

Determining of the effects of paclobutrazol treatments on seedling height control of wild *Gypsophila bicolor* (Freyn & Sint.) Grossh

Fazilet PARLAKOVA KARAGÖZ^{1*}, Atilla DURSUN^{1,2},
Muhammed Melih KOTAN¹

¹Atatürk University, Faculty of Agriculture, Department of Horticulture, TR-25240 Erzurum,
Turkey; f.parlakova@atauni.edu.tr (*corresponding author); kotanmelih25@gmail.com

²Kyrgyzstan -Turkey Manas University, Faculty of Agriculture, Department Horticulture and Agronomy, Bishkek, Kyrgyzstan;
atilladursun@atauni.edu.tr

Abstract

Within the scope of this study, seeds were collected from *Gypsophila bicolor* (Freyn&Sint.) Grossh plants in their natural environment and two different locations (Turkey). The research was carried out to determine the effects of paclobutrazol applied at different concentrations (0.6, 0.9, 1.2, 1.5- and 2.0-ml L⁻¹) on the seedling height control in the cotyledon leaf stage of *G. bicolor*. As a result of the study, it was determined that the decrease in the number of leaves per plant was determined at a dose of 1.2 mg L⁻¹ paclobutrazol. The effect of paclobutrazol treatments on the seedling height control of *G. bicolor* changed according to the genotype as well as the dose. As the dose of paclobutrazol applied increased, the number of side branches decreased. It was determined that the seedling stem thickness increased in 47.71% with the dose of 2.0 mg L⁻¹ when compared to the control treatment. The application in which the highest chlorophyll (SPAD) value was obtained from the application with a dose of 1.5 mg L⁻¹. The chlorophyll value of G2 (Genotype 2 (ERZ) (G2)) was higher than that of G1 (Genotype 1 (VAN)). Seedling biomass of G1 increased in 15.87% in 1.2 mg L⁻¹ treatment when compared to control. In the present study, darker green leaves were obtained from the highest dose of paclobutrazol, 2.0 mg L⁻¹. As a general result, it was concluded that 1.5 mg L⁻¹ dose of paclobutrazol was sufficient for both genotypes for plant height control in the seedling period.

Keywords: compact; *Gypsophila bicolor*; PBZ; plant height control breeding; potted ornamental plant; wild species

Introduction

Annual ornamental plants with compact form and grown in pots has a special place in the production of ornamental plants because it can be grown in viol or plastic bag and small-sized pots and the production cost is low. Especially for small-scale commercial greenhouse producers, the production of small, compact plants can provide more economic benefits; because in plant production facilities, plants can be placed closer to each other and provide more space usage efficiency. Plant height reduction plays an important role as increasing in yield and quality and reducing cost, place and labour (Kim *et al.*, 2010). Annual ornamental plants with compact

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form and grown in pots are showier, less damaged during transportation and easier to transport. This situation also increases in the wholesale opportunities (Atlas, 2006). Also, compact form and potted plants tend to have longer marketability periods than tall and undersized plants (Hojati *et al.*, 2009).

Besides the compact growth achieved by classical breeding, compact growth is achieved by the application of chemicals with plant growth regulators (PGRs) or growth retardants, mainly interrupting gibberellic acid (GA) biosynthesis (Rademacher, 2016). Growth inhibitory chemicals are used to obtain tighter textured, stunted, mature seasonal and potted plants, to darken the colour of green parts (Whipker, 2013), to increase in the strength of flower stalks and to program flowering, and to increase in resistance to adverse environmental effects. In addition, the first application area of most of the growth inhibitory chemicals used in ornamental plants has been ornamental plants (Bhattacharya *et al.*, 2010; Hamza *et al.*, 2019).

In recent studies, growth retardants such as paclobutrazol, chlormequat chloride (CCC), prohexadione ca, uniconazole, morphactins, ancimidol and diaminozid (Rademacher, 2000; Gholampour *et al.*, 2015), which are effective antigibberellins, have been used in a wide range of plants. Plant growth retardants can delay cell division and upper part elongation of the plant. In addition, by limiting the biosynthesis of gibberellins, they cause a decrease in internode lengths and vegetative growth (Magnitskiy *et al.*, 2006). The most common application methods of growth retardants are foliar spraying and addition to growing medium (Al-Khassawneh *et al.*, 2006; Gholampour *et al.*, 2015). Plant growth can be affected by concentration, application time, number of applications, application method, formulation/ingredient, and substrate composition (Ahmad *et al.*, 2015).

Paclobutrazol is one of the growth retardants that has been effective in controlling plant size and increasing in compactness (Bañón *et al.*, 2002), reducing water use (Hickman, 1986; Ahmad *et al.*, 2014) and reducing abiotic stresses (Whipker, 2013). Paclobutrazol can be applied to the plant by spraying a single high dose to provide seasonal growth control or by spraying the plant with a low dose of 0.1 to 1 ppm to provide temporary control of plant growth. With paclobutrazol applications, without causing any phytotoxicity, delay in production or loss of potted flower quality; in zinnia (*Zinnia elegans* Jacq.) (Pinto *et al.*, 2005; Parlakova Karagöz and Dursun, 2022); in marigold (Chen *et al.*, 1993), oleander (*Nerium oleander* L.) (Singh *et al.*, 2004), *Curcuma alismatifolia* Gagnep. (Pinto *et al.*, 2006), *Mussaenda* L. (Cramer and Bridgen, 1998) and lavender (*Lavandula stoechas* L.) (Papageorgiou *et al.*, 2002) were found to effectively control plant overgrowth (Ahmad *et al.*, 2015).

In recent years, it has become increasingly important for many countries to evaluate the plants in their own flora, to cultivate new species, to expand their production areas and to use them in landscape planning. While *Gypsophila* cultivars of foreign origin are used as design plants both in the cut flower sector and in urban design studies in Turkey, many naturally grown *Gypsophila* species cannot be used as ornamental plants yet. This study was carried out to determine the effects of application of paclobutrazol (0.6, 0.9, 1.2, 1.5- and 2.0- ml L⁻¹) on the growth tips of plantlets in the cotyledon stage obtained from *Gypsophila bicolor* (Freyn & Sint.) Grossh seeds collected from their natural environment on seedling height control. The determination of the most appropriate dose of paclobutrazol for seedling height control for this species was another aim of the study.

Materials and Methods

Material

In the study, *Gypsophila bicolor* (Freyn & Sint.) Grossh seeds collected from Erzurum and Van regions (Table 1) were used as plant materials.

Table 1. Location information of *G. bicolor* (Freynd&Sint.) Grossh seeds* used in the experiment and genotype codes

Genotype code	Location (in Turkey)	Height above sea (m)	Coordinates
G1 (Genotype 1 (VAN))	Van - Gürpınar Road, 12 km before Gürpınar	1967	43° 23'605 East 38° 25'481 North
G2 (Genotype 2 (ERZ))	Erzurum - 27. Km from Aşkale	1858	40° 50'401 East 39° 59'208 North

* Seeds and location information of *G. bicolor* (Freynd&Sint.) Grossh seeds used in the experiment was obtained from Kaya *et al.* (2012).

Collected *G. bicolor* (Freynd & Sint.) Grossh seeds were grown in open field conditions (latitude; 39°53'57.4"N, longitude 41°14'14.8"E; altitude 1890 m from sea) in Erzurum, Turkey, in order to increase in the number of seed material. These seeds obtained constitute the plant material of this study. The germination status of these seeds was determined and seedlings were obtained by sowing.

Method

Sorting, cleaning and counting of seeds brought to maturity and grown in Erzurum open field conditions were carried out. The seeds were subjected to germination test by performing surface sterilization, and sowing was done in line with the results obtained (Figure 1A). The seeds were surface sterilized with 2.5% sodium hypochlorite for 10 minutes, passed through distilled water seven times and left to dry (Azizi *et al.*, 2011). Seeds were taken to germination test at 25 °C temperature conditions. The initial viability of the seeds was determined according to the rules of ISTA (1996). Germination tests were carried out on 4 x 50 seeds at 25 °C for 14 days on double-layer blotting papers in petri dishes with a diameter of 90 x 17 mm (ISTA, 1996). *G. bicolor* seeds germinated in petri dishes were transferred to viols in November 2022 (Figure 1B) and grown under greenhouse conditions. Ornamental peat with a pH of 5.06 was used as the plant growing medium. Plant seedlings were grown in viols under greenhouse conditions at an average temperature of 25/15 °C (day/night) at natural day length and light intensity (Figure 1C).



Figure 1. Germinated seed (A), planting the viol (B), stage at which cotyledon leaves emerge from the soil (C)

Applications were made when the seedlings in the viol reached the cotyledonous leaf stage (Figure 2B, Figure 3A). The trial was constructed from control and five different doses of paclobutrazol (0.6, 0.9, 1.2, 1.5- and 2.0-ml L⁻¹) (Figure 2A). Ahmad *et al.* (2015) reported that the optimal concentration of paclobutrazol applied on zinnia flower to control plant growth is 0.5-1.0 mg L⁻¹. Based on these results (Ahmad *et al.*, 2015), different doses were tested on wild *Gypsophila*. Different levels of paclobutrazol were applied to all groups except for the control group; they were applied in growth tips, at the stage of emergence of both cotyledon leaves (Figure 2B). Only distilled water was applied to the plants in the control treatment.

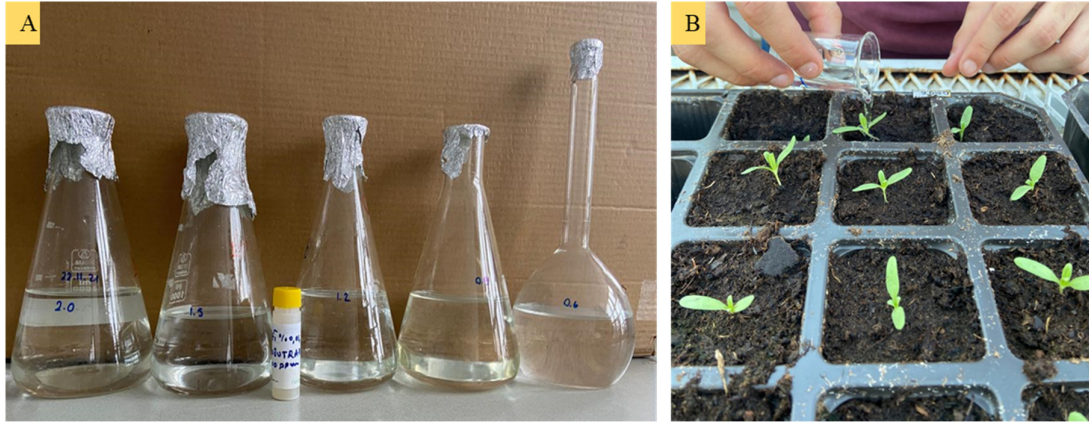


Figure 2. Preparation of five different concentrations of paclobutrazol solutions used in the experiment



Figure 3. Plant development status at the time of application of different levels of paclobutrazol (A), growth stages of seedlings in viols (B, C)

The research was established in a randomized plot design with three replications according to factorial arrangement. There are 10 seedlings in each replication. After paclobutrazol application, seedlings were grown up to the marketing stage (approximately 8 weeks). Seedling height (cm), number of leaves (number/plant), number of side branches, seedling stem thickness (diameter) (mm), leaf area (cm²) (by leaf meter), chlorophyll content (Konica Minolta Spad 502 plus with chlorophyll meter device) seedling biomass (g plant⁻¹) (by method of Khalaj *et al.* (2017)), leaf colour values (L*, a*, b* values with Konica Minolta CR400 chromameter) (McGuire, 1992) were measured in seedlings coming to the marketing stage; chroma and hue angle were also determined. The chroma and hue angle values were calculated with the formulas given below and according to the method of Little (1975) (Siddiq *et al.*, 2010):

$$\text{Chroma: } (C^*) = \sqrt{a^*2 + b^*2}$$

$$\text{Hue Angle } (h^\circ) = \tan^{-1} (b^*/a^*)$$

Statistical analysis

The results were evaluated according to the analysis of variance in the SPSS (Statistical Package for Social Sciences, Version 20.0) statistical program and given in tables after calculating the arithmetic means and standard deviations. Duncan's test ($p=0.05, 0.01$ or 0.001) was used to determine the significance of the difference among the treatments.

Results and Discussion

Seedling height (cm)

In *G. bicolor* seedlings, all applications of different doses of paclobutrazol reduced the seedling plant height when compared to the control application. In general, the decrease in seedling plant height was parallel to the increase in paclobutrazol doses (Figure S1). Seedling height control was most effective in the 1.5 mg L⁻¹ application in Genotype 1 (VAN) (G1) (Figure 4A), the relevant dose reduced the seedling height by 24.45% compared to the control application, and no adverse effects were observed in terms of seedling quality. In Genotype 2 (ERZ) (G2) (Figure 4B), the highest seedling length reduction was determined in 1.5 mg/L application, and 1.2 mg L⁻¹ and 2.0 mg L⁻¹ doses were also included in the same statistical group. In this case, it was concluded that a dose of 1.2 mg L⁻¹ paclobutrazol was sufficient for the control of G2 seedling height (Table 2). It has been reported that the length, internode length, leaf length and width and root length values show a significant decrease with the increase in the doses (50 and 100 g da⁻¹ paclobutrazol) in some grass varieties (*Lolium perenne* 'Ovation' and *Cynodon dactylon* x *Cynodon transvaalensis*) (Baysal and Karagüzel, 2005). Applications of paclobutrazol on ornamental plant species such as carnation (*Dianthus caryophyllus* 'Mondriaan') (Bañón *et al.*, 2002), geranium (*Pelargonium* × *hortorum* 'Mustang') (Cox, 1991), pansy (*Viola* × *wittrockiana* 'Bingo Yellow Blotch') (Magnitsky *et al.*, 2006), pink lady (*Dissotis rotundifolia*) and majestic bush (*Tibouchina fothergillae*) (Hawkins *et al.*, 2015) caused a decrease in plant height. In the present study, a significant decrease in seedling elongation was found for two different *G. bicolor* (Freyn & Sint.) Grossh genotypes; our finding is in line with the results reported by Hawkins *et al.*, (2015), Hwang *et al.* (2008), Bañón *et al.* (2002), Magnitsky *et al.* (2006). The reason for this decrease can be explained as paclobutrazol and other triazoles reduce shoot elongation through inhibition of GA biosynthesis by blocking the P450 enzyme kaurene oxidase (Davis *et al.*, 1991; Yokota, 1999).



Figure 4. At the end of the trial, Genotype 1 (VAN) genotype (G1) (A); A visual of the genotype 2 (ERZ) (B) genotype (G2) from the general plant development conditions after the treatments

Number of leaf (number plant⁻¹)

In terms of its effect on seedling quality, paclobutrazol caused a decrease in the number of leaves per plant in both genotypes of *G. bicolor*. While this decrease was found to be significant ($p < 0.05$) in general application averages, the differences between genotypes were found to be statistically insignificant ($p > 0.05$, Figure S1). According to the general application averages, the minimum number of leaves was determined in the application with a dose of 2.0 mg L^{-1} . With this application, which is the highest dose of paclobutrazol used in the research, the number of leaves per plant was reduced by 23.05% compared to the control application. The decrease in the number of leaves obtained from the 1.2 mg L^{-1} dose application was determined as 19.91% when compared to the control. Considering the effects of paclobutrazol applications on the number of G1 leaves, it decreased by 24.97% in 1.5 mg L^{-1} application when compared to the control. It was determined that there was no statistical difference between this dose and 0.9 mg L^{-1} , 1.2 mg L^{-1} and 2.0 mg L^{-1} doses. When the effects on the number of leaves per plant in G2 were examined, the maximum number of leaf reduction was determined in the application of 2.0 mg L^{-1} , and the doses of 1.2 mg L^{-1} and 1.5 mg L^{-1} were also included in the same statistical group. In this case, a dose of 1.2 mg L^{-1} paclobutrazol was found to be sufficient for G2 (Table 2).

Table 2. The effects of applications on seedling height, number of leaves, number of side branches, and seedling stem diameter in seedlings that are in the marketing

	Treatments	Control	0.6 mg L ⁻¹	0.9 mg L ⁻¹	1.2 mg L ⁻¹	1.5 mg L ⁻¹	2.0 mg L ⁻¹	Mean
Plant height (cm)	G1	9.20±0.41 c*	9.03±0.30 c	9.19±0.42 c	10.66±1.27 a	9.58±0.24 ab	10.02±0.17 ab	9.61±0.77 ***
	G2	31.01±2.87 a**	24.45±2.80 b	24.07±2.52 b	22.14±7.28 b	20.28±0.33 b	13.38±1.27 c	22.55±6.21
	Mean	20.10±12.08 A***	16.74±8.63 B	16.63±8.31 B	16.40±7.83 B	14.93±5.87 B	11.70±2.01 C	
Number of leaf (number plant ⁻¹)	G1	18.22±0.69 a***	16.67±0.88 a	14.89±1.54 b	14.33±1.21 b	13.67±0.67 b	13.89±0.38 b	15.28±1.88 NS
	G2	17.44±1.17 a***	17.11±0.38 a	17.33±0.67 a	14.22±0.39 b	14.22±10.39 b	13.56±1.39 b	15.65±1.85
	Mean	17.83±0.96 A***	16.89±0.66 AB	16.11±1.71 B	14.28±0.80 C	13.95±0.57 C	13.72±0.93 C	
Number of side branches (number plant ⁻¹)	G1	3.09±0.23 c***	2.95±0.23 c	2.93±0.12 c	4.32±0.31 b	5.19±0.12 a	5.37±0.32 a	3.97±1.09 ***
	G2	3.66±0.39 d***	4.40±0.22 bc	5.33±0.05 a	5.59±0.36 a	4.60±0.43 b	3.95±0.09 cd	4.59±0.75
	Mean	3.38±0.43 C***	3.67±0.82 C	4.13±1.32 B	4.95±0.76 A	4.90±0.43 A	4.66±0.80 A	
Seedling stem diameter (mm)	G1	3.22±0.20 c**	3.22±0.20 c	3.41±0.46 bc	3.85±0.48 abc	4.04±0.02 ab	4.38±0.43 a	3.69±0.53 ***
	G2	3.32±0.52 d***	4.15±0.34 cd	3.81±0.15 bc	4.19±0.28 bc	4.59±0.12 b	5.27±0.50 a	4.22±0.69
	Mean	3.27±0.36 D***	3.68±0.56 CD	3.61±0.37 CD	4.02±0.39 BC	4.31±0.31 B	4.83±0.64 A	

ns: NS: insignificant at $p > 0.05$, statistically significant at the * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ probability level. Note: There is no difference at the 5% significance level between the means denoted by the same letter.

In the literature, it is emphasized that paclobutrazol applications in studies conducted with different plant species generally have a reducing effect on the number of leaves. Hazar and Bora (2018) stated that different doses of paclobutrazol in fuchsia (*Fuchsia* spp.) reduced the number of leaves, Berova and Zlatev (2000) stated that paclobutrazol applied to tomatoes caused a decrease of up to 5% in the number of leaves. Collado and Hernández (2021) reported that paclobutrazol applied to petunia and dianthus species reduced leaf numbers by 7% and 6%, respectively; however, they reported that geranium and pansy plants had no effect on the number of leaves. In our study, the effects of the applied doses on the number of leaves per plant varied according to the genotype.

Number of side branches (number plant⁻¹)

Plant branch development, number of branches and flowering characteristics of the branch are important in determining the aesthetic value of potted ornamental plants and in producing compact potted plants. Whipker (2013) stated that growth inhibitory chemicals are widely used to obtain tighter form, in potted plants. According to the general average of paclobutrazol applications, an increase in the number of *G. bicolor* side branches was obtained. The highest number of side branches was found in the 1.2 mg L^{-1} dose

application and the 1.5 mg L⁻¹ and 2.0 mg L⁻¹ dose applications with the mentioned application were in the same statistical group. When the effects of paclobutrazol applications on the number of side branches in G1 were evaluated according to Table 2, the highest number of side branches (5.37 numbers plant⁻¹) was obtained from 1.5 mg L⁻¹ application. There is no statistical difference between the 1.5 mg L⁻¹ dose and the 2.0 mg L⁻¹ dose. In G2, a decrease was detected in the number of side branches with increasing doses. As a result of our study, the effect of paclobutrazol applications on the number of lateral branches per plant of *G. bicolor* varied according to the genotype as well as the dose (Table 2). Based on these results, it is predicted that G2 may have weaker apical dominance than G1. As a matter of fact, it has been reported in previous research results that varieties with weaker apical dominance have better branching characteristics than varieties with strong apical dominance (Hrotkó *et al.*, 1999). Currey and Erwin (2012) stated that the application of paclobutrazol as a leaf spray did not affect the number of branches in *Kalanchoe* species. On the contrary, growth regulators, which are compounds that affect the growth and development of plants by modifying the natural hormonal action of plants; plant species and variety, concentration, application time, number of applications, application method, formulation / component and substrate composition (Ahmad *et al.*, 2015). There are many research findings supporting this situation: Dorajeero (2010) reported that treating paclobutrazol with different concentrations increased in the number of branches per plant. Biswas *et al.* (2018) found that paclobutrazol applied to *Viola x wittrockiana* plants at a concentration of 5 mg L⁻¹ caused an increase in the number of branches. In addition, Ahmade (2019) concluded that in the cultivation of *Chrysanthemum coronarium* as a potted plant, performing 2 tips with paclobutrazol at a concentration of 20 mg L⁻¹ significantly increased in the number of chrysanthemum branches. Paclobutrazol is a retarder and is known to suppress the effect of apical dominance resulting in increased in development of lateral buds that develop into lateral shoots over time (Bhardwaj *et al.*, 2020). A similar increase in the number of lateral branches has been recorded in *Barleria cristata* (Nichal, 2010), Chinese aster cv. 'Poornima' (Mishra and Mishra, 2006), 'Sardinia' (Singh *et al.*, 2016; Abd El-Aal and Mohamed, 2017). Pinching can be defined as the removal of part of the stem, including the shoot tip, in order to reduce the plant's height, eliminate apical dominance, and thus encourage lateral branching (Reiss-Bubenheim and Lewis, 1986; Mutlu and Ağan, 2015). As a result of the present study, it can be concluded that paclobutrazol application can be used to promote *G. bicolor* side-branching and the appropriate dose is 1.2 mg L⁻¹.

Seedling stem diameter (mm)

In this research, it was observed that as the amount of applied paclobutrazol dose increased, the seedling stem diameter increased as well. The highest mean stem diameter value was found in the application with a dose of 2.0 mg L⁻¹ with 4.83 mm. When the application in question and the control application are compared, an increase of 47.71% rate was obtained. The effect of paclobutrazol applications on stem diameter of both genotypes was highest in 2.0 mg L⁻¹ application. Increases in seedling stem diameters were detected at increasing in doses (Table 2). Similar results were also found by Shalaby *et al.* (2022) and the researchers stated that the application of paclobutrazol to tomato seedlings increased in the stem thickness. Treatment of potato plants grown in greenhouse conditions with paclobutrazol resulted in compact plants with wider stems (Tekalign and Hammes, 2004). Paclobutrazol application increased in stem width and number of leaves (Pal *et al.*, 2016). As the amount of paclobutrazol dose increased, the tomato seedling stem thickness increased (Tsegaw *et al.*, 2005). The reason for this increase determined in the current study and previous studies can be explained by the accumulation of a large number of starch granules in the stems of paclobutrazol-treated plants. In line with this, Tsegaw *et al.* (2005) reported the finding that the stems of plants from the control treatment had smaller irregularly shaped core cells almost devoid of starch granules, while the stems of paclobutrazol-treated plants had large symmetrical core cells containing numerous starch granules.

Chlorophyll content (SPAD)

One of the important seedling quality criteria is its chlorophyll content. In particular, it provides a lively and bright appearance of the seedlings. When the general averages of the paclobutrazol applications determined in the current study were examined, the highest chlorophyll (SPAD) value in *G. bicolor* leaves was the application with 1.5 mg L⁻¹. The chlorophyll value of G2 was higher than G1. Chlorophyll values increased in increasing doses up to 1.5 mg L⁻¹ of paclobutrazol applied to the G1 genotype, while a decrease was observed in the application of 2.0 mg L⁻¹ dose (Table 3). The increase in the amount of chlorophyll in the leaves due to the application of paclobutrazol may be associated with the increase in the amount of chlorophyll per unit leaf area due to the stagnation in the seedlings. Sebastian *et al.* (2002) and Khalil (1995) reported that increased in chlorophyll synthesis when they observed more densely packed chloroplasts per unit leaf area in response to paclobutrazol application. The fact that chlorophyll accumulation is less at high doses than at low doses can be explained by the stress of the seedlings due to the long-term retardation or pause of growth and development in seedlings at high doses of paclobutrazol. Baninasab and Ghobadi (2011) applied 0, 25, 50, and 75 ppm paclobutrazol to cucumber seedlings and determined that paclobutrazol application increased in the amount of chlorophyll, and the amount of paclobutrazol increased in up to 50 ppm dose caused a regular increase in the amount of chlorophyll. However, although the increase in 75 ppm dose application was higher than control plants, it was determined that it was lower than 25 and 50 ppm doses. On the other hand, in G2, chlorophyll values decreased with increasing application doses. The highest value was determined in the control application. Paclobutrazol doses used in our study did not cause any abnormal growth in either genotype. There are results in the literature that paclobutrazol can suppress vegetative growth and increase in leaf chlorophyll content without causing abnormal growth (Chaney, 2004; Jungklang *et al.*, 2017). However, as a result of our study, paclobutrazol application doses had different effects on genotypes in terms of chlorophyll parameter.

Seedling biomass (g plant⁻¹)

According to the general application averages, the minimum biomass ratio was determined in the application with a dose of 2.0 mg L⁻¹. In the application with a dose of 2.0 mg L⁻¹, a decrease in seedling biomass of 71.79% was determined when compared to the control application. The seedling biomass of G1 increased in 15.87% in 1.2 mg L⁻¹ application when compared to the control. Since they were in the same statistical group at 1.2 mg L⁻¹ and other increasing doses, they also increased in the seedling biomass. This situation can be explained by the fact that nutrient and water translocation is improved, a better root structure is formed in plants grown under paclobutrazol application increasing in the biomass production of plants (Kamran *et al.*, 2018; Kuai *et al.*, 2015). The highest dose of paclobutrazol used in a previous study increased in the dry matter accumulation of sesame (*Sesamum indicum* L.) by 21% (Mehmood *et al.*, 2021). The dose increases of paclobutrazol applied to G2 decreased the seedling biomass (Table 3). For the petunia, pansy, geranium and clove plant species, paclobutrazol was significantly reduced plant dry mass (Collado and Hernández, 2021). Ruter (1994) stated that shoot dry weight, root dry weight and total biomass (shoot dry weight + root dry weight) decreased as the amount of paclobutrazol applied to the *Pyracantha coccinea* increased. Dorajeero (2010) reported that treating paclobutrazol at different concentrations increased in the number of branches per plant, the number of flowers per plant, the dry matter weight. Barrios and Ruter (2019) emphasized that increasing doses of paclobutrazol (1, 2, 4, 6 mg pot⁻¹) applied on an ornamental plant known as swamp sunflower (*Helianthus simulans*) were increased in its biomass. Abod and Yasin (2002) reported that root dry mass of *Acacia mangium* seedlings was 12% lower than control plants 12 weeks after exposure to paclobutrazol. In this study, it is thought that the difference determined in seedling biomass between the two genotypes may not be due to paclobutrazol applications. When compared the G2 genotype with the G1 genotype; G1 was found to have a greater ability for dry mass accumulation as well as an increased in number of leaves and leaf area, even under paclobutrazol applications. However, it can be inferred that the biomass determined for G2

plants is better distributed between shoots and root system. And the difference between genotypes can be explained in this way.

Table 3. The effects of the applications on leaf chlorophyll, seedling biomass and leaf area of seedlings getting to the marketing stage

	Treatments	Control	0.6 mg L ⁻¹	0.9 mg L ⁻¹	1.2 mg L ⁻¹	1.5 mg L ⁻¹	2.0 mg L ⁻¹	Mean
Chlorophyll content (SPAD)	G1	34.51±1.53 c***	37.85±1.56 b	37.80±0.68 b	38.35±0.77 b	41.53±0.86 a	38.69±0.80 b	38.12±2.30 ***
	G2	45.29±2.76 a**	44.56±1.14 a	43.94±1.63 a	39.99±0.80 c	42.30±1.95 ab	39.48±0.68 c	42.59±2.67
	Mean	39.90±6.23 BC**	41.21±3.87 AB	40.87±3.54 ABC	39.17±1.14 C	41.92±1.42 A	39.08±0.79 C	
Seedling biomass (g plant ⁻¹)	G1	9.20±0.41 c*	9.03±0.30 c	9.19±0.42 c	10.66±1.27 a	9.58±0.24 ab	10.02±0.17 ab	9.61±0.77 ***
	G2	31.01±2.87 a**	24.45±2.80 b	24.07±2.52 b	22.14±7.28 b	20.28±0.33 b	13.38±1.27 c	22.55±6.21
	Mean	20.10±12.08 A**	16.74±8.63 B	16.63±8.31 B	16.40±7.83B	14.93±5.87 B	11.70±2.01 C	
Leaf area (cm ²)	G1	78.89±0.95 a***	78.92±0.90 a	74.69±1.67 b	71.99±1.39 c	69.59±0.75 d	55.38±1.08 e	71.58±8.29 ***
	G2	137.27±1.08 a***	135.76±1.46 a	121.48±6.85 b	116.23±2.74 b	106.33±1.16 c	74.06±0.556 d	115.19±22.07
	Mean	108.08±31.99 A***	107.34±31.15 A	98.09±26.01 B	94.11±24.31 C	87.96±20.14 D	64.72±10.26 E	

ns: NS: insignificant at $p > 0.05$, statistically significant at the * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ probability level. Note: There is no difference at the 5% significance level between the means denoted by the same letter.

Leaf area (cm²)

According to the general application averages, the minimum leaf area value was determined as 64.72 cm² in the application with a dose of 2.0 mg L⁻¹, and a decrease of 40.12% was detected in the leaf area in the application mentioned when compared to the control application. The highest leaf area values were determined in the G1 genotype, 0.6 mg L⁻¹ and in the control application. The lowest leaf area value was determined in 2.0 mg L⁻¹ application (55.38 cm²). Leaf area decreased by 42.45% in 2.0 mg L⁻¹ application compared to the control application (78.89 cm²). Since gibberellin hormone synthesis was not inhibited in plants that were not applied paclobutrazol (0 ppm), a larger average leaf area was formed in order to enable periclinal cell division and growth in plants. This decrease can be explained by increasing doses of paclobutrazol, as the biosynthesis of the gibberellin hormone was suppressed and resulted in smaller leaf areas. According to the results of previous studies, paclobutrazol applications significantly reduced leaf area of petunia, geranium, pansy and clove plants when compared to seedlings without paclobutrazol application (Collado and Hernández, 2021). Bahlebi *et al.* (2017) have found a 26-29% reduction in leaf area of potted geranium (*Pelargonium* spp.) plants in response to paclobutrazol (100 mg L⁻¹) application. Matsoukis *et al.*, (2001) reported that increasing concentrations of paclobutrazol and triapene-thenol in general led to a significant reduction in leaf area of *Lantana camara* at different shading levels. The results of our research showed parallelism with the results of previous studies (Matsoukis *et al.*, 2001; Bahlebi *et al.*, 2017; Collado and Hernández, 2021).

L*(brightness)

Considering the effect of paclobutrazol on seedling leaf colour, it was determined that the differences in L* (brightness) value between applications and genotypes were statistically insignificant. In addition, when the values of the L* parameter were analysed numerically, a decrease was found in their values compared to the control (Table 4). It has been reported that darker green leaves (lower L* value) are formed in cloves (*Dianthus caryophyllus* L. 'Mondriaan') with paclobutrazol application (Bañón *et al.*, 2002). Kim *et al.* (1999) also stated that paclobutrazol application caused darker green leaves in *Dicentra spectabilis*. Although statistically significant differences were not detected in our study, decreasing numbers can indicate that darker green leaves were obtained. No chlorosis or necrosis was found in the leaves of all plants. However, Hamid and Williams (1997) and Dasoju *et al.* (1998) described various symptoms of phytotoxicity (chlorosis, necrosis, etc.) of paclobutrazol when used in other species. As a result of our study, phytotoxicity was not observed at all doses applied on *G. bicolor* genotypes.

a^* : The “a” value measured from the plant leaves of all applications in the experiment was determined in the negative direction and the leaf colour was determined to be closer to green. In the control application, 7.24% darker green leaves were obtained when compared to the highest dose application. The difference between the genotypes in terms of a^* value was not found to be statistically significant ($p>0.05$) (Table 4). One of the uses of growth inhibitory chemicals is to darken the colour of the green parts of the plant (Whipker, 2013). Although it was not statistically significant in our study, darker green leaves were observed with the highest dose of paclobutrazol.

Table 4. The effect of the applications on the leaf colour parameters of the seedlings getting to the marketing stage

	Treatments	Control	0.6 mg L ⁻¹	0.9 mg L ⁻¹	1.2 mg L ⁻¹	1.5 mg L ⁻¹	2.0 mg L ⁻¹	Mean
L*	G1	48.81±0.68 ^{ns}	47.73±2.72	46.55±1.34	48.49±2.80	46.75±1.61	47.62±2.26	47.66±1.92 ^{NS}
	G2	50.07±1.55 ^{ns}	47.37±0.67	47.39±1.40	47.26±1.28	46.91±0.92	49.49±3.18	48.08±1.91
	Mean	49.44±1.27 ^{NS}	47.55±1.78	46.97±1.31	47.88±2.06	46.83±1.18	48.55±2.67	
a^* (-)	G1	16.66±0.15 ^{ns}	15.62±0.45	15.53±0.48	15.95±0.95	15.19±0.58	14.86±1.10	15.63±0.83 ^{NS}
	G2	16.49±0.53 ^{ns}	15.41±0.49	15.28±0.41	14.71±0.47	14.49±0.95	15.90±1.33	15.38±0.96
	Mean	16.58±0.36 A*	15.52±0.44 B	15.40±0.42 B	15.33±0.97 B	14.84±0.80 B	15.38±1.24 B	
b^*	G1	27.01±3.07 ^{ns}	23.61±1.35	22.39±1.64	23.84±2.52	21.02±1.56	21.68±3.32	23.26±2.83 ^{NS}
	G2	24.08±2.10 ^{ns}	21.81±0.79	20.66±1.15	20.23±1.03	20.31±1.77	23.39±3.39	21.75±2.24
	Mean	25.55±2.85 A*	22.71±1.39 B	21.53±1.58 B	22.04±2.62 B	20.67±1.54 B	22.54±3.15 B	
Chroma value	G1	31.77±2.58 ^{ns}	28.31±1.37	27.25±1.60	28.69±2.61	25.94±1.54	26.29±3.32	28.04±2.77 ^{NS}
	G2	29.20±2.03 ^{ns}	26.71±0.93	25.70±1.17	25.01±1.14	24.95±1.98	28.29±3.52	26.64±2.36
	Mean	30.48±2.51 A*	27.51±1.36 B	26.48±1.52 B	26.85±2.70 B	25.45±1.68 B	27.29±3.25 B	
Hue angle	G1	58.17±2.95 ^{ns}	56.49±0.75	55.21±1.21	56.15±1.27	54.11±1.44	55.38±2.46	55.92±2.02 *
	G2	55.53±1.49 ^{ns}	54.75±0.25	53.50±0.80	53.96±0.64	54.46±0.75	55.65±1.99	54.64±1.25
	Mean	56.85±2.54 ^{NS}	55.62±1.08	54.36±1.31	55.05±1.50	54.29±1.05	55.52±2.01	

ns: NS: insignificant at $p>0.05$, statistically significant at the * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ probability level. Note: There is no difference at the 5% significance level between the means denoted by the same letter.

b^*

The “b” value measured from the plant leaf, taken from all the experimental groups, was determined in the positive direction and the leaf colour was determined to be closer to blue. In the control application, 11,78% darker green leaves were obtained when compared to the highest dose application. The difference between the genotypes in terms of b^* value was not found to be statistically significant ($p>0.05$) (Table 4). The leaves of the paclobutrazol-treated *Consolida orientalis* (eastern hazelnut) plants had darker green and darker violet flower colour than the control plants (Mansuroğlu *et al.*, 2009).

Chroma value and hue angle

It was determined that paclobutrazol applications did not have a significant effect on the color saturation (chroma) of *G. bicolor* leaves (Table 4). Hue angle is expressed between 0 and 360 degrees. It is stated that the outer colour is determined by the hue angle and is better perceived by the human eye (Radzevičius *et al.*, 2009). There was no statistical difference between the application and genotypes in terms of hue angle (Table 4). However, Hamid and Williams (1997) and Dasoju *et al.* (1998) were described various symptoms of phytotoxicity (chlorosis, necrosis, etc.) when paclobutrazol used in other species. No phytotoxicity symptoms were encountered in our study.

In the study conducted by Kaya *et al.* (2012), it has been specified that *G. bicolor* (Freynd&Sint.) Grossh species has a woody stem branching from the base, it is not tall enough to be a cut flower, and it may be appropriate to use this species as a border plant and as a design plant in landscaping works due to its flamboyant, white or pink flowers. With this study, the first phase of creating and/or increasing the potential for use as a

potted plant of *G. bicolor* (Freyn&Sint.) Grossh, which is compatible with our natural species and the ecology of the region, has been completed. In the current study, no problems such as phytotoxicity, delay in production, decrease in seedling quality were encountered on the seedlings of paclobutrazol at different doses applied on *G. bicolor*.

Conclusions

The application in which the highest chlorophyll (SPAD) value was obtained from the application with a dose of 1.5 mg L⁻¹. The chlorophyll value of G2 was higher than that of G1. Seedling biomass of G1 increased in 15.87% in 1.2 mg L⁻¹ treatment when compared to control. Increasing in the dose of paclobutrazol applied to G2 was decreased the seedling biomass. In the present study, darker green leaves were obtained from the highest dose of paclobutrazol, 2.0 mg L⁻¹. Application with a dose of 2.0 mg L⁻¹ decreased the leaf area by 40.12% when compared to the control.

As a general result obtained from the study, 1.5 mg L⁻¹ dose of paclobutrazol is sufficient for both genotypes for plant height control in the seedling period. Seedling growth control has been done effectively. Genotype-specific responses were determined. In the current study, no problems such as phytotoxicity, delay in production, decrease in seedling quality were encountered on the seedlings of paclobutrazol at different doses applied on *G. bicolor*. The aspect of our study that can be suggested for the future is to carry out studies that can examine the plant growth parameters after the seedling period.

Authors' Contributions

Conceptualization: FPK; Methodology and Investigation: FPK, MMK; Project administration and funding: FPK, MMK; Project administration and result analysis: FPK, AD; Methodology and result analyses: FPK; Software and statistical analysis: FPK; Supervision AD. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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