Research Article

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Effect of micronutrients and plant growth regulators on fruit set and retention in ber (*Ziziphus mauritiana* Lamk.)

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ABSTRACT

The determination of fruit set has been used in ber as a consistent indicator of the real productivity level. Due to short flower life, prevalence of self and cross incompatibility, pollen sterility and synchronous protandrous dichogamy, fruit set in the ber largely depends on sources of pollination, its related factors like effective pollination period, pollinators, environmental conditions, and the success in fertilization. Hence, any factor that can extend the effective pollination period, pollen germination and pollen tube growth such as the application of micronutrient or plant growth regulators, should be beneficial for fruit production. During 2020– 21, forty-one genotypes of ber were evaluated for fruit set at ICAR-CIAH, Bikaner. Simultaneously, a trial was conducted to find out the response of micronutrients and plant growth regulators on fruit set and fruit retention in the 'Gola' ber. Foliar sprays of micronutrients (Boron at 0.1% and Zinc at 0.5%) and graded doses (10, 20, and 30 ppm) of plant growth regulators (GA_3, NAA, kinetin, and benzyladenine) were applied at BBCH stage 54 (green bud) and 67 (flower fading *i.e.* most petal fall). The genotypes showed large variation in natural fruit sets ranging from 0.34 (Chochal) to 13.89% (Illaichi). About 88 per cent of genotypes under observation exhibited fruit set less than 6 per cent. Intervention with micronutrients and plant growth regulators applied at two different stages bring a significant response on fruit set in Gola ber. Among the various treatments evaluated, the highest fruit set was observed with the application of 0.1% boron (B) at BBCH stage 67 (11.41%), followed closely by the application of 10 ppm gibberellic acid (GA3) at the same stage (10.94%). Both treatments were statistically on par in terms of fruit set. However, the boron treatment demonstrated a superior fruit retention rate (80.38%) compared to GA3 (63.68%). In contrast, the water-sprayed control trees recorded significantly lower fruit set (3.77%) and retention (56.52%). In conclusion, the application of Boron (0.1%) at BBCH stage 67 is an effective approach to enhance fruit set and retention in ber cv. Gola under the hot arid regions of Rajasthan.

Introduction

Ber belongs to the family Rhamnaceae, which consists of about 45 genera and 950 species (Christenhusz and Byng, 2016). The genus *Ziziphus* includes approximately 170 species native to the tropics and subtropics. Of these, approximately 150 species are native to the Old World, including Europe, the Middle East, Africa, the Indian subcontinent, and Asia

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(Liu and Cheng, 1995), while about 25 species originate from the New World, covering the Americas and the Caribbean islands (Schirarend, 1991). Among these, *Ziziphus mauritiana*, *Ziziphus rotundifolia*, and *Ziziphus nummularia* are particularly well-adapted to dry and hot climates, thriving in harsh environments with degraded soils, high temperatures, and limited water availability. However, due to its greater economic significance, *Z. mauritiana* is extensively cultivated in the arid and semi-arid regions of Rajasthan.

Most angiosperm species produce hermaphroditic flowers (Renner, 2014), which favours self- pollination and consequently inbreeding depression. To counter this, plants with hermaphroditic flowers often evolve morphological, molecular, and/ or phenological adaptations that favours cross-pollination. The mechanisms preventing self-pollination in *Ziziphus mauritiana* are well- documented through studies on its morphology, physiology, genetics, and reproduction (Tel-Zur and Schneider, 2009; Asatryan and Tel-Zur, 2013; Asatryan and Tel-Zur, 2014).

Self-incompatibility is the inability of a fertile hermaphroditic plant to form zygotes following self-pollination (Allen and Hiscock, 2008). *Ziziphus mauritiana* exhibits self-incompatibility (Asatryan and Tel-Zur, 2013), which is governed by a gametophytic mechanism (Asatryan and Tel-Zur, 2013). An additional mechanism that reduces self-fertilization in *Ziziphus mauritiana* is dichogamy. Its flowers exhibit synchronous protandrous dichogamy, i.e., flowers of an individual plant mature in synchrony, and anther dehiscence precedes stigma receptivity, with little or no over-lapping between the sexual stages (Islam and Simmons, 2006; Asatryan and Tel-Zur, 2013; Tel-Zur and Schneider, 2009).

Due to the prevalence of self and cross incompatibility and synchronous protandrous dichogamy, fruit set in the ber largely depends on sources of pollination, its related factors like effective pollination period, pollinators, environmental conditions, and the success in fertilization. Further, the life of an individual ber flower is very short and many flowers are not pollinated during their respective period. Consequently, despite a very profuse flowering, fruit set in ber is very low even under open pollination. Natural fruit set in ber varies from 2-18 per cent (Vashishtha and Pareek, 1979). Therefore, the determination of fruit set has been used in ber as a consistent indicator of the real productivity level. Hence, any factor that can extend the effective pollination period, pollen germination and pollen tube growth such as the application of micronutrient or plant growth regulators, could be beneficial for fruit production.

Some micronutrients (Zn & B) and plant growth regulators (auxin, cytokinin, gibberellins) are known for their positive influence on fruit set by affecting pollen germination and pollen tube growth. However, their concentration, targeted stage as well as interaction with climatic variables have an influence on their response. The present experiment was conducted to find out the response of micronutrients and plant growth regulators on fruit set in Gola cultivar of ber under hot arid region.

Material and Methods

The present study was carried out at the ICAR–Central Institute for Arid Horticulture (CIAH), Bikaner, Rajasthan, located at 28°06' N latitude, 73°20' E longitude, and an elevation of 240 m above mean sea level. The site lies within the Western dry region of India, characterized by extremely hot summers, with maximum temperatures reaching up to 50 °C, and moderately cold winters, with minimum temperatures occasionally dropping to −3 °C. During the flowering and fruit set period (September–October) of 2020, the mean monthly temperature was 28.90 °C, about 0.06 °C higher than the decadal average for the same months (2010–2019).

During 2020–21, 41 genotypes of ber were evaluated for fruit set. Three trees of each genotype were selected, and two proleptic branches on each tree were tagged in two directions (East-South and North-West) to observe natural fruit set. Each tree was treated as one replication. Simultaneously, a trial was conducted to find out the response of micronutrients and plant growth regulators on fruit set and fruit retention in the 'Gola' ber. Foliar sprays of micronutrients (Boron at 0.1% and Zinc at 0.5%) and graded doses (10, 20, and 30 ppm) of plant growth regulators (GA_3- gibberellic acid, NAA-naphthaleneacetic acid, kinetin, and benzyladenine) were applied at BBCH stage 54 (green bud) and 67 (flower fading *i.e.* most petal fall). The experiment was set up in factorial randomized block design with three replications of two trees per replicate. For assessing fruit set and retention, two uniform and healthy proleptic branches were selected from each tree in two directions (East-South and North-West). The total number of flowers on each selected branch was counted. At 15 days after the last bloom (DALB), the number of fruits set was counted. Subsequently, the number of fruits retained until harvest was counted. The fruit set and retention data were expressed as percentages using the following formulas for treatment comparison:

$$\text{Fruit set (\%)} = \frac{\text{No. of fruit set per branch at 15 DALB}}{\text{No. of flowers per branch}} \times 100$$

$$\text{Fruit retention (\%)} = \frac{\text{No. of fruit at harvest}}{\text{No. of fruit at 15 DALB}} \times 100$$

The collected experimental data were subjected to statistical analysis based on the analysis of variance (ANOVA) using the programme R Software. Significant differences at p=0.05 level was obtained using Duncan's multiple range test.

Result and Discussion

Experiment 1

The present study revealed a significant variation in natural fruit set among 41 ber (*Ziziphus mauritiana*) genotypes, ranging from as low as 0.34% in the genotype Chochal to as high as 13.89% in Illaichi. This wide range highlights the inherent genetic diversity present within the ber germplasm concerning reproductive success and adaptability under prevailing environmental conditions. The variation in fruit set among different genotypes corroborates earlier findings by Vashishtha and Pareek (1979), who also reported significant genotypic influence on fruit setting patterns in ber.

About 88% of the genotypes exhibited fruit set percentages below 6%, indicating that a substantial proportion of the studied genotypes may have limited reproductive efficiency under natural pollination in the hot arid climate. This low fruit set could be attributed to various factors including floral biology, pollen viability, stigma receptivity, pollinator activity, and environmental stressors such as high temperatures and low relative humidity.

On the other hand, a few genotypes demonstrated relatively higher fruit set, suggesting a better adaptation to arid conditions. Notably, Mehrun (7.07%), Rashmi (7.24%), and Umran (7.85%) recorded satisfactory levels of fruit set. The highest fruit set was observed in Illaichi (13.89%), indicating its potential as promising genotype for cultivation and future breeding programme. These genotypes might possess favorable floral traits or higher compatibility with the existing pollinator fauna, which could be contributing to their better performance.

Among the 41 genotypes evaluated, the average fruit set was significantly higher on the east– south side of the trees (4.7%) compared to the north–west side (3.4%). This variation may be attributed to differences in light interception and canopy microclimate. The east–south orientation receives more favorable sunlight during the morning and early afternoon, promoting optimal photosynthetic activity and maintaining suitable temperature conditions during the critical period of pollination and fertilization. During the fruit set phase (September–October), the sun's apparent path in September remains nearly balanced, rising close to the east and setting in the west, whereas in October it becomes lower in the sky and shifts toward the south in Bikaner. Consequently, improved light availability and moderated thermal conditions on the east–south side likely enhanced pollen viability, stigma receptivity, and overall fruit set.

The determination of natural fruit set serves as a reliable indicator of the reproductive potential and yield capacity in ber. Unlike other yield components that may vary due to management practices or environmental fluctuations, fruit set reflects the inherent reproductive efficiency of a genotype under natural pollination conditions. Therefore, genotypes exhibiting consistently higher fruit set are likely to possess greater yield stability and adaptability across diverse environments.

Table 1. Range of natural fruit set in different ber genotypes under the hot arid region.

Range (%)	Genotype(s)
0 – 2	Chochal, C. Bawal, Nazuk, Khatti
2 – 4	Gola, Banarsi Kadaka, Chhuhara, Sanaur, ZG-3, Jogia, Kissmiss, S. Rohtak, Kala Gola, Dharki No. 1, Safeda Selection, Tikadi, Aliganj, Jogia, Nanki, Laddu, Nilgiri, Ponda
4 – 6	Seb, Banarsi Pavindi, Mundia, Kathaphal, Lakhan, Thar Malti, Kheera, Kali, Dadan, Thornless, Banarsi, Kaithli, Gularvasi, Meharwali
6 – 8	Rashmi, Mehrun, Badami, Umran
>12	Illaichi

Experiment 2

Fruit set

The present study revealed that the fruit set (%) in ber cv. Gola was significantly influenced by the type and concentration of chemical treatments, as well as the stage of application (Figure 1; Table 2). Among the two stages evaluated, the flower fading stage (BBCH 67) exhibited a greater response to chemical treatments, resulting in a significantly (P = 0.0008) higher fruit set (5.89%) compared to the green bud stage (5.49%) (Figure 1). This suggests that the reproductive physiology of ber is more receptive to external stimuli closer to anthesis or immediately post- anthesis, likely due to enhanced metabolic and hormonal activity in the floral tissues during this period. Among the chemical treatments, boron (B) at 0.1% showed the most pronounced effect (10.3%; $P \leq 0.0001$), particularly when applied at BBCH 67, resulting in the highest fruit set of 11.4%, which was significantly superior ($P \leq 0.0001$) to the untreated control (3.77%) (Table 2). This enhanced fruit set aligns with the essential role of boron in reproductive development, as it forms borate cross-links in rhamnogalacturonan-II (RG-II) pectin that are critical for pollen tube growth, transmitting tissue integrity, and successful fertilization. The gene *NpGUT*1, responsible for RG-II biosynthesis and borate cross-linking, has been shown to be crucial for the development of male and female reproductive tissues and for guiding pollen tubes to the ovary during fertilization. Disruption of the boron–RG-II complex or the associated *NpGUT*1 gene results in sterility and defective reproductive tissue development (Iwai *et al*., 2006). Reproductive tissues have a higher demand for boron than vegetative tissues (Rerkasem and Jamjod, 2004), likely due to the elevated concentration of the RG-II complex in the cell walls of pollen tubes (Matoh *et al*., 1997). Consequently, boron deficiency has a more severe impact on reproductive processes than on vegetative growth. In monocots, the putative boron transporter OsBOR4 is specifically expressed in floral tissues, and its disruption leads to impaired reproductive competence due to slower or reduced pollen tube growth in rice (Tanaka *et al*., 2013). Similarly, the expression of VvBOR1 (putative boron transporters in dicot) in floral tissue rises sharply at anthesis and expression can be directly linked to the accumulation of boron in grape berries during development (Pérez-Castro *et al*., 2012). Inadequate boron levels also disrupt microsporogenesis, resulting in reduced pollen production, smaller pollen grains, and lower pollen viability (Huang *et al*., 2000). Moreover, boron promotes pollen germination and pollen tube elongation, as demonstrated in apple (Sharafi and Raina, 2020) and almond (Agnes *et al*., 2000), highlighting its vital role in ensuring successful fertilization and fruit set.

The application of plant growth regulators (PGRs) also showed promising results, with a clear dependence on both the type and concentration used. GA_3 at 10 ppm, when applied at BBCH stage 67, resulted in a fruit set of 10.94%, which was statistically at par with the boron treatment (Table 2). Similarly, benzyladenine (BA) at 30 ppm and 20 ppm, applied at the same stage, produced fruit sets of 10.1% and 9.04%, respectively. Kinetin at 10 ppm, also applied at BBCH 67, was effective as well, resulting in a fruit set of 9.25 per cent.

Higher doses of BA (30 ppm) proved more effective than lower concentrations, indicating a positive response to increased levels. This suggests that higher concentrations of BA may further enhance fruit set in ber. Interestingly, lower concentrations (10 ppm) of GA3, NAA, and kinetin were generally more effective than higher doses, suggesting a threshold beyond which these compounds may not produce favorable physiological responses or could even inhibit normal reproductive development due to hormonal imbalance. However, NAA exhibited contradictory and even negative effects, especially when applied at BBCH 67. The fruit set in these treatments ranged from 0.9 to 1.28%, which was significantly lower than the control. This implies that NAA, at least at the tested concentrations and timings, may interfere with natural hormonal balance or cause excessive abscission in ber. Similarly, the application of zinc (Zn), regardless of timing, did not result in any significant improvement in fruit set, suggesting its limited role under the tested stage and conditions.

These findings are consistent with earlier research demonstrating the importance of plant growth regulators like GA3 (Garmendia *et al*., 2019; Lindo-García *et al*., 2020; Choudhary *et al*., 2020; Das *et al*., 2020; Jayachitra and Richard Kennedy, 2021; Priya *et al*., 2023; Narayan and Deen, 2024) in improving fruit set in fruit crops. The negative response of NAA, however, warrants further investigation into its concentration- dependent effects and interaction with endogenous hormones during the flowering and early fruiting stages in ber.

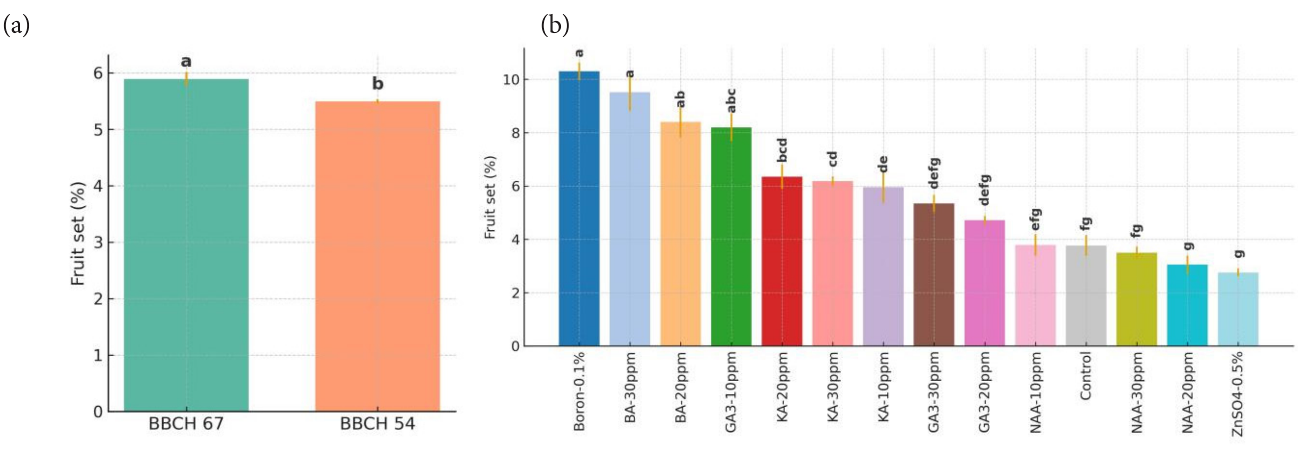

Fig. 1. Effect of targeted phenological stage (a), chemical treatments, and their concentrations (b) on fruit set in ber cv. Gola

Fruit retention

The findings presented in Figure 2 and Table 2 clearly show that fruit retention in ber cv. Gola was also significantly influenced by the stage of application ($P \leq 0.0001$), the type of chemical treatment ($P \leq 0.0001$), and their interaction ($P \leq 0.0001$). The highest fruit retention was recorded with the application of NAA at the flower fading stage (BBCH 67). Interestingly, several treatments that produced lower or even negative effects on initial fruit set subsequently exhibited higher retention at physiological maturity. This inverse relationship between fruit set and retention may reflect an internal self-regulatory mechanism within the tree, wherein an excessive initial fruit load intensifies competition among developing fruits for assimilates, mineral nutrients, and hormonal signaling, ultimately leading to enhanced fruit abscission (Bangerth, 2000; Goren *et al*., 2004). In contrast, a moderate fruit set allows for more efficient assimilate partitioning and stronger sink development in individual fruits, thereby improving their capacity for retention and continued growth. These findings are consistent with established physiological concepts that final yield in fruit crops is determined primarily by sink strength and assimilate availability rather than the initial number of fruits formed (Forshey and Elfving, 1989).

However, two notable exceptions to this trend were observed: the application of boron (B) at 0.1%, whether at the green bud stage (BBCH 54) or the flower fading stage (BBCH 67), and KN (10 ppm) applied at BBCH 67. These treatments not only significantly enhanced fruit set but also maintained a high proportion of fruit retention until harvest. This dual benefit suggests a more balanced influence on both reproductive processes and carbohydrate partitioning.

Additionally, the application of benzyladenine (BA) at either BBCH 54 or 67 effectively improved fruit set, though the retention rate was lower compared to that observed with B (0.1%) and KN (10 ppm). This indicates that while BA may stimulate initial fruit development, its ability to support sustained retention may be limited, possibly due to a lack of support for sink strength or inadequate influence on hormonal balance and resource allocation during the later stages of fruit development.

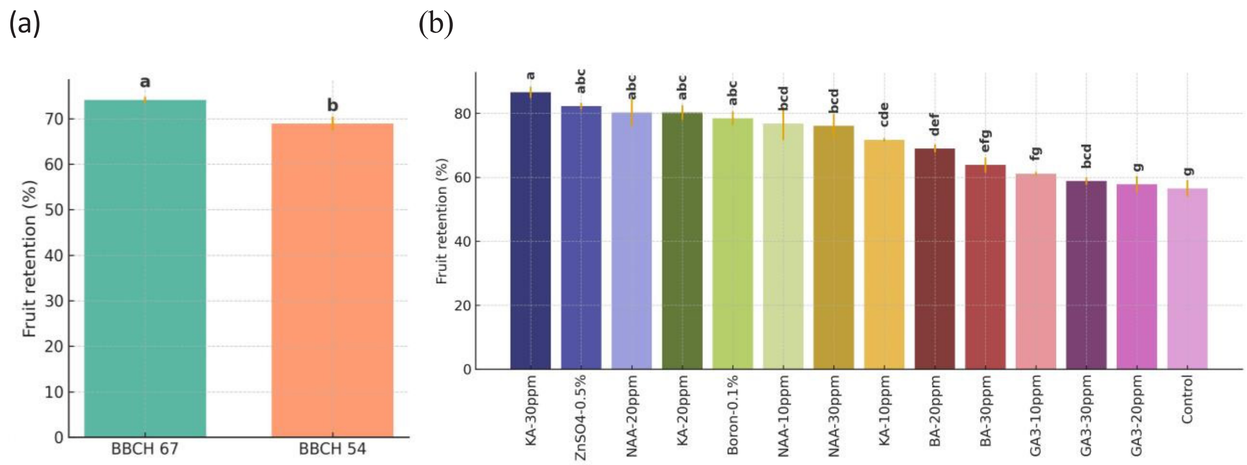

Fig. 2. Effect of targeted phenological stage (a), chemical treatments, and their concentrations (b) on fruit retention in ber cv. Gola

Table 2. Interaction effects of application stages and chemical treatments on fruit set and retention in ber cv. Gola

Stage	Treatments	Fruit set (%)	Fruit retention (%)
	Boron- 0.1 %	9.18 ± 1.04^{d}	76.60 ± 1.87^{efg}
BBCH stage 54	ZnSO4- 0.5%	3.42 ± 0.24^{jk}	79.85 ± 2.28^{def}
	GA3-10 ppm	5.45 ± 0.38^{hi}	58.52 ± 1.35^{mno}
	GA3-20 ppm	3.35 ± 0.16^{jk}	55.53 ± 2.99^{o}
	GA3-30 ppm	3.78 ± 0.32^{j}	57.80 ± 1.37^{mno}
	NAA- 10 ppm	6.45 ± 0.54^{fg}	65.08 ± 2.73^{jkl}
	NAA- 20 ppm	5.19 ± 0.63^{i}	65.36 ± 3.62^{jkl}
	NAA- 30 ppm	5.73 ± 0.61^{ghi}	63.25 ± 2.31^{klm}
	KA- 10 ppm	2.66 ± 0.54^{kl}	71.13 ± 1.77^{ghij}
	KA- 20 ppm	6.98 ± 0.49^{ef}	81.81 ± 2.86^{def}
	KA- 30 ppm	6.13 ± 0.89^{fgh}	90.75 ± 4.48^{ab}
	BA- 10 ppm	3.59 ± 0.42^{j}	75.54 ± 2.01^{fgh}
	BA- 20 ppm	7.77 ± 0.72^{e}	70.22 ± 1.70^{hij}
	BA- 30 ppm	8.93 ± 0.51^{d}	65.71 ± 2.56^{jkl}

BBCH stage 67	Boron- 0.1 %	11.41 ± 0.50[a]	80.38 ± 2.45[def]
	ZnSO4- 0.5%	2.11 ± 0.17[lm]	84.69 ± 4.28[bcd]
	GA3-10 ppm	10.94 ± 0.66[ab]	63.68 ± 2.61[klm]
	GA3-20 ppm	6.10 ± 0.30[fgh]	60.03 ± 2.46[lmno]
	GA3-30 ppm	6.93 ± 0.64[ef]	59.87 ± 1.74[lmno]
	NAA- 10 ppm	1.12 ± 0.30[n]	88.57 ± 10.30[bc]
	NAA- 20 ppm	0.90 ± 0.09[n]	95.24 ± 8.25[a]
	NAA- 30 ppm	1.28 ± 0.15[mn]	88.89 ± 9.62[ab]
	KA- 10 ppm	9.25 ± 0.64[cd]	72.26 ± 2.72[ghi]
	KA- 20 ppm	5.72 ± 0.70[ghi]	78.65 ± 2.52[def]
	KA- 30 ppm	6.23 ± 0.52[fgh]	82.36 ± 1.05[cde]
	BA- 10 ppm	3.51 ± 0.35[jk]	70.37 ± 1.63[ghij]
	BA- 20 ppm	9.04 ± 0.44[d]	67.74 ± 4.24[ijk]
	BA- 30 ppm	10.10 ± 0.99[bc]	62.06 ± 2.87[klmn]
Control		3.77 ± 0.39[j]	56.52 ± 2.61[no]
Pr > F		< 0.0001	< 0.0001

Conclusion

In conclusion, the observed variation in fruit set among genotypes can be primarily attributed to inherent genetic differences. Identifying and utilizing genotypes with superior fruit set potential offer a viable strategy to enhance ber production in challenging environments. The present study demonstrates that the application of micronutrients and plant growth regulators can effectively improve fruit set in ber cultivated under hot arid conditions. These findings highlight the significance of using suitable micronutrient and growth regulator treatments to enhance fruit set and ensure effective fruit retention. Furthermore, internal physiological factors such as crop load and assimilate availability play a crucial role in determining the final yield outcome. Among the treatments evaluated, boron (0.1%) application at the BBCH 67 stage proved particularly effective in improving both fruit set and retention under variable crop load conditions. Future research should focus on elucidating the physiological and molecular mechanisms governing fruit set in diverse genotypes and assessing their performance under different agro-climatic conditions and in controlled pollination trials to confirm their breeding value.

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Conflict of Interest

The authors have no conflict of interest.

Data Sharing

All relevant data are within the manuscript.

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