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Research article

# Kernel size and weight affected by three plant bioregulators applied at bloom to Non Pareil and Carmel almond cultivars

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ABSTRACT

*Background:* The yield of almonds [*Prunus dulcis* (Mill.) D.A. Webb] could be low due to climatic problems and any factor improving kernel size and weight, such as the use of plant bioregulators (PBRs), should be beneficial.
*Results:* Three plant bioregulators: 24-epibrassinolide (BL), gibberellic acid ($GA_3$) and kinetin (KN) were applied at three spray concentrations to Non Pareil and Carmel cultivars, at two phenological stages during bloom, in the 2014 and 2015 seasons. The results showed significant differences ($P < 0.0001$). For total dry weight of Non Pareil, the best treatment was BL (30 mg·$L^{-1}$), with an average of 1.45 g, while the control was 1.30 g, at pink button during 2015. For Carmel, the best dry weight was 1.23 g, achieved with BL (30 mg·$L^{-1}$) at fallen petals in both seasons. The average dry weight of the controls varied between 1.13 and 1.18 g. The greatest almond lengths and widths in Non Pareil were 24.98 mm and 15.05 mm, achieved with BL (30 mg·$L^{-1}$) and KN (50 μL·$L^{-1}$) treatments, respectively, applied at pink button in 2015. In Carmel, the greatest length and width were 24.38 and 13.44 mm, obtained with BL (30 mg·$L^{-1}$) applied at the stages of pink button and fallen petals, respectively, in 2015. The control reached lengths between 22.33 and 23.38 mm, and widths between 11.99 and 12.93 mm.
*Conclusions:* The use of the bioregulators showed significant favorable effects on dry weight, length and width of kernels at harvest, in both cultivars.

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## 1. Introduction

The almond [*Prunus dulcis* (Miller) D.A. Webb] is a fruit species with a low chilling requirement, thus it begins flowering early in the season, coinciding with the end of winter, and consequently, the low temperatures, mists and rains typical in this period, which can have negative effects on pollination, fruit set and yield [1]. It is also important to consider that almond flowers are mostly self-incompatible and thus require cross-pollination [2]. For this reason, almond orchards should be planted with at least two intercompatible cultivars with simultaneous bloom, and will also require pollinators to transfer the pollen [3]. Pollination can be limiting in some areas of production; for example, in Chile, years with irregular spring weather conditions can have fruit set percentages of less than 20%, which has a direct impact on yields [4].

The unfavorable weather conditions during the period of almond flowering, in addition to the lack of adequate technology in managing the cultivars, can in some cases lead to very low yields in Chile, averaging 1400 kg $ha^{-1}$ of kernel, compared with average almond yields in California that easily reach 2800 kg·$ha^{-1}$ [5].

Pollen germination and fertility are affected by environmental factors such as light, temperature and relative humidity. The processes of seed and fruit development are intimately connected and synchronized, and are regulated by phytohormones [6]. Nevertheless, in contrast to the fruit, which can develop in the absence of pollination, seed development is strictly dependent upon successful fertilization. The development of the seed includes both the production of endosperm and the growth of the embryo, both of which have been shown to be under multihormonal regulation by auxins, cytokinins, gibberellins and brassinosteroids [7]. Successful fertilization is particularly important in the case of optimal yield for almonds, as the useful part of the fruit is the seed [8].

Cytokinins are a group of plant hormones that regulate cell division and influence numerous physiological processes of plant development, including: leaf senescence, vascular development, cell differentiation in apical meristems of shoots and roots, distribution and consumption of nutrients, responses to biotic and abiotic stress, regulation of source–sink relationships, and recent studies have also revealed that cytokinin is a key regulator in seed production [9].

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The principal physiological function of gibberellins in higher plants is the stimulation of organ growth by increasing cellular elongation and in some cases, cell division. In addition, gibberellins promote certain changes between seed dormancy and germination, juvenile and adult growth stages, and vegetative and reproductive development. In the performance of their functions, gibberellins act in response to both developmental and environmental signals [10]. Gallego-Giraldo et al. [11] also indicated that gibberellins are a key factor for fruit set and fruit development.

Brassinosteroids act on all parts of the plant, including the roots. These hormones provoke a wide range of physiological responses, including stem elongation, pollen tube growth, epinasty and leaf bending, inhibition of root growth, inducing ethylene synthesis, activating proton pumps, xylem differentiation, photosynthesis, synthesis of proteins and nucleic acids and activation of enzymes. In addition, brassinosteroids are also recognized as having a palliative role in plants that are subjected to abiotic or biotic stressors [12].

Phytohormones play a crucial role in modulating multiple developmental processes and cellular responses to stress of all kinds. Various natural plant hormones, such as gibberellins, salicylic acid, ethylene and brassinosteroids have been associated with cold stress responses in fruit [13].

Currently, the discovery and use of chemical substances that can replace or imitate the action of plant hormones, called plant bioregulators, has allowed growers to correct some deficiencies in order to prevent economic losses [14]. Plant bioregulators were rapidly identified as a means of improving yield, quality and post-harvest shelf-life, and have reached their greatest impact in the area of fruit production [15].

Swain et al. [16] suggested that gibberellins, presumably $GA_1$ and $GA_3$ synthesized in the embryo and/or endosperm, were required for the development of the seeds in the first days after fertilization. The application of gibberellins, and consequently, elevated levels of endogenous gibberellins, were causally associated with early growth of fruitlets and of mature fruit in *Pyrus pyrifolia* [17]. Gibberellic acid ($GA_3$), improved almond fruit set, and maximum fruit retention was observed with a concentration of 200 ppm [18]. Swain et al. [16] suggested two models for explaining the role of gibberellins in the distribution of assimilates; gibberellins could directly promote the consumption of assimilates for the development of seeds, or they could act indirectly through changes in the growth of seeds.

Exogenous application of synthetic cytokinins can induce fruit set and fruit development in various fruit crop species [19]. Zhao et al. [9], evaluated the effect of cytokinins in cotton, finding that a moderate concentration promoted the development of seeds, but an overdose inhibited their development. The application of adequate concentrations promoted seed development and increased seed size. Applications of kinetin, a synthetic cytokinin, in transgenic male-sterile tobacco plants resulted in normal fertilization and development of seeds [20].

In experiments with live plant cells, Vogler et al. [21] observed a five-fold increase in the rates of cell elongation when germination media were supplemented with 10 μM of epibrassinolide. Treatment of crops such as rice, tomato, corn and cucumber with brassinosteroids improved their resistance to low temperatures [12]. Alternatively, Thussagunpanit et al. [22] suggested that the application of brassinosteroids should be useful in increasing rice yields under high temperature conditions in the field.

This study evaluated the effects of three plant bioregulators (currently on the market) on the yields of Non Pareil and Carmel almonds, in an orchard in the area of Paine, in central Chile.

## 2. Materials and methods

This study was carried out during the 2013–2014 and 2014–2015 growing seasons in a commercial almond (*Prunus dulcis*) orchard located in the area of Paine, Metropolitan Region, in central Chile (latitude 33°46′21.3″ S – longitude 70°38′12.5″ W). This experiment evaluated the almond yields, in full production, from 10-year-old Non Pareil and Carmel cultivars grafted onto Nemaguard rootstocks, and planted at 6 m × 4 m. For the treatment applications, 12 trees of each cultivar were selected from alternating rows in a North–South direction.

### 2.1. Treatments

Three bioregulators that are currently on the market were applied to the trees to evaluate their effects on dry weight, length and width of kernels from Non Pareil and Carmel almond trees in the field:

a) Brassinolide 0.1%, wettable powder (WP), with the active ingredient 24-Epibrassinolide (chemical formula: 22R,23R,24R)-2α,3α,22,23-tetrahydroxy-24-methyl-β-homo-7-oxa-5-cholestan-6-one) made and marketed by Green Plantchem Company Limited, in the Republic of China.
b) ProGibb® 4%, soluble concentrate (SL), with the active ingredient Gibberellic Acid ($GA_3$) 3.2% *w/v* (chemical formula: 3S, 3aR, 4S, 4aS, 7S, 9aR, 9bR, 12S)-7, 12- dihydroxy-3-methyl-6-methylene-oxoperhydro-4, 7-methano-9b, 3-propenoazuleno (1,2-b) furan-4-carboxylic acid), made by Valent BioSciences Corporation, in the USA, and imported and distributed by Valen BioSciences Chile S.A.
c) X-Cyte®, soluble concentrate (SL), whose active ingredient is a cytokinin, kinetin, at a concentration of 0.04% *w/v* (chemical formula: 6-furfurylamino-9H-purine), made by Stoller Enterprises Inc. in the USA, and imported and distributed by Stoller Chile S.A.

These three plant bioregulators (PBRs): Brassinolide (BL), ProGibb® ($GA_3$) and X-Cyte® (KN) were sprayed at concentrations of 10, 30 and 50 $mg \cdot L^{-1}$, in the case of BL which is a wettable powder, and 10, 30 and 50 $\mu L \cdot L^{-1}$, in the case of $GA_3$ and KN, which are concentrated solutions. All of the concentrations used in this study correspond to commercially available products.

This experiment followed a random block design with 12 repetitions. For the treatment applications, 12 trees of each cultivar were selected, and then from each of these trees, 20 uniform branches from the middle of the canopy were selected. Ten of the branches were then selected at random to apply the treatments at the phenological stage of pink button, and 10 for treatment application at petal fall. The phenological stages were selected using the floral development scale for almonds proposed by Yi et al. [2], when 50% of the flowers were found to be at the proposed phenological stages used in this study [23].

Of the selected branches, treatments were assigned at random, including the control treatments, which consisted of spraying with water only. Each branch was considered an experimental unit.

### 2.2. Almond dry weight

When the almonds were found to be ripe, at least 80% open mesocarp (hull) [24], they were then harvested and brought to the Deciduous Fruit Crops Laboratory at the Campus of Agronomy and Forestry, of the Pontificia Universidad Católica de Chile. The fruit was then weighed (fresh weight), having been separated from the mesocarp and shell to obtain the almond seeds. Fresh weights were recorded for 10 kernels selected at random from each repetition (120 kernels per treatment). The kernels were then dried at 72°C for 24 h, until reaching a constant dry weight. After this process, the dry weight of the kernels was then recorded. All weighing was done using a digital scale, calibrated to approximately 0.01 g (Radwag® MAC 50/1/WH, Poland).

### 2.3. Almond length and width

After the drying and weighing process, length (polar diameter) and width (equatorial diameter) were measured for 10 kernels selected at random from each repetition, for a total of 120 kernels per treatment. Measurements were done using a digital caliper with a precision of 0.01 mm.

### 2.4. Statistical analysis

An analysis of variance was done for all of the variables evaluated. The averages were compared using a Tukey–Kramer test at $\alpha = 0.05$. The statistical analysis was done using SAS (SAS v.9.1.3) software.

## 3. Results and discussion

### 3.1. Almond dry weight

Almond dry weight results correspond to the average values obtained from the 120 almonds selected at random from each treatment. The application of the PBRs in Non Pareil showed statistically significant differences ($P < 0.0001$) at the two phenological stages in both years (Table 1). The KN (50 $\mu L \cdot L^{-1}$) treatment had the highest kernel dry weight in the 2014 season at both phenological stages, with 1.33 g at pink button, and 1.31 g at fallen petals. Similar studies have shown that application of cytokinins improved seed yield in cotton [9], and also improved seed yield in *Arabidopsis thaliana* [25]. The control dry weights only reached 1.27 g at both phenological stages. It is important to note the effect of the treatment with BL (30 $mg \cdot L^{-1}$) was statistically equivalent to the KN (50 $\mu L \cdot L^{-1}$) treatment, reaching 1.32 g at pink button and 1.31 g at fallen petals. This is in agreement with Zhu et al. [26] who indicated that brassinosteroids also increased dry weight in rice grains. $GA_3$ at low and medium concentrations significantly improved almond dry weight with 1.32 g, and 1.31 g, in the phenological stages of pink button and fallen petals, respectively. This agrees with Huang et al. [27], who improved the weight of rapeseed (*Brassica napus*) with $GA_3$ application.

For Non Pareil almonds in 2015, the BL treatment at a concentration of 30 $mg \cdot L^{-1}$ significantly increased dry weight, reaching 1.45 g, and 1.43 g, in the phenological stages of pink button and fallen petals, respectively. Similar studies have reported that brassinosteroids improved seed-filling in rice and consequently its weight [28]. The treatments with KN at a high concentration (50 $\mu L \cdot L^{-1}$) and $GA_3$ at a low concentration (10 $\mu L \cdot L^{-1}$), significantly improved the dry weight of the almonds at both phenological stages used in this study. The control reached 1.30 g, and 1.34 g average dry weight, at the phenological stages of pink button and fallen petals, respectively. Besides, statistical analysis for kernel dry weight of Non Pareil almond cultivar showed statistically significant differences for phenological stage, bioregulators and concentrations ($P < 0.0000$) and interactions phenological stage × bioregulators, bioregulators × concentrations and phenological stage × bioregulators × concentrations (Table 2).

**Table 2**
Statistical analysis for kernel dry weight of Non Pareil almond cultivar 2015 season.

| Source of variation | Sum of squares | Degrees of freedom | Mean square | F-value | *P*-value |
|---|---|---|---|---|---|
| Total | 0.3200 | 227 | | | |
| Blocks | 0.0022 | 11 | 0.0002 | 0.5700 | 0.8506 |
| Phenological stage (PS) | 0.0400 | 1 | 0.0400 | 117.6471 | 0.0000 |
| Bioregulators (B) | 0.0100 | 2 | 0.0100 | 29.4118 | 0.0000 |
| Concentrations (C) | 0.0100 | 2 | 0.0100 | 29.4118 | 0.0000 |
| PSxB | 0.0032 | 2 | 0.0016 | 4.7059 | 0.0101 |
| PSxC | 0.0018 | 2 | 0.0009 | 2.5882 | 0.0777 |
| BxC | 0.0900 | 4 | 0.0200 | 58.8235 | 0.0000 |
| PSxBxC | 0.0100 | 4 | 0.0013 | 3.8235 | 0.0051 |
| Factors vs control | 0.0900 | 1 | 0.0900 | 261.5700 | 0.0001 |
| Error | 0.0700 | 198 | 0.0003 | | |

For the Carmel cultivar, the application of the PBRs showed statistically significant differences ($P < 0.0001$) on the variable of almond dry weight at both phenological stages, in both growing seasons (Table 3). The KN (50 $\mu L \cdot L^{-1}$) treatment had the highest dry weight in 2014 at both phenological stages, with 1.19 g at pink button and 1.23 g at fallen petals. This is similar to results of Bartrina et al. [25] who reported that cytokinins improved yield of seeds in *A. thaliana*. The control dry weights reached 1.13 g, and 1.18 g, at the phenological stages of pink button, and fallen petals, respectively. It is important to note that the effect of the BL (30 $mg \cdot L^{-1}$) treatment was statistically equivalent to the KN (50 $\mu L \cdot L^{-1}$) treatment, reaching 1.18 g at pink button and 1.23 g at fallen petals. $GA_3$ at high concentrations reached its highest dry weights, with 1.18 g, and 1.22 g, in the phenological stages pink button and fallen petals, respectively. Similarly, Rastogi et al. [29] reported improved dry weight in linseeds (*Linum usitatissimum*) with the application of $GA_3$.

For the Carmel cultivar in 2015, the $GA_3$ treatment at a high concentration (50 $\mu L \cdot L^{-1}$) significantly increased almond dry weight, reaching 1.23 g at pink button, while the BL treatment at the medium concentration (30 $mg \cdot L^{-1}$) reached 1.23 g at the phenological stage of fallen petals. It is also important to note the effect of the BL (30 $mg \cdot L^{-1}$) treatment which reached 1.22 g, and KN (30 $\mu L \cdot L^{-1}$ and 50 $\mu L \cdot L^{-1}$) that also reached 1.22 g at the phenological stage of pink button, and $GA_3$ (30 $\mu L \cdot L^{-1}$ and 50 $\mu L \cdot L^{-1}$) that reached 1.22 g at the stage of fallen petals. The control reached average dry weights of 1.15 g, and 1.17 g, at the phenological stages of pink button, and fallen petals, respectively. Additionally, statistical analysis for kernel dry weight of Carmel almond cultivar showed statistically significant differences for bioregulators and concentrations ($P < 0.0008$) and

**Table 1**
Effect of plant bioregulators on almond dry weight (g) in the Non Pareil cultivar at two phenological stages, during both growing seasons (n = 120 almonds).

| Treatments | Pink button | | | | Fallen petals | | | |
|---|---|---|---|---|---|---|---|---|
| | 2014 | | 2015 | | 2014 | | 2015 | |
| Control | 1.27 | c | 1.30 | f | 1.27 | b | 1.34 | e |
| BL 10 $mg \cdot L^{-1}$ | 1.29 | bc | 1.39 | cde | 1.28 | ab | 1.37 | bcde |
| BL 30 $mg \cdot L^{-1}$ | 1.32 | a | 1.45 | a | 1.31 | a | 1.43 | a |
| BL 50 mg $L^{-1}$ | 1.29 | bc | 1.41 | abcd | 1.29 | ab | 1.36 | de |
| $GA_3$ 10 $\mu L \cdot L^{-1}$ | 1.32 | ab | 1.43 | ab | 1.29 | ab | 1.39 | bc |
| $GA_3$ 30 $\mu L \cdot L^{-1}$ | 1.31 | ab | 1.40 | bcd | 1.31 | a | 1.37 | cde |
| $GA_3$ 50 $\mu L \cdot L^{-1}$ | 1.30 | abc | 1.38 | de | 1.29 | ab | 1.35 | de |
| KN 10 $\mu L \cdot L^{-1}$ | 1.29 | bc | 1.36 | e | 1.27 | b | 1.37 | cde |
| KN 30 $\mu L \cdot L^{-1}$ | 1.31 | ab | 1.40 | bcd | 1.28 | ab | 1.37 | bcd |
| KN 50 $\mu L \cdot L^{-1}$ | 1.33 | a | 1.42 | abc | 1.31 | a | 1.40 | b |

Means followed by the same letter are not statistically different according to the Tukey–Kramer test ($P \leq 0.05$).

**Table 3**
Effect of plant bioregulators on almond dry weight (g) in the Carmel cultivar at two phenological stages, during both growing seasons (n = 120 almonds).

| Treatments | Pink button | | | | Fallen petals | | | |
|---|---|---|---|---|---|---|---|---|
| | 2014 | | 2015 | | 2014 | | 2015 | |
| Control | 1.13 | d | 1.15 | e | 1.18 | d | 1.17 | d |
| BL 10 $mg \cdot L^{-1}$ | 1.18 | ab | 1.21 | abc | 1.21 | abcd | 1.19 | cd |
| BL 30 $mg \cdot L^{-1}$ | 1.18 | ab | 1.22 | ab | 1.23 | a | 1.23 | a |
| BL 50 $mg \cdot L^{-1}$ | 1.16 | abc | 1.20 | bcd | 1.22 | ab | 1.22 | abc |
| $GA_3$ 10 $\mu L \cdot L^{-1}$ | 1.14 | cd | 1.18 | cd | 1.19 | cd | 1.19 | bcd |
| $GA_3$ 30 $\mu L \cdot L^{-1}$ | 1.15 | bcd | 1.21 | abc | 1.22 | abc | 1.22 | abc |
| $GA_3$ 50 $\mu L \cdot L^{-1}$ | 1.18 | ab | 1.23 | a | 1.22 | abc | 1.22 | ab |
| KN 10 $\mu L \cdot L^{-1}$ | 1.16 | abc | 1.18 | d | 1.19 | bcd | 1.18 | d |
| KN 30 $\mu L \cdot L^{-1}$ | 1.19 | a | 1.22 | ab | 1.21 | abcd | 1.19 | bcd |
| KN 50 $\mu L \cdot L^{-1}$ | 1.19 | a | 1.22 | ab | 1.23 | a | 1.21 | abc |

Means followed by the same letter are not statistically different according to the Tukey–Kramer test ($P \leq 0.05$).

interactions bioregulators × concentrations and phenological stage × bioregulators × concentrations (Table 4).

At present, no experimental reports have been published on the application of BL, $GA_3$, or KN in order to improve almond weight in the Non Pareil and Carmel cultivars.

### 3.2. Almond length and width

The PBR treatments showed a statistically significant effect on the variables of almond length and width at harvest, in both the Non Pareil and Carmel cultivars, when applied at the phenological stages of pink button and fallen petals, in the 2014 and 2015 growing seasons.

For the Non Pareil cultivar in 2014, statistically significant differences ($P < 0.0001$) can be observed between the treatments at both phenological stages for the two variables. The KN treatment at a concentration of 50 $\mu L \cdot L^{-1}$ induced the greatest lengths (24.04 mm, and 24.20 mm, at pink button, and fallen petals, respectively) and the greatest widths (13.94 mm, and 14.09 mm, at pink button, and fallen petals, respectively) of almonds at harvest, showing a trend of increasing length and width of almonds with increasing concentrations. This agrees with the study by Zhao et al. [9] who improved the size of cotton seeds with the application of cytokinins. The treatment with BL at a concentration of 30 $mg \cdot L^{-1}$ showed values that were statistically equivalent to KN (50 $\mu L \cdot L^{-1}$), including the trend of improved response with the intermediate concentration. Zhu et al. [26] also reported increased seed size in rice with the application of brassinosteroids. The $GA_3$ treatments showed a positive effect on the length and width of almonds, with a better response of the two variables to the lower concentrations at both phenological stages. This is in agreement with Rastogi et al. [29] and Huang et al. [27] who improved seed size with the application of $GA_3$. All of the treatments were larger than the control, which only reached 23.23 mm in length, and 13.31 mm in width, at the phenological stage of pink button, and 23.49 mm in length, and 13.52 mm in width, at fallen petals.

In 2015, the effect of the PBRs on the length and width of almonds at harvest in the Non Pareil cultivar was similar to that observed in 2014 (Table 5), showing statistically significant differences ($P < 0.0001$) between treatments at the two phenological stages for both variables. The treatment with KN at a concentration of 50 $\mu L \cdot L^{-1}$ was outstanding, similar to the results of Zhao et al. [9] in cotton seeds; the BL treatment at a concentration of 30 $mg \cdot L^{-1}$ was also outstanding, maintaining the trends of 2014 of increasing almond length and width with increasing concentrations in KN, and also in the case of BL, having the best response the intermediate concentration. All of the PBR-treated almonds were larger than the controls.

For the Carmel cultivar in 2014, statistically significant differences ($P < 0.0001$) were shown between the treatments for the two variables at the two phenological stages. The application of KN at a concentration of 50 $\mu L \cdot L^{-1}$ stimulated the greatest length (23.54 mm, and 23.53 mm, at pink button, and fallen petals, respectively) and the greatest width (12.53 mm, and 12.67 mm, at pink button, and fallen petals, respectively) of almonds at harvest, showing a trend of increasing length and width of almonds with increasing concentrations. This agreed with the results of Zhao et al. [9] who also improved the size of seeds in cotton with the application of cytokinins. The application of BL at a concentration of 30 $mg \cdot L^{-1}$ showed values that were statistically equivalent to those of KN (50 $\mu L \cdot L^{-1}$), maintaining the trend of the best response at the intermediate concentration. The treatments with $GA_3$ showed a positive effect on length and width of almonds, with the best response seen at the higher concentrations for both variables at both phenological stages. This was different from the results observed in Non Pareil, where the best response was seen at lower concentrations of $GA_3$. These results are similar to those reported by Huang et al. [27] who improved rapeseed (*B. napus*), and Rastogi et al. [29] who improved linseed (*L. usitatissimum*), with the application of $GA_3$. The average values of the control were 22.33 mm in length, and 11.99 mm in width, at the phenological stage of fallen petals, and 22.69 mm in length and 12.19 mm in width, at the phenological stage of pink button. All of the PBR-treated almonds were larger than the controls.

For the Carmel cultivar in 2015, the effect of the PBRs on almond length and width at harvest showed statistically significant differences ($P < 0.0001$) between the treatments for both variables, at both phenological stages (Table 6). The application of BL at a concentration of 30 $mg \cdot L^{-1}$ stimulated the greatest length (24.38 mm, and 24.32 mm, at pink button, and fallen petals, respectively) and the greatest width (13.34 mm, and 13.44 mm, at pink button, and fallen petals, respectively) of almonds at harvest, demonstrating a trend of increased length and width of the almonds at the intermediate concentration. This agrees with the report by Zhu et al. [26] of

**Table 4**
Statistical analysis for kernel dry weight of Carmel almond cultivar 2015 season.

| Source of variation | Sum of squares | Degrees of freedom | Mean square | F-value | *P*-value |
|---|---|---|---|---|---|
| Total | 0.1800 | 227 | | | |
| Blocks | 0.0100 | 11 | 0.0010 | 2.8800 | 0.0016 |
| Phenological stage (PS) | 0.0003 | 1 | 0.0003 | 0.7714 | 0.3808 |
| Bioregulators (B) | 0.0100 | 2 | 0.0026 | 7.4286 | 0.0008 |
| Concentrations (C) | 0.0400 | 2 | 0.0200 | 57.1429 | 0.0000 |
| PSxB | 0.0033 | 2 | 0.0017 | 4.8571 | 0.0087 |
| PSxC | 0.0001 | 2 | 0.0001 | 0.1914 | 0.8259 |
| BxC | 0.0100 | 4 | 0.0019 | 5.4286 | 0.0004 |
| PSxBxC | 0.0100 | 4 | 0.0024 | 6.8571 | 0.0000 |
| Factors vs control | 0.0400 | 1 | 0.0400 | 105.3300 | 0.0001 |
| Error | 0.0700 | 198 | 0.0004 | | |

**Table 5**
Effect of the plant bioregulators on length and width (mm) of Non Pareil almonds at two phenological stages in the 2015 (n = 120 almonds).

| Treatments | Pink button | | | | Fallen petals | | | |
|---|---|---|---|---|---|---|---|---|
| | Length | | Width | | Length | | Width | |
| Control | 23.91 | d | 14.71 | d | 23.36 | c | 14.21 | c |
| BL 10 $mg \cdot L^{-1}$ | 24.14 | cd | 14.87 | abcd | 23.63 | bc | 14.43 | abc |
| BL 30 $mg \cdot L^{-1}$ | 24.98 | a | 15.04 | a | 24.18 | a | 14.62 | ab |
| BL 50 $mg \cdot L^{-1}$ | 24.19 | bcd | 14.83 | cd | 23.72 | abc | 14.35 | bc |
| $GA_3$ 10 $\mu L \cdot L^{-1}$ | 24.59 | abc | 15.02 | ab | 23.97 | ab | 14.46 | abc |
| $GA_3$ 30 $\mu L \cdot L^{-1}$ | 24.38 | abcd | 14.92 | abc | 23.59 | bc | 14.42 | abc |
| $GA_3$ 50 $\mu L \cdot L^{-1}$ | 24.23 | bcd | 14.90 | abc | 23.39 | c | 14.25 | c |
| KN 10 $\mu L \cdot L^{-1}$ | 24.31 | bcd | 14.85 | bcd | 23.64 | bc | 14.40 | abc |
| KN 30 $\mu L \cdot L^{-1}$ | 24.53 | abcd | 14.96 | abc | 23.84 | abc | 14.43 | abc |
| KN 50 $\mu L \cdot L^{-1}$ | 24.79 | ab | 15.05 | a | 24.20 | a | 14.64 | a |

Means followed by the same letter are not statistically different according to the Tukey–Kramer test ($P \leq 0.05$).

**Table 6**
Effect of plant bioregulators on length and width (mm) of Carmel almonds at two phenological stages in the 2015 (n = 120 almonds).

| Treatments | Pink button | | | | Fallen petals | | | |
|---|---|---|---|---|---|---|---|---|
| | Length | | Width | | Length | | Width | |
| Control | 23.20 | d | 12.78 | c | 23.38 | c | 12.93 | c |
| BL 10 $mg \cdot L^{-1}$ | 24.10 | ab | 13.15 | ab | 24.00 | ab | 13.17 | abc |
| BL 30 $mg \cdot L^{-1}$ | 24.38 | a | 13.34 | a | 24.32 | a | 13.44 | a |
| BL 50 $mg \cdot L^{-1}$ | 23.53 | cd | 12.92 | bc | 24.09 | ab | 13.31 | abc |
| $GA_3$ 10 $\mu L \cdot L^{-1}$ | 23.77 | bc | 13.01 | abc | 23.82 | abc | 13.29 | abc |
| $GA_3$ 30 $\mu L \cdot L^{-1}$ | 24.07 | ab | 13.17 | ab | 24.10 | a | 13.37 | ab |
| $GA_3$ 50 $\mu L \cdot L^{-1}$ | 24.28 | a | 13.31 | a | 24.15 | a | 13.41 | ab |
| KN 10 $\mu L \cdot L^{-1}$ | 23.68 | bcd | 13.12 | abc | 23.57 | bc | 13.02 | bc |
| KN 30 $\mu L \cdot L^{-1}$ | 23.99 | abc | 13.26 | ab | 23.86 | abc | 13.26 | abc |
| KN 50 $\mu L \cdot L^{-1}$ | 24.30 | a | 13.30 | a | 24.24 | a | 13.42 | ab |

Means followed by the same letter are not statistically different according to the Tukey–Kramer test ($P \leq 0.05$).

increased seed size in rice with the application of brassinosteroids. It is important to note that the treatments with KN (50 μL·L$^{-1}$), and also $GA_3$ (50 μL·L$^{-1}$), showed values that were statistically equivalent to BL (30 mg·L$^{-1}$).

At present, no experimental studies have been published on the application of BL, $GA_3$, and KN in order to improve almond size in the Non Pareil and Carmel cultivars.

## 4. Conclusion

The three plant bioregulators used in this study, brassinolide (BL), gibberellic acid ($GA_3$) and X-Cyte (KN), applied at the three concentrations during bloom, in the 2013–2014 and 2014–2015 growing seasons, significantly improved dry weight, length and width of kernels of both the Non Pareil and Carmel almond cultivars compared with the controls. A clear trend was shown by KN at the highest concentration (50 μL·L$^{-1}$) of significantly improving dry weight, length and width of almonds at harvest. The intermediate BL concentration (30 mg·L$^{-1}$) also significantly improved dry weight, length and width of almonds at harvest. In the case of $GA_3$, for the Non Pareil cultivar, the lower concentrations showed a positive effect on the three variables at both phenological stages in both years, while in the Carmel cultivar, the best response was seen at the highest concentrations of this bioregulator.

## Conflict of interest

The authors declare that they have no conflict of interest.

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