

## PACLOBUTRAZOL AND CYTOKININ TO PRODUCE IRIS (*Iris x hollandica* Tub.) IN POTS

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### ABSTRACT

Because of the increasing and diversified demand for potted plants, there is interest in Argentina in commercial iris production (*Iris x hollandica* Tub.). Two experiments were performed at the Instituto Nacional de Tecnología Agropecuaria (INTA), San Pedro Agricultural Experimental Station (33°41' S, 59°41' W), Buenos Aires Province. The effects of increasing concentrations of paclobutrazol (PBZ) and combinations of PBZ and 6-benzylaminopurine (BA) were compared. Height, length of the cycle and rate of flowering were registered. Experiment 1: the bulbs were subjected to immersion in nine concentrations of PBZ: 0, 10, 20, 30, 40, 50, 60, 70 or 80 mg L<sup>-1</sup> for 24 h. Experiment 2: the bulbs were immersed in three concentrations of PBZ: 0, 10 or 20 mg L<sup>-1</sup> for 24 h and then in BA solutions: 0 or 5 mg L<sup>-1</sup> for 24 h. In both cases, the cultivars evaluated were 'Casablanca' and 'Professor Blaauw'. The bulbs were planted immediately after the treatments in 1 L capacity and 12 cm height pots, on 22 July 2008. PBZ allowed for reducing height in all treated plants. A concentration of 20 mg L<sup>-1</sup> would be sufficient to achieve harmonious plants with the containers used: 33 cm tall, average reduction of 41 to 44% compared to the respective control (57 cm). For this concentration, the delay in the duration of the cycle was minimal: 3 to 4 days. There was no effect of BA on the variables. The rate of flowering was not affected by the treatments.

**Key words:** *Iris x hollandica*, bulbous, growth regulator, height.

### INTRODUCTION

Irises have been used for centuries, not only ornamentally, but also as a source for making perfumes and in heraldry (Köhlein, 1987). Most of the species of the genus belong to the rhizomatous plant group that are used in gardening for potting and bedding (De Munk and Schipper, 1993).

A small part of the *Iris* species is bulbous and is produced commercially in greenhouses as cut flowers and potted plants (Le Nard, 1983). Although several related sub-genera are recognized botanically, the commercial *Iris* cultivars are classified as *Iris x hollandica* (Austin, 2005).

The iris is not a cultivar of great importance in Argentina, especially because of the lack of information about its handling and because of the lack of supply of bulbs nationally. However, in the context of the increased

and diversified demand for containerized plants, the beauty of the iris, with flowers that are similar to those of the orchid, the diversity of its colors and tones (Austin, 2005), its relatively short cycle (Le Nard, 1983), the possibility of producing flowering plants during the cold season (Koopman, 2005), and its slow sanitary and nutritional requirements (De Munk and Schipper, 1993), position the cultivar as an excellent candidate for commercialization as a potted plant.

Although currently there are a few cultivars or varieties of dwarf irises available, the majority was originally selected as cut flowers (Koopman, 2005). Different factors can alter producers' decisions in this regard: consumer preferences, availability of bulbs, rusticity and adaptation to cultivation conditions, among others. Consequently, potted plant production requires methods to reduce plant height.

The search for compounds that can influence the regulation of physiological functions of iris plants has led to derivatives of gibberellic acid (Aung *et al.*, 1969; Alpi *et al.*, 1974); diverse growth inhibitors (Okubo and Uemoto, 1981; Doss *et al.*, 1983); sterols (Staby and De Hertogh, 1972), cytokinins (Gregorini, 1983), and ethylene (Marissen *et al.*, 1991). However, little is known

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about the absorption of these compounds, their receptivity and effects (De Munk and Schipper, 1993).

It has been demonstrated that cytokinins stimulate the transportation and distribution of assimilates toward the floral buds, and that the deficiency of cytokinins, in conditions of cultivation with low luminosity, can lead to "blasting" or flower abortion (Vonk *et al.*, 1986). Lee *et al.* (2005) found a direct relationship between the combination of gibberellins (GA 4+7), and cytokinins (6-bencilaminopurine [BA]) and increased rates of flowering of the iris cultivar 'Blue Magic'.

It has been demonstrated with diverse floral species that cytokinins favor all the processes associated with growth and development, decreasing the loss of photosynthetic pigments and proteins to positively modify enzymatic activities related to oxidative stress (Divo de Sesar, 2005).

When considering producing cut flower species in pots a potential problem of many cultivars of great commercial interest is that the stalk is longer the most appropriate values to maintain a harmonious relationship with the container (Krug, 2004). Several studies involving paclobutrazol (PBZ) and other growth regulators have concentrated on finding efficient methods of application and minimal doses (Dole and Wilkins, 1999; Barret, 2002) in order to overcome the growth problem.

Stalk elongation is reduced by PBZ, (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol, which inhibits gibberellin synthesis (Saniewski, 1989), and by others growth retardants, such as ancimidol (De Hertogh, 1989), flurprimidol and uniconazole (Krug *et al.*, 2003), which have been used in bulbous species to obtain plants with lower heights for use as potted plants. PBZ has been used in the United States to widen the market bulbous plants in potted plant market (Latimer, 2001; Miller, 2004).

The objective of this study was to determine if the use of two growth regulators that are available on the Argentine market produces desirable effects on the production of iris plants in plants, in particular in shortening the stalk without altering the parameter related to flowering. The effects of increasing concentrations of PBZ were compared to the combination of PBZ and BA, in the context of cultivation conditions of the north of Buenos Aires Province, temperate winters and unheated greenhouses.

## MATERIALS AND METHODS

The experiments were conducted in 2008 at the San Pedro Agricultural Experimental Station of the Instituto Nacional de Tecnología Agropecuaria INTA (33°41' S, 59°41' W), Buenos Aires Province, Argentina. The iris cultivars used were 'Casablanca' and 'Professor

Blaauw', both from Holland (Van den Bos Flowerbulbs BV, Honselersdijk, Netherland). Number 12 bulbs were employed (measured in cm of the widest circumference), provided by the supplier with cold storage conditions sufficient to be planted in the 30th week of the year.

### Experiment 1: Increasing concentrations of PBZ

The treatments for the two cultivars consisted of the application of PBZ (commercial product Kultar SC™, 25% active ingredient, AgriMarketing, Buenos Aires, Argentina) by immersion of the bulbs prior to transplanting. Different concentrations were compared: 10, 20, 30, 40, 50, 60, 70 and 80 mg L<sup>-1</sup>, with an immersion time of 24 h. In all cases, the control (0 mg L<sup>-1</sup>) consisted of the immersion of the bulbs in potable water for the same amount of time as the treated bulbs. The bulbs were placed in mesh bags (30 per treatment and per cultivar) and were submerged in solutions of 15 L per treatment and per cultivar, a sufficient quantity to ensure maximum absorption. Afterward, the bags were hung until completely drained of the solutions.

### Experiment 2: Combinations of BA-PBZ

The treatments for the two cultivars consisted of the application of PBZ (commercial product Kultar SC™, 25% active ingredient) and BA (reactive *pro analisis*) by immersing the bulbs prior to transplanting. These growth regulators were applied separately: first the bulbs of the two cultivars were submerged in solutions of 0 (water), 10 or 20 mg L<sup>-1</sup> of PBZ for 24 h. They were then submerged in a solution of 0 (water) or 5 mg L<sup>-1</sup> of BA for 24 h. The bulbs were placed in mesh bags (30 per combination of treatments and per cultivar) and submerged in corresponding solutions with an available volume of 500 cm<sup>3</sup> for each one. Following the indicated times, the bags were hung until completely drained of the solution).

After receiving the corresponding treatments, the bulbs were planted on 22 July 2008, one per pot, in black polyethylene containers of 1 L capacity, 12 cm high and 12 cm in diameter (N° 12). The pots were taken to a metallic greenhouse with a polyethylene cover (25% of effective ventilation), without temperature control. The bases of the bulbs were located at a depth of 8 cm. An artisanally produced substrate was used, consisting of earth (70%), peat (25%) and perlite (5%) (bulk density 0.8 kg m<sup>-3</sup>, porosity 22%, water retention 58.2%, pH 4.8, electrical conductivity 0.9 dS m<sup>-1</sup>). The pots were placed on benches at a density of 35 plants m<sup>-2</sup>. The water was supplied by drip individual irrigation according to demand (the same conditions for all the treatments).

A randomized complete block design was used in both experiments, with three replicates. The experimental unit was 10 plants.

The following records were kept of the plants in each plot: days and height at appearance of visible flower buds, days and height at appearance colored buds (harvest, moment of sale), and the flowering rate was calculated (number of plants that reached the moment of sale/total number of plants). Photosynthetically active radiation (PAR), temperature and relative humidity of the air throughout the cycle of the cultivars were obtained (frequency 15 min) with an automatic meteorological mini-station (Watch Dog™, Seedmech, Vienna, Austria).

The SAS program (SAS Institute, 1989) and its GLM, Mean and Reg procedures were used for the statistical treatment of the data. The non-activity Tukey test was applied to confirm the normal distribution, which was submitted to analysis of variance ( $\alpha = 0.05$ ). The treatments were compared using the Tukey test for adjusted means ( $\alpha = 0.05$ ).

**RESULTS AND DISCUSSION**

**Climatic Situation**

Considering the duration of the cycles of the cultivars (85 days), the average temperature and humidity in which the plants developed were 15.9 °C and 72%. The optimal mean temperature for iris production has been established between 14 and 20 °C, the most recommendable being between 15 and 16 °C, and ambient humidity between 70 and 80% (Casañ, 1997). Consequently, the conditions recorded were not limiting for the development of irises. Accumulated PAR until the stage of formed buds was 905 mol photosynthetically active photon flux (PPF) m<sup>2</sup> s<sup>-1</sup> and until harvest, 1061 mol PPF m<sup>2</sup> s<sup>-1</sup>. Mae and Vonk (1974) showed the sensitivity of the cultivar to light in the initial stage of flowering. No information was found about the minimal quantities of PAR required for the cultivar.

**Plant height**

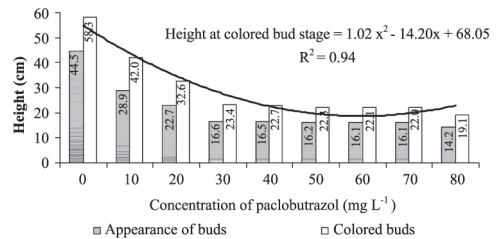
**Effects of increasing concentrations of PBZ (Figures 1 and 2):** Plant height at visible appearance of buds and at colored buds was reduced by the treatments of PBZ for both ‘Casablanca’ and ‘Professor Blaauw’. Height at the moment of sale showed a negative quadratic tendency with the increased concentration of the regulator, with a very high adjustment for both cultivars. In accordance with the statistical differences found, the response to PBZ in ‘Professor Blaauw’ was more scaled to the increasing concentrations of PBZ than it was with ‘Casablanca’. There was a reduction in height in ‘Casablanca’ compared to the control (58.3 cm) of 27% with 10 mg L<sup>-1</sup>, of 44% with 20 mg L<sup>-1</sup> and of 65% on average between 30 and 80 mg L<sup>-1</sup> of PBZ. There was a reduction in height in ‘Professor Blaauw’ compared to the control (56.9 cm) of 34% with 10 mg L<sup>-1</sup>, of 41% with between 20 and 30 mg

L<sup>-1</sup>, of 51% with between 40 and 50 mg L<sup>-1</sup> and 56% with between 60 and 80 mg L<sup>-1</sup> of PBZ.

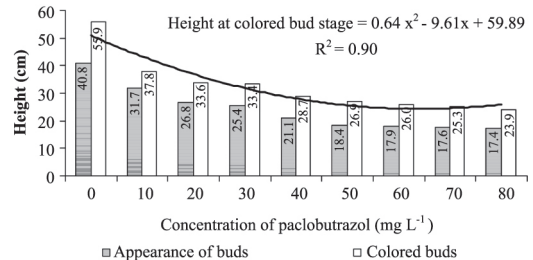
**Effects of the BA-PBZ combination:** The effects of PBZ were similar to those of Experiment 1. The presence of BA in the combinations did not affect plant height. On average among the combinations of 0 and 5 mg L<sup>-1</sup> of BA, at the colored bud stage and only through the effect of PBZ, height reductions were registered in comparison to the control of 30% for the concentration of 10 mg L<sup>-1</sup> and 46% for 20 mg L<sup>-1</sup> for ‘Casablanca’ (control: 59.0-58.9 cm) (Figure 3); and 27% for the concentration of 10 mg L<sup>-1</sup> and 40% for 20 mg L<sup>-1</sup> for ‘Professor Blaauw’ (control: 58.2-58.1 cm) (Figure 4).

**Duration of the cycle**

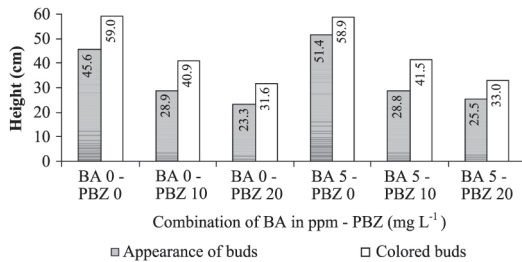
**Effects of increasing concentrations of PBZ:** The treatments affected the duration of the cycle. A delay was observed in both cultivars in the visible appearance buds and the stage of colored buds in almost all the plants treated with PBZ. ‘Casablanca’ plants were more affected by the delay at the moment of sale, with a maximum dilation of approximately 7 days between the controls and the plants with concentrations of 70 and 80 mg L<sup>-1</sup>. ‘Professor Blaauw’ showed a maximum delay at the moment of sale



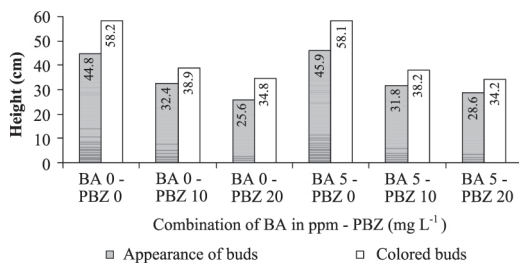
**Figure 1. Height of iris ‘Casablanca’ plants treated with increasing concentrations of paclobutrazol. Regression model with the highest determination coefficient that relates treatments to plant height at the moment of sale (5%).**



**Figure 2. Height of iris ‘Professor Blaauw’ plants treated with increasing concentrations of paclobutrazol. Regression model with the highest determination coefficient that relates treatments to plant height at the moment of sale (5%).**



**Figure 3. Height at different times of the cycle of iris 'Casablanca' plants treated with combinations of 6-benzylaminopurine (BA) and paclobutrazol (PBZ).**



**Figure 4. Height at different periods in the cycle of iris 'Professor Blaauw' treated with combinations of 6-benzylaminopurine (BA) and paclobutrazol (PBZ).**

of almost 5 days in comparison between the control and plants with concentrations of 60 to 80 mg L<sup>-1</sup> (Table 1). The plants of this cultivar treated with 10 mg L<sup>-1</sup> of PBZ were not affected in the total duration of the cycle (from planting to the stage of colored buds). Although there was a delay in the visible appearance of buds, there was acceleration between this stage and that of colored buds.

**Effects of the BA-PBZ combination:** With 'Casablanca' it was observed that the treatments with PBZ affected

the duration of the cycle, independently of whether BA was present in the combination of treatments. The plants from bulbs exposed to 10 or 20 mg L<sup>-1</sup> of PBZ in the combinations required 4 days more than the controls to reach the stage of colored buds. The plants from bulbs exposed only to BA (BA 5 - PBZ 0) had a cycle duration similar to that of the absolute control (BA 0 - PBZ 0). 'Professor Blaauw' exposed to cytokinin in the BA-PBZ combinations (BA 5 - PBZ 10 and BA 5 - PBZ 20) had a delay of 2 to 3 days to reach a state suitable for sale. When the bulbs of this cultivar were treated with only PBZ (BA 0 - PBZ 10 and BA 0 - PBZ 20), with a lower concentration (10 mg L<sup>-1</sup>). Likewise, there was no delay in the cycle in Experiment 1 compared to the absolute control (BA 0 - PBZ 0). With 20 mg L<sup>-1</sup> of PBZ, the dilation of the cycle was 3 days. As with 'Casablanca', 'Professor Blaauw' plants treated only with BA (BA 5 - PBZ 0) (Table 2) were not affected in this variable.

### Flowering rate

The treatments of increasing concentrations of PBZ and the BA-PBZ combination did not affect the flowering rate, which in all cases averaged 99.3%.

One of the primary objectives in the production of potted ornamental plants is to obtain plants of high quality, with ensured flowering and a high degree of harmony with the size of the container. An acceptable proportion has been established for other bulbous species of 1:3 between the height of the container and the plant height (De Hertogh, 1996). For an N° 12 pot (12 cm in diameter, 12 cm high) plants with a maximum height of 36 cm can be considered acceptable.

The height values observed in iris plants at the moment of sale suggest that a concentration of 20 mg L<sup>-1</sup> of PBZ would be sufficient to reach an adequate size in

**Table 1. Effects of concentrations of paclobutrazol on flowering time of iris 'Casablanca' and 'Professor Blaauw'.**

Concentration of paclobutrazol (mg L <sup>-1</sup> )	Casablanca		Professor Blaauw	
	Days until visible appearance of buds	Days until colored buds appear	Days until visible appearance of buds	Days until colored buds appear
0	74.4e	80.3g	73.0d	77.9d
10	79.1d	83.9f	77.8c	78.4d
20	82.6c	84.6ef	77.8c	80.7c
30	84.4b	85.2de	78.2bc	82.1bc
40	84.6b	85.6cde	79.0b	82.3bc
50	84.7b	86.1bcd	79.1b	82.9b
60	84.6b	86.4bc	79.3ab	83.7a
70	85.0ab	87.1ab	80.0a	83.8a
80	85.6a	87.9a	80.1a	83.8a
CV	3.5	1.3	2.8	2.1

CV: variation coefficient. Different letters in columns indicate differences among treatments according to Tukey test (5%).

**Table 2. Effects of 6-benzylaminopurine (BA) and paclobutrazol (PBZ) combinations on flowering time of iris 'Casablanca' and 'Professor Blaauw'.**

Treatments (mg L <sup>-1</sup> )	Casablanca		Professor Blaauw	
	Days until visible appearance of buds	Days until colored buds appear	Days until visible appearance of buds	Days until colored buds appear
BA 0 - PBZ 0	71.9d	78.4b	70.0c	76.9c
BA 0 - PBZ 10	78.0c	82.1a	75.2b	78.0c
BA 0 - PBZ 20	81.1a	82.4a	76.4a	80.1a
BA 5 - PBZ 0	71.9d	78.1b	70.6c	76.8c
BA 5 - PBZ 10	77.8c	82.2a	75.4b	79.0b
BA 5 - PBZ 20	79.7b	82.4a	76.5a	80.3a
CV	2.8	0.7	2.1	2.1

CV: variation coefficient. Different letters in columns indicate differences among treatments according to Tukey test (5%).

'Casablanca' or 'Professor Blaauw' to accommodate the aforementioned container size.

Although height was not measured at the end of flowering in these experiments, it has been observed with other bulbous plants that stalks continue to grow (Zagabria *et al.*, 2006), because of which this possibility should be kept in mind.

While the treatments with PBZ produced a delay in the cycle (up to 5 or 8 days, depending on the cultivar), considering the total duration of the same (around 85 days in both cultivars), this represented a maximum of only 8%. With minimum effective concentrations to reduce the height of each cultivar (20 mg L<sup>-1</sup>) the extension of the observed cycle was 4 days in 'Casablanca' and 3 days in 'Professor Blaauw'.

Considering the information on cytokinin in the prevention of floral abortion caused by low levels of sunlight (Vonk *et al.*, 1986) and the increased rate of iris flowering (Lee *et al.*, 2005); the treatments with BA were included in the experiments to confirm that BA modifies the responses of 'Casablanca' and 'Professor Blaauw' in the climatic situation of the cultivation and/or the use of PBZ.

However, with the evaluated forms of application and BA concentrations, no responses were observed in any of the registered parameters (cycle duration, plant height, flowering rate), and PBZ did not affect the flowering rate. There is not much information on the use of cytokinins with iris plants. Mae and Vonk (1974) and Vonk and Ribot (1982) found that injecting the bulbs with cytokinins (BA, zeatin or kinetin) promoted the development of the floral bud and prevented abortion. However, this treatment is not practical on a large scale.

Bulbs of very good quality were used (suppliers who guaranteed an adequate thermal regime during storage) and appropriate climatic conditions for the species, it

is reasonable to expect a very high flowering rate (De Hertogh, 1989).

However, under conditions of low sunlight levels, floral abortion has been noted in iris plants (Vonk *et al.*, 1986), but a minimum threshold has not been established. The PAR that was registered in the greenhouses in the northern area of Buenos Aires Province during the winter was possibly sufficient to avoid this problem.

## CONCLUSIONS

The study confirmed the responses of the iris cultivars 'Casablanca' and 'Professor Blaauw' to PBZ in reducing height at the moment of sale.

For cultivation conditions similar to those evaluated, a concentration of 20 mg L<sup>-1</sup> de PBZ applied by immersion of bulbs for 24 h prior to transplanting, would be sufficient to achieve plants that are harmonious with commercialization in 12 cm-high containers.

The detected effects of the suggested concentration on delaying the cycle were minimal. The combination of PBZ with BA at 5 mg L<sup>-1</sup> applied by immersion of bulbs for 24 h did not produce effects on the evaluated parameters.

The use of PBZ opens the possibility of including iris cultivars as potted plants, among bulbous plant cultivated in the cold season in temperate zones, thus increasing the diversity in the supply of potted flowers.

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## RESUMEN

**Paclobutrazol y citoquinina para la producción de iris (*Iris x hollandica* Tub.) en macetas.** En Argentina, ante el aumento y la diversificación de la demanda de plantas en maceta, el iris (*Iris x hollandica* Tub.) se presenta atrayente para la producción comercial. En la Estación Experimental Agropecuaria INTA San Pedro (33°41' S, 59°41' O), Provincia de Buenos Aires, se compararon los efectos de concentraciones crecientes de paclobutrazol (PBZ) y combinaciones de PBZ y 6-bencilaminopurina (BA), sobre la altura de la planta, la duración de las etapas del ciclo fenológico y la tasa de floración. Para ello se realizaron dos experimentos, Experimento 1: los bulbos se sometieron a inmersión durante 24 h en soluciones de PBZ de 0, 10, 20, 30, 40, 50, 60, 70 u 80 mg L<sup>-1</sup>; y Experimento 2: los bulbos se sumergieron 24 h en soluciones de 0 ó 20 mg L<sup>-1</sup> de PBZ y luego otras 24 h en soluciones de 0 ó 5 mg L<sup>-1</sup> de BA, en ambos casos, los cultivares evaluados fueron Casablanca y Professor Blaauw. Los bulbos se plantaron el 22 de julio inmediatamente después de los tratamientos, en macetas de 1 L de capacidad y 12 cm de altura. El PBZ provocó la reducción de la altura al estado de pimpollo con color o momento de la venta en todas las plantas tratadas. Una concentración de 20 mg L<sup>-1</sup> sería suficiente para lograr plantas armoniosas con el contenedor utilizado: altura 33 cm, reducción promedio 41 a 44% en relación a los respectivos testigos (57 cm). Para esta concentración el atraso detectado sobre la duración del ciclo fue mínimo, 3 a 4 días. No se registraron efectos de la BA sobre las variables evaluadas. La tasa de floración no fue afectada por los tratamientos.

**Palabras clave:** *Iris x hollandica*, bulbosas, regulador de crecimiento, altura.

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