



## Article

# Effects of Time of Pruning and Plant Bio-Regulators on the Growth, Yield, Fruit Quality, and Post-Harvest Losses of Ber (*Ziziphus mauritiana*)

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**Abstract:** Indian jujube or ber (*Ziziphus mauritiana* Lam.) is a deciduous fruit tree typically cultivated in several semi-arid areas of Asia because of its adaptability to yield-limiting conditions. The present study aimed to assess the effect of four pruning times (i.e., the fourth week of March, second week of April, fourth week of April, and second week of May) and four treatments using stress-mitigating plant bio-regulators (thiourea at 500 ppm and 1000 ppm; salicylic acid at 100 ppm and 150 ppm) as a means to improve both fruit yield and quality post-harvest. To this end, a full factorial experiment lasting two growing seasons was carried out under field conditions in the representative semi-arid region of Rajasthan, the state with the largest production in India. We assessed the vegetative growth of the trees, the fruit size and yield, and some quality parameters (soluble content, acidity, ascorbic acid, and total sugars) as well as the main post-harvest traits (fruit weight loss and spoilage). Overall, pruning during the second week of April had the greatest positive influence on most of the variables studied. For instance, it induced the highest vegetative vigor, allowing the maintenance of relatively higher chlorophyll and relative water content in the leaves. The fruit parameters also responded most positively to the second week of April pruning, a treatment that, compared to the others, induced a higher diameter; a higher amount of TSS (19.6 °Brix), ascorbic acid (86.5 mg/100 g), and total sugar (10.4%); and a better post-harvest shelf-life. Among the plant bio-regulators, the application of thiourea at 1000 ppm had the highest positive influence on the growth parameters, yield, quality, and reduction in spoilage post-harvest. The differences between the doses of PBRs were limited.

**Keywords:** pruning; PGRs; thiourea; salicylic acid; fruit quality; yield; shelf-life

## 1. Introduction

The Indian jujube (*Ziziphus mauritiana* Lam.), better known as ber, is a vigorous, medium-sized fruit tree belonging to the *Rhamnaceae* family. This species is appreciated for its ability to cope with harsh environmental conditions (i.e., extreme temperatures, water-logging, and dry environments) [1], therefore gaining the epithet of “king of the arid-zone fruits” [2]. The easily perishable fruits are typically eaten fresh, but they can be also stewed, pickled, transformed into jam, or dried to a powder and used in beverages [3]. Ber fruits are a rich source of vitamins, as well as carotenoids, pectin, and mineral elements. It is noteworthy that ber fruits have an ascorbic acid (vitamin C) content higher than citrus and second only to guava among the tropical fruits [2]. Other uses of the plant are related to its medicinal properties. For instance, the smoke of its burning leaves is used to cure skin rashes, coughs, and colds [4]. Ber fruits are also increasingly employed for food products such as chutney, murabba, candy, and nectars [2,5]. Finally, ber wood can also be used for

various purposes [6] and is also a good source of charcoal and tannin, while the leaves can be fed to farm animals and lac insects [7].

Indian jujubes are cultivated in several Asian countries, mainly China, Pakistan, Thailand, Korea, Iran, and Vietnam. India is the world's top producer [8] and this species is mainly present in its arid and semi-arid regions [5]. According to the statistics of the Department of Agriculture (available at <https://agriculture.rajasthan.gov.in>, accessed on 25 July 2022), Rajasthan is the leading producing Indian state, with a cultivated area of 954 ha, an annual production of 8578 Mt, and a productivity of 8.99 Mt/ha. Ber farming is gaining popularity in areas characterized by poor-quality soils (e.g., saline) and low rainfall. Its high tolerance for severe climates, its adaptability to a variety of soils, and its fast growth result in low maintenance costs and high fruit yield even under resource-limited conditions [9].

The Indian jujube bears flower and fruits at the axil of the leaves of new shoots (current-season shoots) and annual pruning is necessary to maintain tree vigor and to produce high-quality fruits [10]. Pruning can influence several horticultural aspects in ber trees such as tree architecture, bud sprouting, flower initiation, fruit setting, yield, and quality [11,12]. In ber plants left unpruned for a long time, the canopy becomes overcrowded, and branchlets become weaker, with diminished flowering, higher fruit drop, smaller fruits, and more severe infestations of the fruit-fly and stone weevil [13,14]. Eventually, unpruned tree becomes horticulturally unproductive, reaching very low fruit yield and producing fruits of scarce quality.

Ber trees often experience water deficits because they are typically grown in less-than-favorable soils and climate conditions. This issue is expected to be exacerbated by the current climate change, further encouraging the implementation of measures to increase plant adaptation to an unfavorable environment. Plant bio-regulators (PBRs) are a wide group of chemically diverse molecules that can be used to improve the growth and production of cultivated plants facing abiotic stress [15]. Nowadays, PBRs are employed in perennial fruit trees for a variety of purposes, such as fruit induction and shaping, branching, rooting, dormancy breaking, the control of pre-harvest drop, and the extension of post-harvest life [16]. Among the exogenously applied PBRs, thiourea and salicylic acid (SA) are typically used to boost plant signaling and redox homeostasis, ultimately improving stress tolerance [17,18]. The application of thiourea has multiple effects. To name a few, it increases vegetative growth, nutritional quality and yield [19], net photosynthesis, and leaf chlorophyll content [20]. Moreover, thiourea application can mitigate the effects of low or high temperatures [21,22]. Salicylic acid also endogenously plays a key function in modulating the stress response in plants [18]. Although typically associated with plant resistance to pathogens, SA has also been employed as a PGR to increase tolerance against abiotic stress such as extreme temperatures [23,24]. Moreover, SA has been used to improve fruit quality using pre-harvest or post-harvest treatments [25,26].

Considering the potential benefits of PBRs, the aim of this work was to study the effects of thiourea and SA, two stress-mitigating PBRs, in relation to four different pruning times. Using a full factorial design, we investigated the vegetative growth parameters as well as fruit yield and quality, because ber is attracting increasing attention in sustainable agriculture as a more exploitable source of nutritious fruits in urban areas [27,28]. Moreover, we also examined the fruit's main post-harvest characteristics, and physiological fruit loss and spoilage. The study was conducted at the field level in the state of Rajasthan over two consecutive seasons to provide useful information for the Indian jujube cropping system.

## 2. Materials and Methods

### 2.1. Location, Materials, and Experimental Design

The present research was carried out over two consecutive growing seasons (2018–19 and 2019–20) on six-year-old ber trees (*Ziziphus mauritiana* Lam.) cv. 'Gola' grafted onto *Z. rotundifolia* rootstocks. Plants were planted with 6 × 6 m spacing (278 trees ha<sup>-1</sup>) at the Horticulture Farm, S.K.N. College of Agriculture, Jobner, Rajasthan state, India (26°05' N, 75°28' E; 427 m above sea level). The climate of this region is semi-arid, characterized by

dry air, a scarcity of water, and extreme temperatures during summer (48 °C) and winter (−1.5 °C or below). In this cultivation area, the average rainfall and relative humidity during the growing season vary between 300 and 400 mm (mainly concentrated between July and September) and between 43% and 87%, respectively. Weather data were obtained from the meteorological station of the S.K.N. College of Agriculture and are presented in Figure S1. The mean temperatures during the growing seasons 2018–19 (March 2018 to February 2019) and 2019–20 (March 2019 to February 2020) were 24.8 and 24.4 °C, respectively. The soil was loamy sand in texture with a pH of 8.3 and an EC<sub>e</sub> of 1.26 dSm<sup>−1</sup>.

The experiment consisted of 20 treatments derived from the factorial combination of four pruning times and five PBR applications. The pruning times were: PR-13: trees were pruned on standard meteorological week (SMW) 13 (4th week of March); PR-15: trees were pruned on SMW 15 (2nd week of April); PR-17: trees were pruned on SMW 17 (4th week of April); PR-19: trees were pruned on SMW 19 (2nd week of May). PBRs treatments were as follows: PBR-C: control (trees were sprayed only with water); PBR-TL: thiourea was applied at a concentration of 500 ppm (lowest dose); PBR-TH: thiourea was applied at concentration of 1000 ppm (highest dose); PBR-SAL: salicylic acid was applied at a concentration of 100 ppm (lowest dose); and PBR-SAH: salicylic acid was applied at concentration of 150 ppm (highest dose). Bio-regulators were applied twice: at the beginning of flowering (SMW 36–37, corresponding to the 1st–2nd week of September, respectively) and at pea-sized fruit stage (SMW 40–41, corresponding to the 1st–2nd week of October, respectively). The experimental design was a factorial randomized block design with three replications. Each treatment employed a total of nine trees (three trees per replicate). Irrigation, the use of fertilizers, and insect-pest and disease management were performed following standard cultural practices.

## 2.2. Tree Vegetative Growth

The impact of the treatments on tree vegetative growth was assessed by measuring the increase in trunk diameter between mid-March and the end of October, and the increase in canopy perimeter during the period of maximum vegetative growth (between September, right before the application of the PBR treatments, and the end of October). Canopy perimeter was estimated as the perimeter of an ellipse, having as its axes the canopy width measured in the North–South and East–West directions. Trunk diameter was measured at 10 cm above the graft union.

## 2.3. Physiological Parameters

In both years, the leaf area, total leaf chlorophyll content, and relative leaf water content (RWC) were measured at the end of October. For each treatment, nine 20 cm-long secondary or tertiary branches (three branches per tree) were selected, and their total leaf area was measured using a leaf area meter (LICOR-3000, Lincoln, NE, USA). Leaf chlorophyll content was determined on a sample of leaves per replicate, essentially as described in [29]. Leaf dry weight was measured after drying the leaves in an oven set at 65 °C (UF110, Memmert, Schwabach, Germany) until at a constant weight. The RWC was calculated as:

$$\text{RWC (\%)} = \frac{\text{Leaf fresh weight} - \text{Leaf dry weight}}{\text{Leaf turgid weight} - \text{Leaf dry weight}} \times 100$$

## 2.4. Fruit Yield and Quality at Harvest

Fruit harvest was carried out on five trees per treatment in six to eight pickings between 15 January and 10 February 2019 and between 12 January and 15 February 2020. The fruits harvested in all the pickings from the five trees were weighed separately using a digital scale. Total fruit yield per tree was calculated by adding the yields of the different pickings. In both experimental seasons, twenty fruits from each treatment were randomly selected on the third picking (which is the most representative in terms of the amount of fruit harvested) and used for the assessment of quality traits. Their diameter was individually measured

using a digital caliper, whereas total soluble solid (TSS) concentration in fruit juice was determined on five of these fruits per tree using a digital refractometer (0–50 °Brix; Atago Co., Ltd., Tokyo, Japan). The total acidity and ascorbic acid of freshly harvested fruits were analyzed according to previously published protocols [30,31]. Total sugar content in fruits was determined using already described procedures [32].

### 2.5. Fruit Shelf-Life

Fruit shelf-life was assessed by placing the fruits at room air temperature and relative humidity ( $65 \pm 5\%$ ) for nine days. Thirty fully mature fruits were selected for each treatment and maintained separately. The number of spoiled fruits (e.g., with mold, soft areas, unpleasant odor, etc.) was counted at three-day intervals (i.e., 3, 6, and 9 days after fruit harvest) and spoilage was expressed as a percentage, following the equation:

$$\text{Spoilage (\%)} = \frac{\text{Number of spoiled fruits}}{\text{Total number of fruits}} \times 100$$

Thirty additional fruits were individually marked and weighted on subsequent dates for the determination of physiological fruit weight loss. Individual fruit fresh weight was measured at the beginning of the shelf-life experiment (initial fresh weight) and at three additional times at three-day intervals (3, 6, and 9 days after fruit harvest). The percentage of weight loss (PWL) was calculated as follows:

$$\text{PLW}_i (\%) = \frac{\text{FW}_0 - \text{FW}_i}{\text{FW}_0} \times 100$$

where  $\text{PLW}_i$  is the percentage of fruit weight loss measured after  $i$  days of shelf-life,  $\text{FW}_0$  is the initial fruit fresh weight (at 0 days of shelf-life), and  $\text{FW}_i$  is the fruit fresh weight measured after  $i$  days of shelf-life.

### 2.6. Statistical Data Analysis

The significance of the effect of the growing season (GS), the pruning time (PR), the bio-regulator application (PBR), and their three-way and two-way interactions (GS  $\times$  PR, GS  $\times$  PBR, PR  $\times$  PBR, and GS  $\times$  PR  $\times$  PBR) on the measured parameters was assessed via three-way ANOVA. The Duncan test was used as a post hoc test for mean separation ( $p \leq 0.05$ ). All the analyses were performed using the statistical software SPSS v. 27 (IBM, Chicago, IL, USA).

## 3. Results

The growing season (GS), the pruning time (PR), and the plant bio-regulator application (PBR) significantly influenced all the parameters measured in this study (Tables 1–4). The interactions of the three independent variables (GS  $\times$  PR  $\times$  PBR, GS  $\times$  PR, GS  $\times$  PBR, and PR  $\times$  PBR) with all the dependent variables were not significant, with the only exception being the titratable acidity of the fruit juice at harvest, which was affected only by the PR  $\times$  PBR interaction (Table 2).

### 3.1. Effects on Tree Vegetative Growth and Physiological Parameters

The vegetative growth parameters indicated that tree vigor was higher in the second growing season (2019–2020) (Table 1). Specifically, the annual trunk diameter growth, canopy perimeter growth, and leaf area per branch were 8%, 13%, and 6% higher in the second growing season, respectively. Conversely, the relative leaf water content was higher in the first season (53.56%) compared to the second (49.52%). The total leaf chlorophyll content did not differ in the two seasons, with an average of  $1.49 \text{ mg g}^{-1}$ .

**Table 1.** Effect of the growing season, pruning time, and bio-regulator application, and their interactions on vegetative and physiological parameters of ber trees. For each source of variation, means within each column are significantly different according to the Duncan test ( $p \leq 0.05$ ) if followed by different letters.

Source of Variation	Trunk Diameter Growth (cm)	Canopy Perimeter Growth (m)	Leaf Area ( $\text{cm}^2 \cdot \text{branch}^{-1}$ )	Total Leaf Chlorophyll Content ( $\text{mg} \cdot \text{g}^{-1}$ )	Relative Leaf Water Content (%)
<b>Growing season (GS)</b>					
2018–2019	2.70 ± 0.05 b	8.11 ± 0.13 b	330.3 ± 5.6 b	1.47 ± 0.03 a	53.56 ± 0.67 a
2019–2020	2.92 ± 0.04 a	9.16 ± 0.16 a	351.7 ± 6.5 a	1.51 ± 0.02 a	49.52 ± 0.64 b
Significance	***	***	***	n.s.	***
<b>Pruning time (PR)</b>					
PR-13	2.72 ± 0.07 b	7.63 ± 0.54 c	300.3 ± 5.1 d	1.28 ± 0.02 b	48.22 ± 0.80 d
PR-15	2.90 ± 0.09 a	9.16 ± 0.24 a	383.7 ± 8.3 a	1.60 ± 0.03 a	53.97 ± 1.18 a
PR-17	2.84 ± 0.08 ab	8.97 ± 0.21 ab	362.4 ± 6.3 b	1.56 ± 0.03 a	52.46 ± 0.88 ab
PR-19	2.79 ± 0.07 ab	8.77 ± 0.19 b	317.6 ± 5.2 c	1.53 ± 0.03 a	51.51 ± 0.80 c
Significance	*	***	***	***	***
<b>Bio-regulators (PBR)</b>					
PBR-C	2.12 ± 0.04 c	7.44 ± 0.19 d	315.7 ± 8.6 d	1.33 ± 0.03 c	47.17 ± 1.00 c
PBR-TL	3.01 ± 0.05 ab	9.16 ± 0.23 ab	351.4 ± 9.4 b	1.58 ± 0.04 a	54.02 ± 1.06 a
PBR-TH	3.06 ± 0.05 a	9.39 ± 0.24 a	372.7 ± 10.6 a	1.60 ± 0.04 a	54.57 ± 0.99 a
PBR-SAL	2.96 ± 0.05 ab	8.77 ± 0.21 bc	336.5 ± 8.6 bc	1.49 ± 0.03 b	51.22 ± 0.95 b
PBR-SAH	2.90 ± 0.05 b	8.41 ± 0.19 c	328.4 ± 8.0 cd	1.46 ± 0.03 b	50.70 ± 0.91 b
Significance	***	***	***	***	***
<b>GS × PBR</b>	n.s.	n.s.	n.s.	n.s.	n.s.
<b>GS × PR</b>	n.s.	n.s.	n.s.	n.s.	n.s.
<b>P × PBR</b>	n.s.	n.s.	n.s.	n.s.	n.s.
<b>GS × PR × PBR</b>	n.s.	n.s.	n.s.	n.s.	n.s.

\*, \*\*\*, and n.s. indicate significant differences at  $p \leq 0.05$  and  $p \leq 0.001$  and no significant difference ( $p > 0.05$ ) according to the three-way ANOVA, respectively. SMW = standard meteorological week.

**Table 2.** Effect of the growing season, pruning time, and bio-regulator application and their interactions on fruit yield and quality traits. For each source of variation, means within each column are significantly different according to the Duncan test ( $p \leq 0.05$ ) if followed by different letters.

Source of Variation	Fruit Diameter (cm)	Fruit Yield ( $\text{kg} \cdot \text{tree}^{-1}$ )	TSS ( $^{\circ}\text{Brix}$ )	Titrateable Acidity (%)	Ascorbic Acid ( $\text{mg} \cdot 100\text{g}^{-1}$ )	Total Sugar (%)
<b>Growing season (GS)</b>						
2018–2019	3.02 ± 0.04 b	45.4 ± 0.7 b	17.9 ± 0.3 b	0.41 ± 0.01 a	81.5 ± 1.0 b	9.06 ± 0.14 b
2019–2020	3.19 ± 0.04 a	56.7 ± 1.0 a	19.1 ± 0.3 a	0.35 ± 0.01 b	85.5 ± 1.1 a	10.17 ± 0.14 a
Significance	***	***	***	n.s.	**	***
<b>Pruning time (PR)</b>						
PR-13	3.03 ± 0.06 b	49.3 ± 1.3 c	16.7 ± 0.3 c	0.42 ± 0.01 a	78.7 ± 1.3 b	8.67 ± 0.16 c
PR-15	3.31 ± 0.07 a	56.6 ± 1.8 a	19.6 ± 0.4 a	0.36 ± 0.01 c	86.5 ± 1.8 a	10.38 ± 0.22 a
PR-17	3.10 ± 0.06 b	52.1 ± 1.5 b	19.3 ± 0.3 a	0.37 ± 0.01 b	85.3 ± 1.5 a	10.27 ± 0.18 a
PR-19	2.98 ± 0.05 b	46.2 ± 1.1 d	18.2 ± 0.3 b	0.39 ± 0.01 b	83.5 ± 1.3 a	9.16 ± 0.16 b
Significance	***	***	***	***	***	***
<b>Bio-regulators (PBR)</b>						
PBR-C	2.70 ± 0.06 d	46.4 ± 1.5 c	16.6 ± 0.4 d	0.40 ± 0.02 b	75.4 ± 1.4 c	8.96 ± 0.23 c
PBR-TL	3.28 ± 0.06 ab	54.4 ± 1.8 a	19.4 ± 0.4 ab	0.36 ± 0.01 c	88.0 ± 1.6 a	9.96 ± 0.25 a
PBR-TH	3.34 ± 0.06 a	55.9 ± 1.8 a	19.7 ± 0.4 a	0.36 ± 0.01 c	88.9 ± 1.5 a	10.10 ± 0.25 a
PBR-SAL	3.14 ± 0.05 bc	49.7 ± 1.6 b	18.6 ± 0.4 bc	0.39 ± 0.01 b	83.7 ± 1.4 b	9.63 ± 0.23 ab
PBR-SAH	3.05 ± 0.05 c	48.8 ± 1.5 bc	18.1 ± 0.4 c	0.43 ± 0.01 a	81.5 ± 1.3 b	9.43 ± 0.23 bc
Significance	***	***	***	***	***	***
<b>GS × PBR</b>	n.s.	n.s.	n.s.	***	n.s.	n.s.
<b>GS × PR</b>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<b>P × PBR</b>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<b>GS × PR × PBR</b>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

\*\*, \*\*\*, and n.s. indicate significant differences at  $p \leq 0.01$  and  $p \leq 0.001$  and no significant difference ( $p > 0.05$ ) according to the three-way ANOVA, respectively. SMW = standard meteorological week.

**Table 3.** Effect of the growing season, pruning time and bio-regulator application, and their interactions on percentage of fresh weight loss of ber fruit during 3-, 6-, and 9-day storage. For each source of variation, means within each column are significantly different according to the Duncan test ( $p \leq 0.05$ ) if followed by different letters.

Source of Variation	Fruit Fresh Weight Loss (%)		
	3 Days	6 Days	9 Days
<b>Growing season (GS)</b>			
2018–2019	2.67 ± 0.14 b	5.37 ± 0.13 b	12.06 ± 0.10 b
2019–2020	3.27 ± 0.14 a	6.51 ± 0.12 a	12.95 ± 0.10 a
Significance	***	***	***
<b>Pruning time (PR)</b>			
PR-13	2.67 ± 0.17 c	5.66 ± 0.19 c	12.29 ± 0.15 c
PR-15	3.66 ± 0.20 a	6.60 ± 0.19 a	13.02 ± 0.15 a
PR-17	3.24 ± 0.18 b	6.25 ± 0.18 b	12.74 ± 0.14 b
PR-19	2.31 ± 0.16 c	5.25 ± 0.20 d	11.97 ± 0.15 d
Significance	***	***	***
<b>Bio-regulators (PBR)</b>			
PBR-C	2.18 ± 0.16 d	4.94 ± 0.22 c	11.73 ± 0.16 c
PBR-TL	3.51 ± 0.20 a	6.47 ± 0.20 a	12.92 ± 0.16 a
PBR-TH	3.79 ± 0.18 a	6.71 ± 0.18 a	13.10 ± 0.14 a
PBR-SAL	2.82 ± 0.20 b	5.90 ± 0.19 b	12.47 ± 0.15 b
PBR-SAH	2.57 ± 0.21 bc	5.68 ± 0.21 b	12.30 ± 0.16 b
Significance	***	***	***
<b>GS × PBR</b>	n.s.	n.s.	n.s.
<b>GS × PR</b>	n.s.	n.s.	n.s.
<b>P × PBR</b>	n.s.	n.s.	n.s.
<b>GS × PR × PBR</b>	n.s.	n.s.	n.s.

\*\*\* and n.s. indicate significant differences at  $p \leq 0.001$  and no significant difference according to the three-way ANOVA, respectively. SMW = standard meteorological week.

**Table 4.** Effect of the growing season, pruning time, and bio-regulator application and their interactions on the spoilage of ber fruit during 3-, 6-, and 9-day storage. For each source of variation, means within each column are significantly different according to the Duncan test ( $p \leq 0.05$ ) if followed by different letters.

Source of Variation	Fruit Spoilage (%)		
	3 Days	6 Days	9 Days
<b>Growing season (GS)</b>			
2018–2019	0.00 ± 0.00 a	5.41 ± 0.08 b	11.64 ± 0.44 b
2019–2020	0.00 ± 0.00 a	6.64 ± 0.12 a	12.80 ± 0.45 a
Significance	n.s.	***	***
<b>Pruning time (PR)</b>			
PR-13	0.00 ± 0.00 a	6.57 ± 0.17 a	13.40 ± 0.69 a
PR-15	0.00 ± 0.00 a	5.21 ± 0.14 c	11.40 ± 0.61 c
PR-17	0.00 ± 0.00 a	5.91 ± 0.17 b	11.79 ± 0.61 bc
PR-19	0.00 ± 0.00 a	6.41 ± 0.16 a	12.29 ± 0.62 b
Significance	n.s.	***	***
<b>Bio-regulators (PBR)</b>			
PBR-C	0.00 ± 0.00 a	6.79 ± 0.25 a	18.65 ± 0.41 a
PBR-TL	0.00 ± 0.00 a	5.77 ± 0.18 b	10.52 ± 0.24 b
PBR-TH	0.00 ± 0.00 a	5.72 ± 0.17 b	10.33 ± 0.23 b
PBR-SAL	0.00 ± 0.00 a	5.84 ± 0.18 b	10.70 ± 0.22 b
PBR-SAH	0.00 ± 0.00 a	6.01 ± 0.18 b	10.89 ± 0.24 b
Significance	n.s.	***	***
<b>GS × PBR</b>	n.s.	n.s.	n.s.
<b>GS × PR</b>	n.s.	n.s.	n.s.
<b>P × PBR</b>	n.s.	n.s.	n.s.
<b>GS × PR × PBR</b>	n.s.	n.s.	n.s.

\*\*\* and n.s. indicate significant differences at  $p \leq 0.001$  and no significant difference ( $p > 0.05$ ) according to the three-way ANOVA, respectively. SMW = standard meteorological week.

The pruning time also had a significant effect on the vegetative growth. Pruning on SMW 15 significantly stimulated vegetative growth compared to the earlier pruning time (Table 1). Compared to PR-13, PR-15 induced a 7%, 20%, and 28% increase in the annual trunk diameter growth, canopy perimeter growth, and leaf area per branch, respectively. PR-13 also resulted in the lowest RWC (48.22%). The trees of the other pruning treatments (PR-17 and PR-19) had intermediate values of vegetative traits. In addition, compared to PR-13, leaves of the trees pruned at later times had a higher total chlorophyll content. RWC was highest for tree pruned at SMW 15 (53.97%).

Compared to the control, all the PBR treatments positively influenced the vegetative growth of the trees (Table 1). The differences among the treatments with PBRs were limited. Only the highest dose of SA provided modest, yet significantly lower trunk and canopy growth and a reduced leaf area compared to the two thiourea treatments. Considering all the parameters, higher effects were obtained with both doses of thiourea. For this PBR, the highest dose tested provided a significant difference only in the leaf area compared to the lowest dose (+6%). The differences between the two doses of salicylic acid were not significant. Irrespective of the doses, the thiourea treatments differed from the SA treatment in the physiological parameters under investigation. Specifically, thiourea induced a higher chlorophyll content and RWC in leaves than salicylic acid.

### 3.2. Effects on Fruit Yield and Quality

Consistently with larger growth of the trees, both the fruit diameter at harvest and fruit yield were 6% and 25% higher in the second growing season compared to the first (Table 2). Fruit quality was also higher in the 2019–2020 season. Specifically, fruits had a higher TSS, total sugar, and ascorbic acid amounts and a lower titratable acidity compared to 2018–2019. Additionally, taking into consideration the vegetative growth, all of this suggests that in the second growing seasons, the plants consistently experienced better environmental conditions.

All the fruit parameters under investigation were significantly affected by the pruning time. Pruning on SMW 15 induced the highest fruit yield, and this was associated with increased quality. In PR-15, fruits were the largest and heavier, with higher TSS and total sugar and lower titratable acidity compared to those pruned at the earliest and latest dates (PR-13 and PR-19). The differences in ascorbic acid were more limited, with only PR-13 associated with a significantly lower amount of this water-soluble vitamin in fruits compared to the other pruning times (on average,  $-0.8\%$ ). While PR-15 was associated with the best yield and quality, PR-13 ranked at the bottom for all the parameters but the fruit yield, which was lowest for the latest pruning time.

Bio-regulator application significantly improved fruit yield and quality (larger fruits; increased TSS, total sugar, and ascorbic acid; and lower titratable acidity) compared to the untreated control (Table 2). These positive effects were larger in trees treated with thiourea than those treated with salicylic acid. The dose of application for each bio-regulator did not yield significant differences, but the titratable acidity did (it was higher in PBR-SAH than in PBR-SAL trees. A higher SA dose also induced the highest acidity level in the fruits compared to the control treatment.

### 3.3. Effects on Fruit Shelf-Life

As expected, the relative weight loss of the fresh fruits (RWL) increased over time irrespective of the experimental factors and it was more pronounced between the latest two dates (Table 3). The RWL was higher in the second growing season. Pruning also significantly affected weight loss. The earliest pruning induced the lowest fruit fresh weight loss. This parameter peaked in PR-13, and then, declined to the minimum value for PR-19 (after 9 days: 12.29% in PR-13 compared to 13.02%, 12.74%, and 11.97% in PR-15, PR-17, PR-19, respectively) (Table 3). The application of the bio-regulators also induced an increase in fruit fresh weight loss compared to the control. This negative effect was significantly stronger in trees treated with thiourea (an average of 13.1% after 9 days) than with salicylic

acid (an average of 12.4% after 9 days). The effect of the bio-regulators' application on fruit fresh weight loss was not affected by their dose.

In all the treatments, fruit spoilage was detected for the first time after 6 days of storage under controlled conditions (Table 4). The percentage of spoiled fruit more than doubled after three additional days of storage (Table 4). The highest spoilage percentages were found in the fruit of trees pruned at the earliest and latest dates (after 9 days: 13.40% and 12.29% in PR-13 and PR-19, respectively), whereas the lowest values for this parameter were measured in PR-15 fruits. Independently of the product used and its application dose, the two bio-regulators significantly decreased fruit spoilage compared to control, reaching an interesting decrease of 8% after 9 days.

#### 4. Discussion

The cultivation of ber is becoming increasingly popular in different Asian countries, mainly because of its adaptability to sub-optimal environments and the possibility of obtaining interesting economic results under yield-limiting conditions. Consequently, there is a need for information on technically undemanding horticultural strategies that can improve stress tolerance and hence, production, both in quantitative and qualitative terms. In this work, we aimed to address the potential of two stress-mitigating PGRs. Considering that pruning is a mandatory annual operation that is particularly useful under dry growing conditions [33], we also evaluated the possible interactions between the type and dose of foliar treatments and the time of pruning. An important result was that the two independent technical factors did not interact in influencing the vegetative growth and the fruit quality parameters under investigation. Moreover, their effect was not modulated by the growing season. Likewise, the post-harvest influence of one technical factor was not affected by the level of the second one, and their effects were not influenced by the growing season. All of this indicates that an additive model would be adequate to explain the relationship between the independent experimental factors. A significant main effect was detected for the two technical factors, the time of pruning and the application of PGRs, in the two growing seasons. Collectively considering the vegetative growth, yield, and fruit quality, the best performances were obtained using the PR-15 treatment. This result deepened an early report limited to plant height and length of primary branches [34], and broadened our knowledge by also addressing fruit quality and post-harvest spoilage. Early pruning (PR-13, March) may result in the loss of reserve material and, consequently, it may negatively affect the subsequent vegetative growth, probably also altering the following sink–source relationship during fruit development [35,36]. The improved performance in terms of the vegetative growth of ber pruned in early April may be attributed to the promotion of bud sprouting in the presence of adequate resources for subsequent phenological states. Late pruning may result in a delay in bud sprouting [14,37]. This may be associated with a more limited period for vegetative growth, which also has an influence on the reproductive phase. This can account for the lowest fruit yield observed in PR-19. A direct relationship between vegetative growth and fruit size and yield was previously described in ber [38]. The reason for the better yield, yield components, and quality parameters for the April pruning could be related to an optimal fruit setting [14,37]. It is well established that late pruning induces delays in flowering. As a consequence, an appropriate pruning time could lead to more time for fruit development on the tree when unsuitable environmental conditions are expected to limit the fruiting season, thus allowing fruits to reach their maximum growth potential. On the contrary, in late-pruned trees, the delayed and reduced vegetative re-growth, as well as the delayed onset of flowering, result in a shorter duration of the interval between flowering and fruit maturation. Fruit spoilage was also significantly lower in trees pruned in April compared to those pruned in late March or May. This may be due to more appropriate fruit maturation, as indirectly indicated by a higher sugar-to-TAA ratio [39]. Considering that the earliest and latest pruning times tested provided the least interesting results, it is also unlikely that the examination of an extended calendar for pruning will provide horticulturally valuable results. Our research is consistent with

previous studies that indicated that April is the optimal pruning time for ber in Indian tropical regions, where ber cultivation is diffused [14,40,41]. Our work adds that this period can also guarantee superior fruit quality in terms of post-harvest spoilage. Finally, it is always necessary to add that the pruning time of ber must be adjusted in arid conditions, mainly considering a monthly rainfall analysis [42].

Plant bio-regulators can provide significant advantages when used appropriately because they influence the physiological processes involved in the stress response to harsh environmental conditions. The application of either bio-regulators, thiourea, or SA, significantly improved most of the variables under investigation compared to the untreated control. Regardless of the applied concentrations, thiourea provides more interesting results than SA. Specifically, thiourea better promoted the attributes related to tree growth (leaf area and canopy spread), physiological traits (chlorophyll and RWC), fruit chemicals (TSS, ascorbic acid, and total sugar), and yield (per fruit, plant, and ha), as well as shelf-life (less PLW and fruit spoilage). Although the difference between doses was limited, the most prominent effect was with application at 1000 ppm. It is known that the application of thiourea increases chlorophyll content [43] and photosynthetic efficiency [20], which are main factors in sustaining the wide-ranging positive effects recorded for this treatment. Moreover, the high relative water content in leaves can be explained by considering that thiourea affects different physiological parameters to ameliorate the plant response to drought- and heat-stress [44]. For instance, the application of thiourea increased the relative leaf water content in sunflower leaves [45]. In addition to the quantitative aspects, thiourea also increased fruit quality. This PGR affects the carbohydrate metabolism, protein synthesis, and neutralization of organic acids [45,46]. For example, Thiourea increased TSS in garlic and mango [47,48] and carbohydrate in wheat [49]. It is notable that the foliar application of PGRs played a beneficial role in improving the ascorbic acid content of the fruits. Ascorbic acid is an essential nutrient for humans, and fruits and vegetables are the main dietary source [50]. The highest increases in total ascorbic acid were obtained using thiourea, irrespective of the dose. Previous works also indicate that this PGR increases the accumulation of ascorbic acids in edible products [47,51].

The difference between thiourea and SA could be due to several factors, which may include both direct (bio)chemical interactions of the compounds [52,53] and specific plant physiological alterations [44,54]. The application of SA also had a positive influence on most of the variables under study; however, its influence was not as effective as that of thiourea. Considering its endogenous role in plant physiology [55], it is likely that the foliar application of SA promoted vegetative growth, yield, and other physiological parameters because of its ability to mitigate environmental stress. Statistical differences between the doses were also limited because the tested doses did not encompass ample differences in concentration. Overall, the lower dose of SA provided the most suitable results. It should be added that an excessive dose of SA is typically associated with less beneficial effects. For example, SA treatments at similar concentrations (100 and 200 ppm) had a positive effect on the vegetative growth parameters, chlorophyll content, TSS, and yield in maize compared to higher concentrations (400 ppm) [56].

## 5. Conclusions

This study allowed us to conclude that pruning in the first half of April is the most appropriate for ber in the semi-arid conditions of Rajasthan. PR-15 provided the best performance in terms of tree growth, fruit yield, and compositional traits (i.e., the highest total sugar-to-titratable acidity ratio). Quality wise, it is worth noting the strong increase in ascorbic acid, a molecule with well-known dietary importance. Moreover, the positive influence on the reduction in fruit weight loss and spoilage represents the added value of selecting the appropriate pruning time. Our study also allowed us to highlight the wide-ranging effects pre- and post-harvest of the foliar spray of thiourea. According to our statistical analysis, its implementation is expected to provide additional benefits irrespective of the cultural practices and growing seasons. More generally, it is notable

that the effects of the two technical factors (pruning and the application of PGRs) did not display significant interactions, and neither was different in the two successive growing seasons; however, we cannot exclude that more extreme doses, different PGRs, and an extended calendar of pruning in other varieties may yield a different outcome.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae8090809/s1>, Figure S1: Minimum and maximum air temperatures and relative air humidity between March 2018 and February 2019 (A) and between March 2019 and February 2020 (B).

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