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Article

# Effects of paclobutrazol on stomatal density and height in *Chenopodium quinoa*

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## Abstract

Paclobutrazol (PBZ) is a growth retardant that, in addition to reducing plant height, may increase stomatal density and pore area. This research evaluated the effects of five PBZ concentrations on the number of stomata, stomatal pore area, plant height, and the visual appearance of *Chenopodium quinoa* cv. Suyana and Tunkahuan. The experiment was conducted from July to December 2024 at the Iztacala Faculty of Higher Studies, UNAM. Sowing was carried out in 50 pots containing 2.5 L of peat moss:expanded perlite (1:1). After 25 days, thinning was performed, and the following treatments were applied foliarly: 0, 25, 50, 100 and 200 mg $L^{-1}$ of PBZ. Single-factor analysis of variance and mean comparison tests (Tukey, $p \leq 0.05$) were applied to the number of stomata and pore area. The results showed that the epidermal cells of both leaf surfaces had anticlinal walls with sinuous contours and anomocytic stomata. 'Suyana' had greater height (109.25 cm), more stomata $mm^{-2}$ (98.4), and larger pores (21.28 $\mu m^2$) than 'Tunkahuan'. The application of PBZ increased the number of stomata and the opening of the stomatal pore starting at 25 mg $L^{-1}$, and its effect was more pronounced at 100 or 200 mg $L^{-1}$. PBZ also reduced the height of the plants and made them more colorful for possible ornamental use.

## Keywords:

epidermal cells, number of stomata, stomatal pore area, Suyana, Tunkahuan.

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## Introduction

*Chenopodium quinoa* Willd. (Amaranthaceae Juss.) is native to the Andean region, has lanceolate or rhomboidal leaves and its grains are rich in proteins, lipids and carbohydrates, with values ranging from 9.1 to 15.7, 4 to 7.6 and 48.5 to 69.8%, respectively (Nowak *et al*., 2016; Martínez-Villaluenga *et al*., 2020). The height of quinoa plants fluctuates from 0.9 to 1.8 m, which, added to the length of their panicles (0.4 m), exceeds 2 m. This condition makes it difficult to harvest the grains (Espitia *et al*., 2021).

On the other hand, González *et al*. (2014); Hussain *et al*. (2022) mention that quinoa species with amphistomatic leaves and wide pores may have greater efficiency for $CO_2$ fixation, as occurs in *Ch. quinoa* 'CICA-17' or 'Pichaman'. In these, the density of stomata mm$^{-2}$ on the adaxial and abaxial surfaces ranges from 161.9 to 183.5 and from 217.5 to 233.9, respectively. In 'Pichaman', the opening of the stomatal pore has also been reported to range between 14.5 and 15.4 µm$^2$.

In this regard, growth retardants have been used to reduce plant height and facilitate harvesting, or to increase stomatal number and stomatal pore opening to favor $CO_2$ fixation. One of these retardants is paclobutrazol (PBZ) (Rademacher, 2000). PBZ is a triazole that inhibits gibberellin biosynthesis by blocking the action of the ent-kaurene oxidase enzyme and preventing the oxidation of ent-kaurene into ent-kaurenoic acid (Taiz and Zeiger, 2010).

Thus, PBZ not only shortens the length of the internodes and makes the plants more compact and striking, but it also alters the density and opening of the stomatal pore by increasing the concentration of abscisic acid in the guard cells (Waqas *et al*., 2017; Xia *et al*., 2018; Torres-Pio *et al*., 2021).

For example, in *Beta vulgaris*, the number of stomata in control plants was 5.6 mm$^{-2}$ and increased to 350.1 with the application of 500 mg L$^{-1}$ of PBZ (Shevchuk *et al*., 2019). In *Paeonia lactiflora*, the pore area increased from 4.07 to 7.34 µm$^2$ with 100 mg L$^{-1}$ PBZ (Xia *et al*., 2018). Research on the application of PBZ as an enhancer of the visual appearance and stunting of plants, with no effect on flowering, has been reported in *Lilium* 'Arcahon', 'Armandale', 'Tresor', and 'Litouwen', among others (Torres-Pio *et al*., 2021; Rios-Florida *et al*., 2022; Velasco-Lara *et al*., 2024).

In these cultivars, height decrease, and their potential for ornamental use increases. However, in *Lilium* or *Ch. quinoa* cultivars, the effect of PBZ on stomatal anatomy or density has not been evaluated, which could be relevant if its relationship with $CO_2$ fixation and possible grain yield is considered.

Therefore, this research aimed to evaluate the effects of five PBZ concentrations on the description, number of stomata, stomatal pore area, plant height, and visual appearance of *Ch. quinoa* 'Suyana' and 'Tunkahuan'.

## Materials and methods

The seeds of 'Suyana' and 'Tunkahuan' were harvested in November 2023 from the experimental plots of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP, by its Spanish acronym). For this research, cultivation was carried out in a greenhouse with a plastic cover from July to December 2024 at the Iztacala Faculty of Higher Studies, UNAM. Sowing was carried out in 50 pots containing 2.5 L of peat moss:expanded perlite (1:1) brought to 100% usable moisture with 1.25 L of tap water.

At 25 days after sowing, the plants were thinned to one plant per pot. The experimental units consisted of a pot containing 2.5 L of substrate and a 'Suyana' or 'Tunkahuan' plant, which were randomly placed inside the greenhouse (Figure 1).

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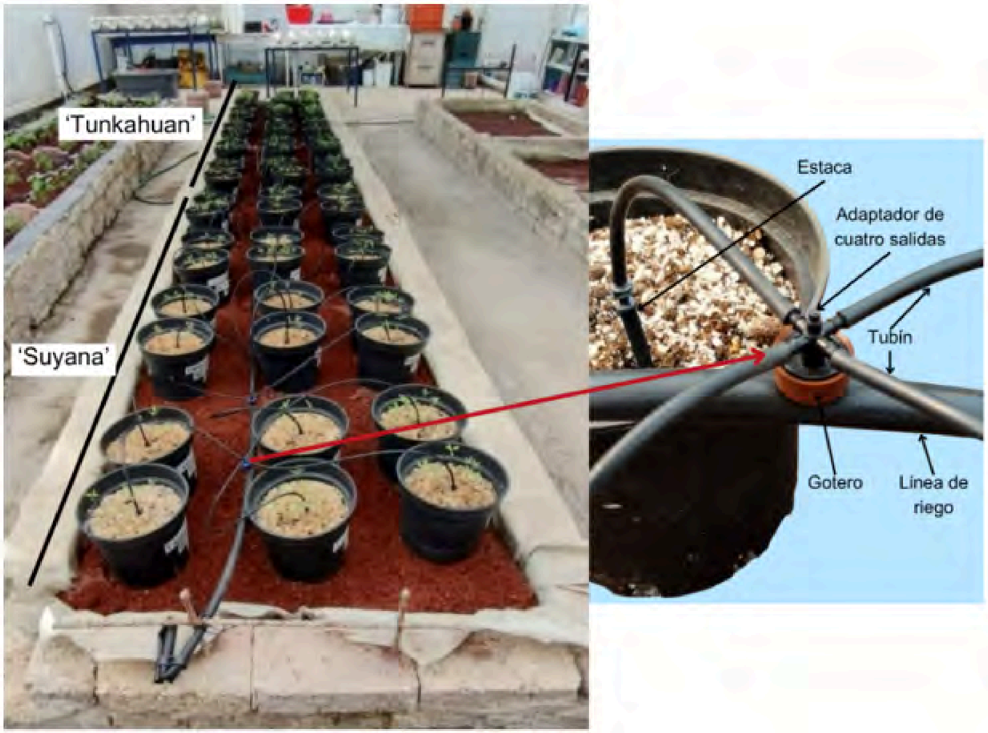
**Figure 1. Experimental units with drip fertigation, randomly distributed within the greenhouse.**




From this moment until 76 days after sowing, on Tuesdays and Fridays each week, between 10 am and 12 pm, the following treatments were applied foliarly with five replications: 0 (control), 25, 50, 100 and 200 mg $L^{-1}$ of PBZ (2RS, 3RS)-1(4-chlorophenyl-4,4-dimethyl-2(1H1, 2,4-triazol-1-yl)-pentan-3-ol (Cultar® 23.42%, Syngenta, Switzerland).

Moisture was recorded every third day using a tensiometer (Irrometer® Co, USA), and when it had values of 56 cbars (equivalent to 80%), it was restored with Steiner solution (1961) applied at 8, 14 and 18 h via a semi-automated system with 4 L $h^{-1}$ drippers (Figure 1). The pH and electrical conductivity of the nutrient solution were 6.2 and 2 dS $m^{-1}$, respectively. To prevent salt accumulation, moisture was restored every 15 d with acidulated water (pH, 6.1), and plants were monitored to prevent deficiencies, diseases or pests.

The following were evaluated: epidermal anatomy: description, number of stomata, and pore area. In the middle part of each plant, samples of leaves were taken and fixed in FAA (10% formaldehyde, 5% acetic acid, 50% absolute ethanol, and 35% distilled water), and epidermal scrapings were taken and placed on slides to obtain semi-permanent preparations (Aguilar-Rodríguez, 1998).

These preparations were used to perform the anatomical description of the epidermal cells and stomata on the adaxial and abaxial surfaces of the control treatments. An optical microscope (Nikon, E200) coupled with an image analyzer (NIS-Elements, BR5.21.01, Nikon Instruments) was used to evaluate the anatomy, stomatal number and stomatal pore area.

## Height and visual appearance of plants

The height was recorded with an FH-5M tape measure (Truper®), using the substrate surface and the apex of each plant as references. The visual appearance was obtained by taking pictures with a Nikon D3500 digital camera. The images were processed using the free software GIMP v. 3.0.4 (http://www.gimp.org.es/).

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### Statistical analysis

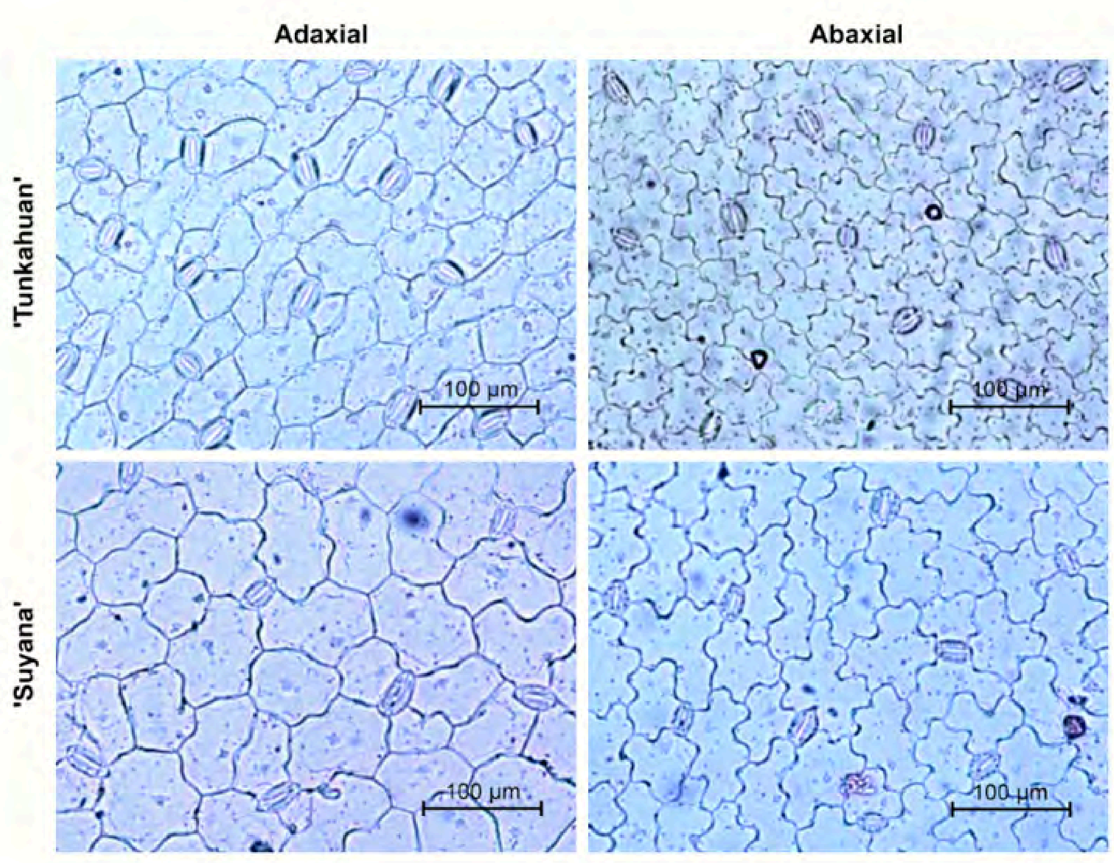
The results were tested for normal distribution (Anderson-Darling, p> 0.05) and homogeneity of variances (Bartlett, p> 0.05) before being analyzed with one-factor analysis of variance and mean comparison tests (Tukey, p≤ 0.05). The analysis was performed using SAS® 9.0 for Windows (SAS Institute Inc., 2013).

## Results and discussion

### Description of epidermal cells and stomata on the adaxial and abaxial surfaces of the leaf

In the surface view, the adaxial epidermal cells of 'Suyana' and 'Tunkahuan' showed smooth, thin anticlinal walls with slightly sinuous to straight contours; in contrast, deeper undulations were observed in the abaxial epidermis. Both cultivars have amphistomatic leaves with anomocytic stomata on both surfaces, That is to say, there are no differentiated cells around the guard cells (Figure 2).

**Figure 2. Surface view of the adaxial and abaxial epidermis of the leaves of *Ch. quinoa* 'Suyana' and 'Tunkahuan'.**




Anomocytic stomata on both surfaces are present in different species of *Ch.* (Gaafar, 2019; Buedo *et al*., 2024) and in cultivars such as 'CICA-17# and 'Real' (González *et al*., 2022), as well as in other Amaranthaceae such as *Atriplex halimus* and *Aerva javanica* (Tantawy *et al*., 2023). Nonetheless, anisocytic stomata have also been described for the genus *Chenopodium*; that is, the guard cells are surrounded by three epidermal cells, one of which is smaller than the other two (Gaafar, 2019).

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From a taxonomic perspective, there were no qualitative anatomical differences in the leaf that are evident to distinguish 'Suyana' from 'Tunkahuan', but the quantitative characters do contribute to the separation of other cultivars of *Ch. quinoa*.

Undulations in the anticlinal walls of abaxial epidermal cells, present in 'Suyana' and 'Tunkahuan', may contribute to resistance to shaking by predators or environmental conditions. According to Vőfély *et al*. (2019), this trait is common in many dicots, and its function is to improve the contact surface between adjacent cells for greater structural resistance to mechanical stresses in the leaves (Sapala *et al*., 2018; Panteris and Adamakis, 2022).

## Number of stomata and pore areas

Both cultivars have amphistomatic leaves. Nevertheless, 'Suyana' had a higher number of stomata on the adaxial and abaxial surfaces of the leaf (75.3 and 98.4) than 'Tunkahuan', which had 48.1 and 70.8, respectively. These numbers increased with the application of PBZ, being more noticeable with 100 or 200 mg $L^{-1}$ treatments.

A similar response was observed for pore area, since 'Suyana' had greater adaxial and abaxial areas (18.06 and 21.08 $\mu m^2$) than 'Tunkahuan', which had 12.79 and 15.89 on the upper side and underside of the leaf, respectively. This area increased with the doses of PBZ applied (Table 1).

**Table 1. Number of stomata $mm^{-2}$ and pore area in the middle part of both leaf surfaces of 'Suyana' and 'Tunkahuan' sprayed with five concentrations of PBZ during cultivation.**

| Cultivars | PBZ (mg $L^{-1}$) | Adaxial | | Abaxial | |
|---|---|---|---|---|---|
| | | Stomata $mm^{-2}$ | Pore area ($\mu m^2$) | Stomata $mm^{-2}$ | Pore area ($\mu m^2$) |
| 'Suyana' | 0 | 75.33 c$^z$ | 18.06 c | 98.4 b | 21.28 c |
| | 25 | 100.13 b | 22.57 bc | 101.87 b | 24.97 b |
| | 50 | 93.2 b | 28.09 b | 113.87 ab | 25.41 b |
| | 100 | 118.73 a | 52.27 a | 115.47 ab | 29.63 a |
| | 200 | 123.8 a | 49.04 a | 127.6 a | 29.99 a |
| HSD | | 14.91 | 7.9 | 22.42 | 2.9 |
| CV (%) | | 20.44 | 32.49 | 28.21 | 15.49 |
| 'Tunkahuan' | 0 | 48.07 c | 12.79 c | 70.8 c | 15.89 d |
| | 25 | 59 b | 18.25 b | 84.67 b | 20.98 c |
| | 50 | 64.8 b | 20.57 b | 92.53 b | 22.25 c |
| | 100 | 112 a | 23.87 a | 109.07 a | 28.27 b |
| | 200 | 114.87 a | 24.56 a | 115.4 a | 31.91 a |
| HSD | | 8.85 | 3.27 | 12.5 | 2.85 |
| CV (%) | | 15.56 | 22.92 | 18.55 | 16.74 |

Z= different letters in each column for each cultivar, indicate significant differences (Tukey, $p \leq 0.05$). HSD= honestly significant difference; CV= coefficient of variation. Each data point is the average of 30 repetitions.

In 'Suyana', the number of stomata (98.4 $mm^{-2}$) fell within the range reported by Rasouli *et al*. (2023); García-Parra *et al*. (2024) for *Chenopodium quinoa* 'Q20' and 'Titicaca', with 85 and 153 stomata $mm^{-2}$ on the abaxial surface. In 'Tunkahuan', this number (70.8 $mm^{-2}$) was below the three cultivars; however, starting at 25 mg $L^{-1}$ of PBZ, it matched 'Q20', and at 200 mg $L^{-1}$, it was slightly below 'Titicaca'; this shows that, in 'Suyana' and 'Tunkahuan', the number of stomata increases with the application of PBZ.

It is important to note that the higher density of stomata on the abaxial surface is a protective response to desiccation and saving water (Taiz and Zeiger, 2010). Regarding the stomatal pore area, Waqas *et al*. (2017); Waqas *et al*. (2021) observed that, in *Ch. quinoa* '2 Want' and 'Pichaman', the pore opening was 10.78 and 15.36 $\mu m^2$, respectively, which is below what is reported here for 'Suyana' (21.28 $\mu m^2$) or 'Tunkahuan' (15.89 $\mu m^2$) without PBZ.

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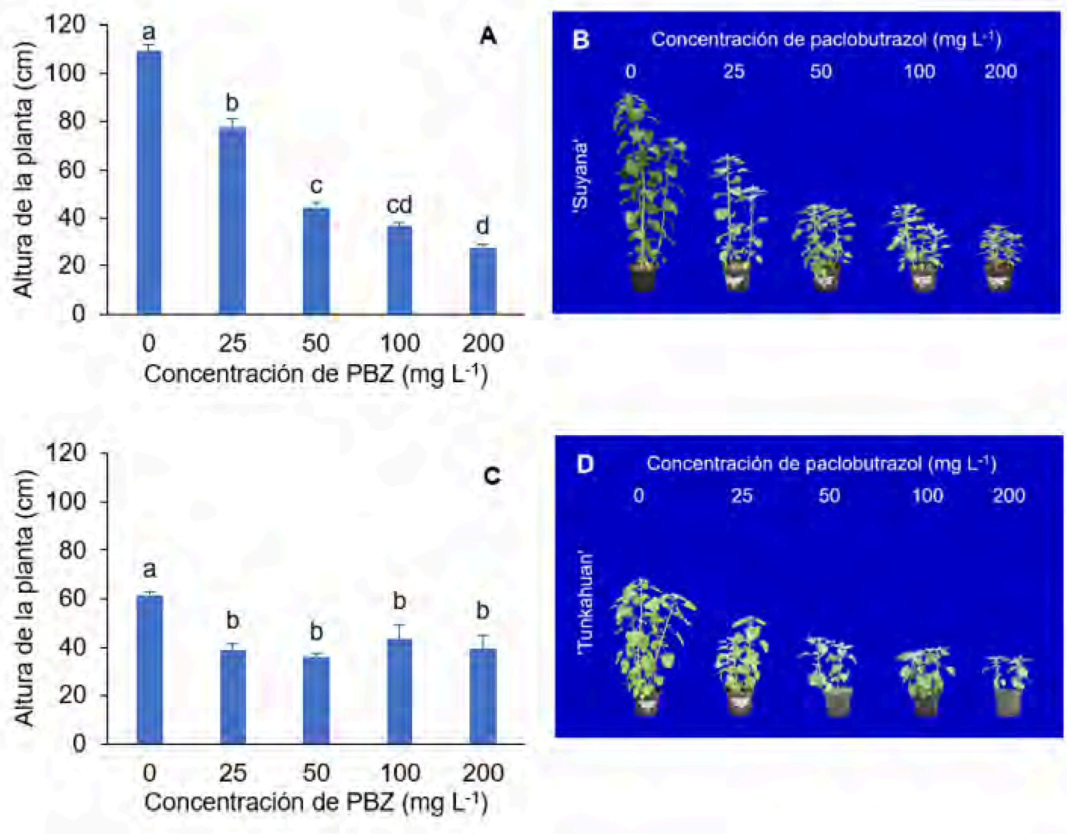
Nonetheless, at 200 mg $L^{-1}$ of PBZ, the pore area increases to 30.9 $\mu m^2$, corroborating that PBZ has an effect that favors crop physiology. The increase in stomatal number and pore opening favors stomatal conductance, which facilitates $CO_2$ capture and improves photosynthetic efficiency for a potential increase in grain yield (Gaafar, 2019; Xiong and Flexas, 2020).

## Height and visual appearance of plants

Plant height was greater in ‘Suyana’ (109.25 cm) than in ‘Tunkahuan’ (61.58 cm). In addition, in ‘Suyana’, height decreased proportionally with PBZ concentration, whereas in ‘Tunkahuan’, the decrease was similar at any concentration of the retardant (Figure 3).

**Figure 3. Height and visual appearance of plants grown with five concentrations of PBZ. A, B) ‘Suyana’; C, D) ‘Tunkahuan’. Different letters on the bars of each cultivar indicate significant differences (Tukey, p≤ 0.05) (n= 5 ± standard error).**




Both cultivars had a more striking visual appearance, giving them potential use as ornamental plants in addition to the proven contribution of their grains to food security (Romero-Benavides *et al*., 2023). The improving effect that PBZ has on the visual appearance of plants has been reported by Torres-Pio *et al*. (2021); Rios-Florida *et al*. (2022) in *Lilium* ‘Arcachon’, ‘Armandale’ and ‘Tresor’.

In these cultivars, height decreases while the number of flower buds is maintained or increased, giving them a very striking appearance for marketing as cut flowers or potted plants.

In *Ch. quinoa* and other Amaranthaceae, such as *Celosia* and *Amaranthus*, the concentration of pigments such as betalains is increased by the effect of PBZ by 1.6 to 6 times (Srinivasan, 2017; Xie *et al*., 2025), giving the leaves more striking colors, not only for possible ornamental use but also for protection against environmental stress due to its antioxidant capacity or as a natural colorant due to its concentration of betacyanins (Martínez et al., 2024).

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## Conclusions

The leaves of ‘Suyana’ and ‘Tunkahuan’ are amphistomatic with anomocytic stomata. On both leaf surfaces, ‘Suyana’ had a greater number of stomata with larger pores than ‘Tunkahuan’. Paclobutrazol increases the number of stomata and the opening of the pore starting at 25 mg $L^{-1}$, having its limit at 100 mg $L^{-1}$; that is, higher concentrations will no longer increase the stomatal conductance for possible $CO_2$ fixation.

‘Suyana’ had a larger size than ‘Tunkahuan’ and with paclobutrazol, its height decreased proportionally with the doses applied. ‘Tunkahuan’ is more sensitive to paclobutrazol; applying 25 mg $L^{-1}$ is sufficient to have a significant reduction in plant height and to facilitate cultivation work. In addition, due to the intensity of color conferred by paclobutrazol, both cultivars improve their visual appearance to be used as ornamental plants or as sources of vegetable dyes.

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# Effects of paclobutrazol on stomatal density and height in *Chenopodium quinoa*

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