Horticulture, Srinagar (Jammu and Kashmir),

India. The mean temperature data of the study year is presented in Figure S1 in electronic supplementary material (ESM). Rootstocks (M.9) harvested from the stoolbeds were transplanted at 15 cm (plant)  $\times$  30 cm (row) spacing in a nursery plot in February 2018, and scions with 4–5 buds were grafted onto the rootstocks measuring 20 cm in height by wedge grafting in March 2018. Routine standard nursery management practices were followed. The nursery trees were lifted in the last week of February 2019, and sorted trees measuring 75–80 cm in height were used as the experimental material. The nursery trees were planted at 60 cm (plant)  $\times$  90 cm (row) in the experimental block as per the technical programme.

**Experimental details.** The experiment consisted of 20 treatment combinations comprising two methods [central leader method (CLM) and renewal leader method (RLM)] and ten genotypes ('Red Chief', 'Red Spur, 'Starkrimson,' 'Oregon Spur,' 'Red Velox,' 'Top Red, 'Red Gold,' 'Gala Mast", 'Golden Delicious,' and 'Shireen'). In the CLM, the nursery trees were allowed to grow naturally (unpruned) (Figure 1A). Meanwhile, in the RLM, the nursery trees were headed back during late winter (1st week of March) at 70 cm above the ground, which resulted in the development of 2-4 proleptic shoots below the cut during spring. One vigorously growing proleptic shoot was selected as a renewal leader, and the remaining were removed (Figure 1B). In both methods, four sprayings of 600 ppm of BA (6-benzyladenine, extrapure AR, 99%; Sisco Research Laboratories Pvt. Ltd., India) were applied to the actively growing central and renewal leader of the nursery tree. The first spraying was applied when the central and renewal leader attained about 15 cm new growth, i.e. on May 7th, 2019 in the CLM and on May 20<sup>th</sup>, 2019 in the RLM. The remaining three sprayings were applied at a one-week interval. The treatment employed a factorial randomised complete block design with three replications of four apple nursery trees per replicate (12 trees per treatment). Uniform cultural practices like irrigation, fertiliser applications, and pest and disease management were followed in the nursery.

Variables of the study. During the fall, various observations were recorded to assess the quality of the apple nursery trees. The observations included the tree height, trunk diameter, number of sylleptic shoots on the tree (those longer than 10 cm), height from the ground level to each induced sylleptic shoot, length and crotch angle of each sylleptic shoot, and sylleptic shoot zone. Considering the length, the syl-

leptic shoots were grouped into short (10–20 cm), medium (21–40 cm), and long (> 40 cm). The total sylleptic shoot length was worked out by aggregating the length of each sylleptic shoot on the tree.

**Statistical analysis.** The collected experimental data were subjected to statistical analysis based on an analysis of variance (ANOVA) using the SAS software version 9.3. The significance of differences

at a P = 0.05 level was tested using Duncan's multiple range test (DMRT). The correlations among the component traits were assessed in the R 4.2.0 statistical software version RStudio 2023.03.1 Build 446 and visualised by correlation matrix using the ggcorrplot version 0.1.4 package. The correlogram displays the significance level and computes a matrix of correlation P-value at a 0.05% level of significance.

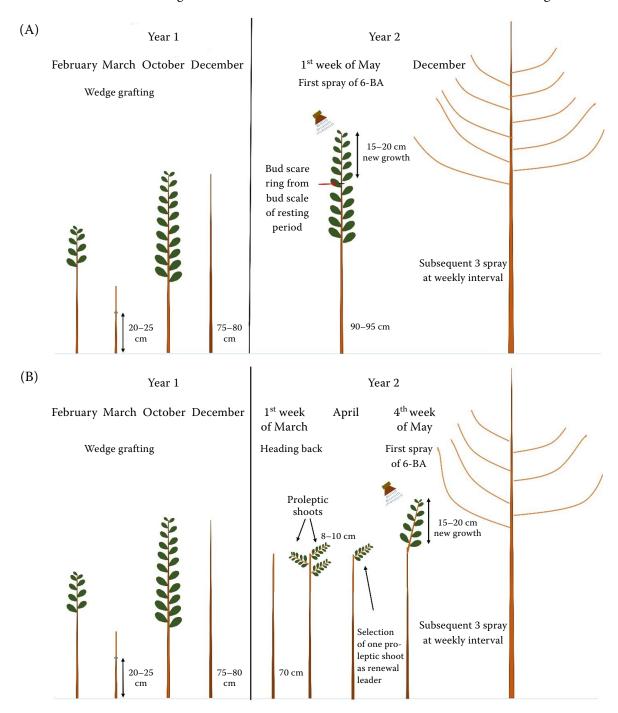


Figure 1. Schematic illustration of the (A) central and (B) renewal leader methods used in the experiment 6-BA – 6-benzyladenine

#### RESULTS

The method, as well as the genotype, significantly responded differently for most of the studied variables to the repeated BA application to the apple nursery trees. The data of the main and interaction effect of the method and genotype are presented in Figure 2 and Table 1. The sylleptic branching response of some apple genotypes using central a renewal leder methods with BA is shown in Figure S2 in ESM. The standard deviation for the main and interaction effect of the method and genotypes for the different variables under study is presented in Table S1 in ESM.

#### Main effect

*Method.* In both methods, the BA application produced considerable lateral bud break on the current season growth and resulted in acceptable sylleptic branching. However, the CLM had a more pronounced effect on most of the studied variables compared to the RLM and produced a significantly higher

number of sylleptic shoots (P=0.0014) (Figure 2A) with a higher sylleptic shoot zone (P=0.0010) and total sylleptic shoot length ( $P\le0.0001$ ). Furthermore, the trees produced by the CLM were significantly taller ( $P\le0.0001$ ) with a higher trunk diameter ( $P\le0.0001$ ). On the other hand, the RLM produced feathers with a significantly wider crotch angle ( $P\le0.0001$ ). In both methods, no significant differences were observed for the average length of the sylleptic shoot (Table 1).

Genotype. The effect of the 6-BA application on the nursery tree quality determinants depended significantly on the genotype. Among the different apple genotypes, a significantly ( $P \le 0.0001$ ) higher number of sylleptic shoots (9.41) were observed in 'Shireen' followed by 'Red Gold' (8.29). On the other hand, the minimum response in terms of the number of sylleptic shoots (5.25) was observed in 'Top Red'. However, it remained statistically at par with 'Red Velox' (Figure 2B). A significant effect of genotype on the number of short ( $P \le 0.0001$ ) and medium sylleptic

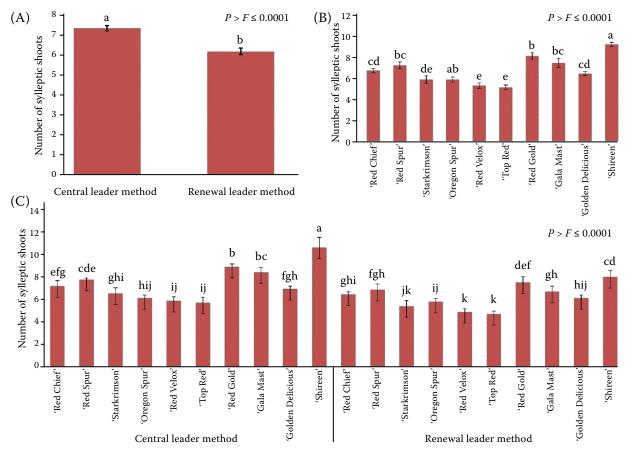


Figure 2. Effect of the (A) method, (B) genotype and (C) interaction of the method and genotype on the number of sylleptic shoots produced on the apple nursery trees

The bars represent  $\pm$  SD; a-k vertical bars with the same letter are not significantly different (P = 0.05)

Table 1. Main and interaction effect of the methods and genotypes on the quality determinants of the apple nursery trees

Treatment		SS (10–20 cm)	MS (21–40 cm)	LS (> 41 cm)	ASL (cm)	TSL (cm)	CA (°)	SZ (cm)	TH (cm)	TD (mm)
Met	hod (A)		· · · · · · · · · · · · · · · · · · ·							
CLM		$2.49^{a}$	$3.33^{a}$	1.65 <sup>a</sup>	$30.74^{a}$	226.37 <sup>a</sup>	$50.20^{b}$	27.43 <sup>a</sup>	169.86ª	12.56 <sup>a</sup>
RLM		2.31 <sup>a</sup>	$2.80^{b}$	$1.16^{b}$	$29.40^{a}$	$184.35^{b}$	53.13 <sup>a</sup>	$22.57^{b}$	$152.98^{b}$	$11.77^{b}$
Genotype (B)										
'Red Chief'		$2.16^{bcd}$	$3.12^{bc}$	$1.58^{\mathrm{abc}}$	$29.86^{b-f}$	$205.96^{bcd}$	$50.55^{\rm cde}$	$23.45^{d}$	$158.79^{bc}$	$12.01^{ab}$
'Red Spur'		$2.29^{bc}$	$3.25^{\rm bc}$	1.83 <sup>ab</sup>	$30.99^{ab}$	$227.60^{b}$	$51.14^{b-e}$	$24.45^{\mathrm{dc}}$	$161.83^{abc}$	$11.74^{\rm b}$
'Starkrimson'		$1.95^{\rm cd}$	$2.66^{cd}$	$1.37^{ m abc}$	$32.33^{ab}$	$192.20^{bcd}$	$52.15^{a-d}$	$22.20^{de}$	$164.16^{abc}$	$12.10^{ab}$
'Oregon Spur'		$2.66^{ab}$	$2.20^{d}$	$1.12^{\mathrm{bc}}$	$27.75^{ef}$	$169.56^{d}$	$52.98^{a-c}$	$20.79^{de}$	152.66 <sup>c</sup>	$12.21^{ab}$
'Red Velox'		$2.20^{bcd}$	$2.25^{d}$	$0.95^{c}$	$31.76^{abc}$	$171.71^{d}$	$52.89^{a-c}$	18.83 <sup>e</sup>	$160.75^{abc}$	$11.70^{b}$
'Top Red'		$1.58^{d}$	$2.20^{d}$	$1.45^{\mathrm{abc}}$	$33.34^{a}$	174.85 <sup>cd</sup>	$54.27^{a}$	$18.54^{e}$	$173.20^{a}$	$12.39^{ab}$
'Red Gold'		$3.25^{a}$	$3.62^{b}$	$1.41^{\mathrm{abc}}$	$26.86^{\rm  f}$	$223.76^{b}$	$49.61^{\mathrm{de}}$	$31.79^{ab}$	$155.04^{\rm c}$	12.71 <sup>a</sup>
'Gala Mast'		$2.79^{ab}$	$3.58^{b}$	$1.25^{ m abc}$	$28.35^{\mathrm{def}}$	$212.96^{bc}$	$50.72^{b-e}$	$28.50^{\mathrm{bc}}$	$159.54^{\mathrm{bc}}$	$11.95^{ab}$
'Golden Delicious'		$2.12^{bcd}$	$3.29^{bc}$	$1.16^{\mathrm{abc}}$	$29.10^{c-f}$	$192.53^{bcd}$	$53.37^{ab}$	$27.87^{bc}$	$159.25^{bc}$	$12.18^{ab}$
'Shireen'		3.00 <sup>a</sup>	4.50 a	1.91ª	$30.36^{a-e}$	282.49 <sup>a</sup>	48.95 <sup>e</sup>	33.85 <sup>a</sup>	169.00 <sup>ab</sup>	12.73 <sup>a</sup>
A×	В									
CLM	'Red Chief'	$2.42^{b-f}$	$2.92^{c-h}$	$1.92^{c-h}$	$30.52^{a-g}$	$223.05^{bcd}$	$47.75^{i}$	$26.08^{bcd}$	168.33 <sup>cde</sup>	$12.45^{b-e}$
	'Red Spur'	$2.33^{b-f}$	$3.33^{b-f}$	$2.17^{b-f}$	$32.15^{a-d}$	$250.61^{b}$	$49.88^{f-i}$	$27.25^{bc}$	$163.33^{\mathrm{def}}$	$11.75^{efg}$
	'Starkrimson'	$1.83^{d-f}$	$3.08^{c-g}$	$1.67^{c-g}$	$33.21^{abc}$	$215.46^{b-e}$	$50.16^{e-h}$	$24.58^{\rm cde}$	$173.08^{bc}$	12.69 <sup>a-d</sup>
	'Oregon Spur'	$2.58^{a-f}$	$2.50^{d-h}$	$1.08^{d-h}$	$28.61^{c-g}$	$178.42^{e-i}$	$50.35^{e-h}$	$22.67^{\text{def}}$	$161.83^{ef}$	$12.27^{c-f}$
	'Red Velox'	$2.50^{a-f}$	$2.33^{d-h}$	$1.08^{d-h}$	$33.51^{ab}$	$197.32^{c-g}$	$51.56^{d-g}$	$20.83^{ef}$	$168.42^{\rm cde}$	$11.86^{efg}$
	'Top Red'	$1.50^{f}$	$2.42^{d-h}$	$1.83^{d-h}$	$34.94^{a}$	$199.00^{c-g}$	$53.49^{a-d}$	$20.75^{ef}$	183.67 <sup>a</sup>	$12.86^{abc}$
	'Red Gold'	$3.50^{a}$	$3.75^{bc}$	$1.75^{\rm bc}$	$27.78^{d-g}$	$247.96^{b}$	$48.64^{hi}$	35.50 <sup>a</sup>	$162.58^{\mathrm{def}}$	$13.23^{ab}$
	'Gala Mast'	$3.08^{ab}$	$4.33^{ab}$	$1.08^{ab}$	$27.20^{e-g}$	$227.61^{bc}$	$49.53^{ghi}$	$30.17^{b}$	$170.42^{\rm cd}$	$12.69^{a-d}$
	'Golden Delicious'	$2.08^{b-f}$	$3.42^{b-e}$	$1.50^{b-e}$	$29.73^{b-g}$	$209.85^{c-f}$	$52.30^{b-e}$	$28.83^{b}$	$168.25^{cde}$	12.51 <sup>b-e</sup>
	'Shireen'	$3.08^{ab}$	5.25 <sup>a</sup>	$2.42^{a}$	$29.81^{b-g}$	314.46 <sup>a</sup>	$48.41^{hi}$	37.67 <sup>a</sup>	$178.75^{ab}$	13.35 <sup>a</sup>
RLM	'Red Chief'	$1.92^{c-f}$	$3.33^{b-f}$	$1.25^{b-f}$	29.22 <sup>b-g</sup>	$188.88^{d-h}$	53.36 <sup>a-d</sup>	20.83 <sup>ef</sup>	$149.25^{hi}$	11.56 <sup>fg</sup>
	'Red Spur'	$2.25^{b-f}$	$3.17^{c-f}$	$1.50^{c-f}$	$29.83^{b-g}$	$204.59^{c-g}$	$52.40^{b-e}$	$21.67^{ef}$	$160.33^{gef}$	$11.73^{efg}$
	'Starkrimson'	$2.08^{b-f}$	$2.25^{e-h}$	$1.08^{e-h}$	$31.46^{a-f}$	$168.93^{g-i}$	$54.16^{abc}$	$19.83^{fg}$	$155.25^{gfh}$	$11.51^{\mathrm{fg}}$
	'Oregon Spur'	$2.75^{a-e}$	$1.92^{h}$	1.17 <sup>h</sup>	$26.89^{fg}$	$160.69^{hi}$	55.62ª	$18.92^{fg}$	$143.50^{i}$	$12.16^{c-f}$
	'Red Velox'	$1.92^{c-f}$	$2.17^{fgh}$	$0.83^{fgh}$	$30.01^{b-g}$	$146.10^{i}$	$54.23^{\mathrm{abc}}$	$16.83^{g}$	$153.08^{hi}$	$11.52^{\mathrm{fg}}$
	'Top Red'	$1.67^{\rm ef}$	$2.00^{gh}$	$1.08^{gh}$	$31.76^{a-e}$	$150.70^{i}$	55.06 <sup>a</sup>	16.33 <sup>g</sup>	$162.75^{\mathrm{def}}$	$11.94^{d-g}$
	'Red Gold'	$3.00^{\mathrm{abc}}$	$3.50^{\mathrm{bcd}}$	$1.08^{\mathrm{bcd}}$	$25.94^{g}$	199.57 <sup>c-g</sup>	$50.60^{e-h}$	$28.08^{\mathrm{bc}}$	$147.50^{\:hi}$	$12.18^{c-f}$
	'Gala Mast'	$2.50^{a-f}$	$2.83^{c-h}$	$1.42^{c-h}$	$29.51^{b-g}$	198.31 <sup>c-g</sup>	$51.93^{c-f}$	$26.83^{bc}$	$148.67^{\;\mathrm{hi}}$	$11.21^{g}$
	'Golden Delicious'	$2.17^{b-f}$	$3.17^{c-f}$	$0.83^{c-f}$	$28.48^{d-g}$	$175.22^{f-i}$	$54.46^{ab}$	$26.92^{bc}$	$150.25^{\:hi}$	$11.85^{\rm efg}$
	'Shireen'	$2.92^{a-d}$	$3.75^{bc}$	$1.42^{\mathrm{bc}}$	$30.90^{a-f}$	$250.51^{b}$	$49.50^{ghi}$	$29.50^{b}$	$159.25^{\rm fg}$	$12.11^{c-f}$
$\overline{P} > 1$	F									
A		ns	0.0315	0.0016	ns	< 0.0001	< 0.0001	0.0010	< 0.0001	< 0.0001
В		< 0.0001	< 0.0001	ns	0.0003	< 0.0001	0.0006	< 0.0001	0.0494	ns
$A \times B$		0.0034	< 0.0001	< 0.0001	0.0023	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

SS – short sylleptic; MS – medium sylleptic; LS – long sylleptic; ASL – average sylleptic shoot length; TSL – total sylleptic shoot length; CA – crotch angle; SZ – sylleptic shoot zone, TH – tree height; TD – trunk diameter; CLM – central leader method; RLM – renewal leader method; ns – non-significant;  $^{\rm a-i}$ means followed by the same superscript letter within the columns are not significantly different (P=0.05) as established by Duncan's multiple range test

shoots ( $P \le 0.0001$ ), average sylleptic shoot length (P = 0.0003), and crotch angle (P = 0.0006), total sylleptic shoot length ( $P \le 0.0001$ ), sylleptic shoot zone ( $P \le 0.0001$ ) and tree height (P = 0.0494) was also observed (Table 1).

#### **Interaction effect**

For most of the studied variables, all the genotypes under study performed better with the CLM compared to the RLM. The maximum number of sylleptic shoots (10.75) was produced by 'Shireen' with the CLM, whereas the minimum number of sylleptic shoots (4.75) was produced by 'Top Red' with the RLM. As far as the comparison between the two methods for the number of sylleptic shoots is concerned, the most pronounced effect was observed on 'Shireen' (2.67 more sylleptic shoots with the CLM compared to the RLM), followed by 'Gala Mast' (1.75) and 'Red Gold' (1.42). On the other hand, the difference was less than one in 'Oregon Spur' (0.33), 'Red Chief' (0.75), 'Golden Delicious'

(0.83), and 'Red Spur' (0.92) (Figure 2C). Furthermore, irrespective of the genotypes, the sylleptic shoots produced on the RLM attained a higher crotch angle. However, considering the overall quality in terms of the tree height, trunk diameter, sylleptic shoot zone, etc. the CLM produced better-quality nursery trees (Table 1).

The correlation matrix related to the interrelationships among the different variables is presented in Figure 3. In the present experiment, the number of sylleptic shoots exhibited a highly significant positive (P < 0.05) correlation with the number of medium-length sylleptic shoots (r = 0.936), total sylleptic shoot length (r = 0.938) and sylleptic zone (r = 0.937).

Moreover, it also exhibited a significant positive (P < 0.05) correlation with the trunk diameter (r = 0.615), number of short (r = 0.744) and long (r = 0.649) sylleptic shoots. Conversely, a negative correlation was found between the number of sylleptic shoots and the crotch angle (r = -0.793).

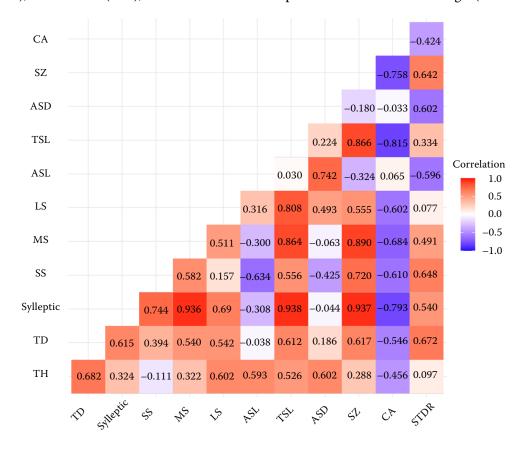


Figure 3. Pearson's correlation matrix among the nursery tree quality variables TH – tree height; TD – trunk diameter; SS – short sylleptic; MS – medium sylleptic; LS – long sylleptic; ASL – average sylleptic shoot length; TSL – total sylleptic shoot length; ASD – average sylleptic shoot diameter; SZ – sylleptic shoot zone; CA – crotch angle; STDR – trunk-to-sylleptic shoot diameter ratio

#### **DISCUSSION**

The current study has conclusively shown that the method and genotypes have a significant impact on the efficacy of the BA treatment for sylleptic branching in apple nursery trees. The BA application resulted in a significant lateral bud break on the current season growth for both the methods, resulting in acceptable branching. However, the CLM had a more pronounced effect on most of the studied variables and resulted in a more uniform distribution of bud break and sylleptic shoots along the length of the leader. It might be due to the fact that, in the CLM, the nursery trees got more period of rapid growth as the trees were ready for the first spraying on May 7th (about 15 cm of new growth), whereas, with the RLM, the tree attained the required height for the first spraying on May 20<sup>th</sup>.

Under the climatic conditions of this study, the growth of nursery trees usually begins in the 3<sup>rd</sup> to 4<sup>th</sup> week of April and continues until the 3<sup>rd</sup> week of July. From the last week of July to the 3<sup>rd</sup> week of August, the tree growth slows down and even stops in some trees. However, the tree growth resumes in the last week of August, leaving a bud scare of a rest period. Most nursery trees completely cease growth by the 3<sup>rd</sup> week of September. The sylleptic shoot formation usually stops after the 3<sup>rd</sup> week of July. However, some trees produce 2-4 sylleptic shoots even after this period. These sylleptic shoots develop spontaneously rather than as a result of the BA effect and disturb the hierarchy of nursery trees as they are produced on the upper part of the tree after leaving a considerable gap with the BA-induced sylleptic shoots. Moreover, due to the limited available growth period, they only attain 5–15 cm in length; therefore, they were not considered useful sylleptic shoots and thus were not included in the observation.

Considering the above fact about the growth pattern under the climatic condition of the study area, in the present investigation, compared to the RLM, an extra period of about two weeks of rapid growth of the apical section in the CLM seems to be the key contributing factor to the current findings, as the branching usually occurs in periods when the growth vigour of the parent shoot is highest (Wertheim, Webster 2003). The ability to produce sylleptic shoots later in the season may be compromised because part of the carbohydrates and nutrients required were used up in the future growth of the already

existing sylleptic shoots (Tromp 1996). Moreover, the presence of a higher accumulated reserve due to the uncut terminal could contribute to better outcomes in the CLM compared to the RLM.

The tree height was higher in the CLM compared to the RLM, possibly because the renewal leader had less time to grow than the central leader, and heading back the leader in the RLM negatively impacted the nursery tree growth. Similarly, the trunk diameter was also higher in the CLM, which can be explained by the positive correlation between the number of sylleptic shoots and the trunk diameter, as reported in the present study. The presence of more sylleptic shoots in the CLM positively influenced the trunk diameter. Moreover, Jacyna (2007) also stated that the presence of a higher number of sylleptic shoots on nursery trees can have a positive effect on the trunk diameter.

In the present study, the different apple genotypes responded differently to the BA application for growth and branching, which might be due to the different degrees of apical dominance and growth behaviours. The results indicated that each genotype has a specific growth behaviour. Even with the exogenous application of BA, the sylleptic induction can also be influenced by the endogenous auxin: cytokinin ratio, which can vary with the genotype. The greater level of endogenous auxin can inhibit bud break by antagonising the effects of the exogenously applied BA.

Thus, in the present study, the variable response of different genotypes to the exogenously applied BA might be due to their different endogenous auxin levels, and the genotype that has produced lower numbers of sylleptic shoots with 600 ppm might require higher doses of BA to combat the antagonising effect of the endogenous auxin. The findings of this study are supported by the works of Lordan et al. (2017) and Nečas et al. (2020). They also observed a significant variation in the sylleptic branching response of different apple genotypes with the BA application.

In the present study, with some exceptions, it was a noticeable trend that the genotype induced a higher number of sylleptic shoots, also induced the sylleptic shoots to have narrower crotch angles, and the present study reports a highly significant negative correlation between the number of sylleptic shoots and the crotch angle of sylleptic shoots. A similar pattern between the number of sylleptic shoots and the crotch angle was also reported by Rufato et al. (2019)

in 'Maxi Gala' and Kumawat et al. (2022) in 'Gala Mast' apple nursery trees.

Furthermore, the RLM produced sylleptic shoots with wider crotch angles than the CLM, which might be attributed to the presence of fewer sylleptic shoots on the trees. Thus, it seems that the presence of varying numbers of sylleptic shoots on nursery trees corresponds with the results in the current study. Regardless of the genotype, sylleptic shoots initially emerged at a very narrow angle from the vertical, which increases with the advancement of the sylleptic shoot growth. The final crotch angle of the sylleptic shoots in the lower tier is generally wider than those in the upper tier, as they got a comparatively longer growth period. Thus, the genotype that induces a higher number of sylleptic shoots also produced a higher number of sylleptic shoots in the later part of the season in the upper tier with a narrower crotch angle, and this is the probable reason for their lower average crotch angle in the trees that induces a higher number of sylleptic shoots.

In opposition, the researchers (Elfving, Visser 2006; Dorić et al. 2016) reported that the presence of a varying number of sylleptic shoots on nursery trees did not influence the average crotch angle of sylleptic shoots. Thus, their findings indicate that, in the present study, the variation in the crotch angle also might be due to the genotype and not solely due to the presence of a varying number of sylleptic shoots on the nursery trees. In addition, in the present study, some exceptions were also observed in the trend between the number of sylleptic shoots and the crotch angle. For instance, 'Golden Delicious' produced a higher number of sylleptic shoots (6.58) than 'Red Velox' (5.41), 'Oregon Spur' (6.0), and 'Starkrimson' (6.0), yet they produced sylleptic shoots with a wider crotch angle. Thus, it indicates that both the number of sylleptic shoots as well as the genotype have an influence on the crotch angle of sylleptic shoots.

With the exception of the crotch angle, apple nursery trees of different apple genotypes performed better with the CLM. However, the method's effect was not consistent across all the genotypes studied. As far as the number of sylleptic shoots, the most and the least influence of the method was observed on 'Shireen' and 'Oregon Spur', respectively. 'Shireen' produced 2.67 more sylleptic shoots, while 'Oregon Spur' produced only 0.33 more sylleptic shoots with the CLM than with the RLM, suggesting that genotype growth behaviour is one factor that affects

the branching response to the method used. Although the responses varied among the genotypes, all the genotypes under study still produced a higher number of sylleptic shoots with the CLM.

It is important to mention that, in both methods, branches have induced on the current season growth, thus apart from genotypes, it seems that the response largely depends on the available period of rapid active growth of the terminal section, which, in the present study, is primarily influenced by the method used as described earlier.

#### **CONCLUSION**

In the present investigation, it was found that the effect of the BA application on the sylleptic shoot production in apple nursery trees is significantly dependent on the genotypes. Furthermore, the methods employed for inducing sylleptic shoots in apple nursery trees have a substantial effect on the branching and other nursery tree quality determinants. The CLM outperformed the RLM in terms of the sylleptic shoot production and overall tree quality. This suggests that the CLM is a good alternative method to RLM, particularly in those climatic conditions where the rapid growth period of the parent shoot is a limiting factor in the sylleptic branching of apple nursery trees. Moreover, compared to RLM, the CLM can result in cost savings in the production of branched apple nursery trees by escaping the need of heading back and the subsequent selection of one proleptic shoot and the removal of other proleptic shoots.

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