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HORTICULTURAL STUDIES

RESEARCH PAPER

Effects of Different Embryo Development Stages and GA_3 Doses on Germination in Clementine Mandarin × Carrizo Citrange Immature Embryos

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Abstract

Citrus rootstock breeding is important to improve their resistance to diseases, pests and adverse environmental conditions. The majority of citrus species have nuceller embryony. Although Clementine mandarin is monoembryonic, in some cases (intercrossing etc.) abortive seed formation can be seen frequently. In this study, Clementine mandarin × Carrizo citrange were crossed. In the study, interspecies hybridization (*Citrus* × *Poncirus*) was used. Embryo rescue technique was used to prevent the loss of hybrid individuals due to abortive seed formation. The seeds were gathered from the crossed plants 80, 100, and 120 days after pollination. The immature embryos were removed from the seeds and these embryos were transferred to control, 0.5, and 1.0 mg l^{-1} GA_3 containing medium to germination. The highest germination rate with 100% was observed from 1.0 mg l^{-1} GA_3 containing media harvested 120 days after pollination. Additionally, the highest rate of trifoliate seedlings was obtained from embryos gathered after 120 days of the pollination.

1. Introduction

The citrus, which has major importance in the world and Türkiye, is propagated by vegetative and generative methods. However, in particular diseases and because of some soil and climatic conditions rootstock uses is essential in citrus. Therefore, almost all types of citrus are grafted on rootstocks which are grown from seeds and the rootstock has a significant effect on some properties of grafted cultivars. The Mediterranean basin, with world citrus production by 22%, is under threat because of sour orange rootstock uses (Pestana et al., 2005). If CitrusTristeza virus and its pest vector *Toxoptera citricida* is spread, the uses of sour orange rootstock, tolerant to salinity and calcareous soil, will be limited in the Mediterranean region. Therefore, new rootstocks urgently are required as alternatives to the sour orange (Ollitrault et al., 2006).

Monoembryonic diploid varieties are effective when used as the female parent in crosses (Xie et al., 2019). However, the presence of a few monoembryonic parents causes problems in intraspecies or interspecies hybridizations. Although this problem is partially reduced by breeding studies, there is a need to obtain new individuals (Spiegel-Roy and Goldschmidt, 1996). With some exceptions, hybrid breeding is the most used method in rootstock breeding, which includes the same methods as variety breeding, which is difficult, costly and needs in long duration (Barrett, 1985; Cheng and Roose, 1995). Citrus rootstock breeding has focused on crossing a selected male or female parent with a trifoliate, which is still important as a genetic resource recently (Castle, 2010).

Although embryos are produced from such inter species and genus hybridizations in citrus, seed development is halted because the normal embryo

to endosperm ratio is not achieved. In such crosses, different types of seeds are obtained and multiple small embryos are also observed exclusively in partially developed seed (abortive seeds). Embryo rescue is necessary to obtain these genotypes from abortive seeds. In addition, Monoembryonic female parents are widely used in interploid hybridizations in citrus, and then genotypes are obtained by embryo rescue technique (Oiyama and Kobayashi, 1990; Oiyama et al., 1991; Shen et al., 2011).

One of the major problems in citrus breeding is competition between zygotic and nucellar embryos (Soost and Roose, 1996). Generally, to determine hybrid embryo some additional experiments require such as cytological, flow cytometry, isoenzyme analysis or molecular analysis (Tusa et al., 2002). This negative situation is eliminated by *in vitro* embryo rescue techniques for developing embryos. The success of embryo rescue depends on the ingredients of medium and embryo developing stages (Jaskani et al., 2005). The germination capacity of citrus embryos can be affected by the embryo's genetic structure and embryo developing stage (Viloria et al., 2005). Embryos of some citrus species have developed more easily than others in culture, and sometimes there are differences between varieties (Collins and Grosser, 1984; Rangan, 1984; Jia, 1993).

Various studies reported that the addition of 0.01 mg l^{-1} GA_3 (Riberio et al., 2000; Chagas et al., 2005); 0.1 mg l^{-1} GA_3 (Pasqual et al., 1990; Jumin and Nito, 1996; Singh et al., 2020); 1.0 mg l^{-1} GA_3 (Ollitrault et al., 2007; Zhang et al., 2013; Kurt and Ülger, 2014); 1.5 mg l^{-1} GA_3 (Perez-Tornero and Porras, 2008; Soni et al., 2019) and 2.0 mg l^{-1} GA_3 (Gmitter et al., 1990; Turgutoğlu et al., 2015) in growing media for embryos developing of citrus is to be appropriated.

Rangan et al. (1969) studied nuceller embryos developing in common sour orange. They indicated that nuceller embryos developing were not seen in growing seeds in 120 days after anthesis. It was determined depending on the examined species and varieties of citrus that 50 days (Wang et al., 1999); 80 days (Tan et al., 2007); 85 days (Xie et al., 2019); 95 days (Singh et al., 2020); 100 days (Tusa et al., 1996; Deng et al., 1996), 105 days (Scarano et al., 2005; Ferrante et al., 2010), 118 days (Chagas et al., 2005); 120 days (Perez-Tornero et al., 2011; Kurt and Ülger, 2019); 130-140 days (Soni et al., 2019) and 135-150 days (Perez-Tornero and Porras, 2008) after pollination was found to be suitable for embryo rescue.

The objective of the study to determine the effect of different embryo development stages (80, 100 and 120 days after pollination-DAP) and GA_3 concentrations (control, 0.5, and 1.0 mg l^{-1} GA_3) in the culture medium of Clementine mandarin (*Citrus clementina* Hort. ex. Tanaka) × Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] hybrid seeds.

2. Materials and Methods

2.1. Plant materials

Clementine mandarin and Carrizo citrange in the Citrus Genetic Resources Collection located in Batı Akdeniz Agricultural Research Institute were used as plant materials. The study was conducted in 2012. Carrizo citrange was used as the male parent and Clementine mandarin was used as the female parent in the crossing combinations.

2.2. In vitro experiments

Murashige and Tucker (1969) medium was used as a basic culture medium and 50 g l^{-1} sucrose, 25 mg adenine sulfate, and 500 mg l^{-1} malt extract were put in medium. Then, control (0), 0.5, and 1.0 mg l^{-1} GA_3 were supplemented to the prepared medium and medium pH was adjusted to 5.7 and 8.0 g l^{-1} agar was added. After sterilization, the prepared medium was distributed in petri dishes as 40 ml medium containing.

The fruits were taken 80, 100 and 120 days after crossing, were washed with water and detergent, and the fruits were soaked in 70% ethyl alcohol for 5 min and 20% sodium hypochlorite for 30 min to make surface sterilization (Ollitrault et al., 2007). Then, the fruits were cut horizontal. The seeds were removed from the fruit by forceps and immature embryos were taken from the microphyl parts of the seeds by cutting with a surgical blade under binocular (Figure 1a). Two embryos were placed into each petri dishes containing a culture medium. And then, the petri dishes were incubated at 25ºC under 1000 lux light intensity and 16 h photoperiod in a growth chamber. Germinated embryos were counted and the germination rate of embryos was calculated (Figure 1b).

Germinated embryos were sub-cultured Murashige and Skoog (1962) medium containing 0.02 mg l^{-1} NAA and 20 mg l^{-1} sucrose in culture tubes for seedling growing (Perez-Tornero and Porras, 2008). Then the plantlets in culture tubes were incubated at 25ºC under 1000 lux light intensity and 16 h photoperiod in a growth chamber (Figure 2).

The developing plants in the sub-culture were transferred to plastic pots (Figure 3). The plastic pots were put in a chamber with 25-26ºC temperature and 80-85% humidity for two weeks.

Trifoliate seedlings in sub-culture were counted and the rate of trifoliate was calculated. Trifoliate is controlled by two dominant genes in citrus and this feature is shown in the hybrids of zygotic dominant. This feature was taken into consideration when the trifoliate rate was determined. Heterozygous and recessive zygote seedlings are not taken into consideration as they have no trifoliate features.

The developing plants in the sub-culture were measured at 15 days intervals to observe growing

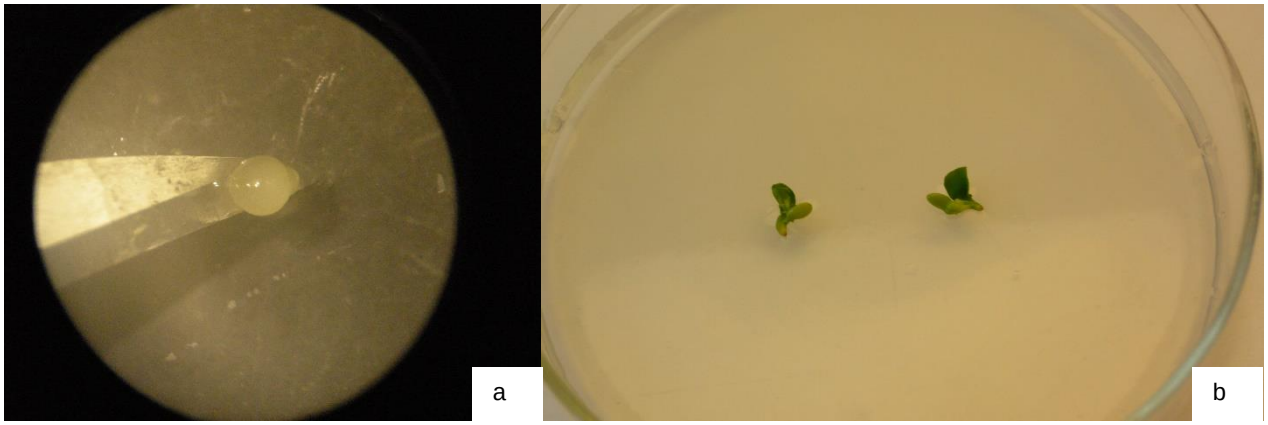

Figure 1. Immature embryo under binocular (a) and germinated embryos (b).

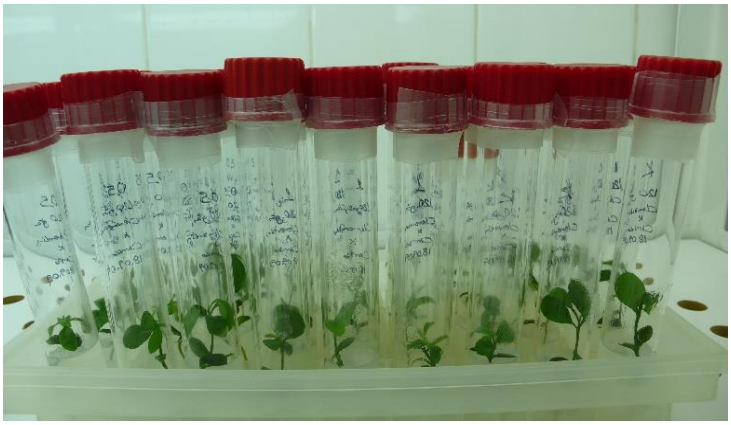

Figure 2. Developed plantlets in the culture tubes.

Figure 3. Hybrid bifoliate and trifoliate seedlings transplanted into plastic pots.

seedlings. As a result of the measurements, the plant height development was evaluated.

2.3. Experimental design and data analysis

The experiment was conducted as random plots with 10 replications and each replication have two embryos. Data were subjected to analysis of variance with mean separation by Least significant difference (LSD) test. Square root transformation was made to data before the compare the percentage values with variance analysis.

3. Results and Discussion

3.1. Germination of embryos

Embryo development stages, GA_3 concentrations in the medium and their interactions were significant to the germination rate of *Clementine mandarin* × *Carrizo citrange* hybrid embryos ($p \leq 0.05$). The highest germination rates in embryos were obtained 120 days later taken after pollination on all embryo stages. The germination rate of embryos that were taken 120 days after

pollination and germinated 1.0 mg l^{-1} GA_3 containing media was found as 100%. The lowest germination rate in embryos was obtained 80 days later taken after pollination with 20% and 22%. (Table 1).

3.2. The rate of trifoliate in plantlets

Trifoliate is controlled by two dominant genes in citrus and this feature is shown in the hybrids of zygotic dominant. The highest trifoliate rate was observed in 120th days with 74% and it was followed by 100th days later taken after pollination with 18%. The lowest rate was found in the 80th day with 8%. (Figure 4). In the study, this feature was taken into consideration when the trifoliate rate was determined. Heterozygous and recessive zygote seedlings are not taken into consideration since they have no trifoliate features.

3.3. The growth of the seedling's height

According to Figure 5, the growth of seedlings height occurred as a linear increase at all embryo development stages and all GA_3 doses. It observed that the growth of seedlings' height taken from 120 days after pollination was higher than other DAP at all GA_3 doses.

Table 1. The effect of embryo development stages and GA_3 concentration on germination rate.

Days after pollination (DAP)	GA_3 doses (mg l^{-1})			Average of days after pollination
	Control	0.5	1.0	
80th DAP	20.00 g *	22.00 g	30.00 f	24.00
100th DAP	15.00 h	42.50 d	35.00 e	30.83
120th DAP	82.50 c	92.50 b	100.00 a	91.67
Average of GA_3 doses	39.17	52.33	55.00	

*Different letters indicate significant differences (P<0.05) according to the Least Significant Difference test (LSD: 3.2346).

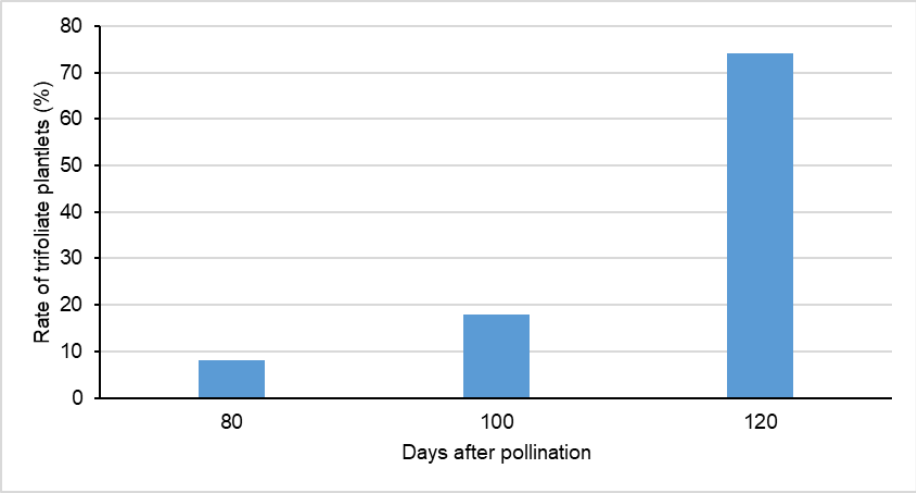

Figure 4. The effect of embryo development stages on the rate of trifoliate plantlets.

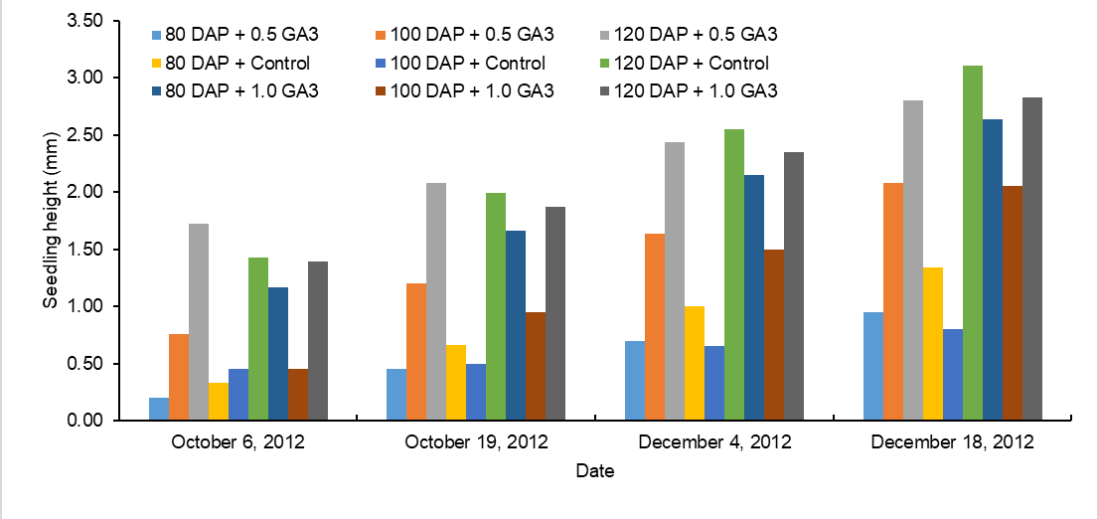

Figure 5. The effect of embryo development stages and GA_3 concentration on seedling height.

According to the experiment results, it was postulated that there was a relation between embryo development stages and embryo germination since the highest germination results were obtained 120 days after pollination in all GA_3 doses. In accordance with the results, Carimi et al. (1998), Viloria et al. (2005), Perez-Tornero et al. (2011) and Kurt and Ülger (2014) had good embryo germination from 120 DAP has taken embryos and they were indicated that genetic and embryo developing stages were effected the germination capacity of embryos. On the other hand, there were some results that good embryo germinations were obtained in 50 days (Chen and Wang 1986; Wang et al., 1999), 80 days (Tan et al., 2007), 85 days (Xie et al., 2019), 95 days (Singh et al., 2020), 100 days (Deng et al., 1996; Tusa et al., 1996), 105 days (Ferrante et al., 2010) and DAP has taken embryos after pollination. This may be due to the growing location and cultivars used.

Since the best results were obtained from 1.0 mg l^{-1} GA_3 containing medium 120 days after pollination has taken embryos, it showed that this dose and embryo development stage was appropriated for germination of embryos. Similarly, Button and Kochba (1977), Kunitake et al. (1991), Carimi et al. (1998), Das et al. (2000), Wakana et al. (2004), Jaskani et al. (2005), Ollitrault et al. (2007) and Zhang et al. (2013) studied in different citrus species and cultivars and they indicated that adding of 1 mg l^{-1} GA_3 to the medium was given good results in the germination of embryos in citrus. Some experiments reported that 0.01 mg l^{-1} GA_3 (Ribeiro et al., 2000; Chagas et al., 2003), 0.1 mg l^{-1} GA_3 (Pasqual et al., 1990; Jumin and Nito 1996; Singh et al., 2020) and 2.0 mg l^{-1} GA_3 (Gmitter et al., 1990; Turgutoğlu et al., 2015) appropriated for embryo germination of citrus.

4. Conclusion

In this study was found that 1.0 mg l^{-1} GA_3 dose and 120 DAP development stage appropriated for immature embryo rescue. In addition, it was determined that the best embryo rescue time was 120 days after pollination since germination and trifoliate seedling rate were higher than others. Immature embryo culture, which is a preferred and valuable method in parallel with the advances in classical and genetic breeding studies in citrus, creates an important potential in the development of superior new varieties and shortening the breeding period.

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RESEARCH PAPER

Earliness, Yield, and Fruit Quality Attributes of Low-Chilling Peach-Nectarine Cultivars with the Application of Low Biuret Urea and Calcium Nitrate

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Abstract

This study aimed to determine the effects of low biuret urea and calcium nitrate application on earliness, fruit set, yield, and fruit quality characteristics in 'Astoria', 'Maya' peaches, and 'Garbaja' nectarine cultivars. In the study, Bud Feed (low biuret urea 15%) and calcium nitrate (calcium oxide 12% and total nitrogen 7%) were applied 35 days before bud break. In this study, flowering and fruit set, harvest times, fruit yield, and quality characteristics were evaluated. The source of temperatures used to calculate chill accumulation (expressed as hours below 7°C and chill unit) and growing degree hours was investigated. Bud Feed application provided earliness of 2 days in 'Astoria' and 'Garbaja' cultivars and 3 days in 'Maya' cultivar. This application was showed positive effect on flowering and final fruit set in all cultivars compared to control plants. The application was more effective in increasing the yield per tree by 33.72% ('Astoria'), 41.00% ('Maya'), and 52.18% ('Garbaja'). Bud Feed application was improved fruit size in 'Garbaja' and 'Astoria' cultivars, whereas provided more intense fruit skin color in 'Maya' and 'Garbaja' cultivars. These results showed that bud feed and calcium nitrate application can be used to prevent yield and fruit quality losses in peach-nectarines in warm winter under Mediterranean climate conditions.

1. Introduction

Türkiye has a wide range of products in terms of horticultural crops due to its geographical location and different climate and soil characteristics (Tüzel and Öztekin, 2015). It has wide ecological differences from the temperate climate to the Mediterranean climate, allowing for early, mid-season, and late-season cultivation (Bayazit et al., 2021; Çalışkan et al., 2021a).

Peach-nectarines are one of the most produced fruits in the world after apples. Early peach-nectarine cultivars with attractive fruits and regular yields have increased remarkably, in recent years. The Southern Aegean and Mediterranean Regions of Türkiye have a subtropical climates. Due to their climatic characteristics, these regions have large areas and advantages in early fruit growing with low chilling requirements. Early peach production leads to high economic value in April and May under both protected and open areas in this region. Currently, the open vase system is commonly used as the orchard system for peach cultivation in Türkiye. However, diverse high-density systems are used for commercial peach production (Çalışkan et al., 2021a).

Türkiye produces 3.23% of the world's peach-nectarine production with 830,577 tons. (FAO, 2022). The Mediterranean region produces 25.76% of this production. The Mediterranean coastline of Turkey is quite a favorable location for early fruit cultivation because of the advantages of its favorable ecology. In this region, the cultivar of some stone fruit species such as peach, apricot,

and plum ripen about 10-15 days earlier than other areas of Türkiye as well as from important fruit growing countries of Europe such as Spain and Italy. (Caliskan et al., 2012). This is due to differences in latitute, considering that one latitute degree leads to 4-5 days of earliness or delay. (Kaşka et al., 1981).

Fruit consumers prefer cultivars having high quality in the fresh fruit market. The most crucial point is that the fruits that arrive early to the market are preferred because they do not have alternatives and, as a result, are sold at high prices (Erez et al., 2000).

Shortly, it is predicted that peach-nectarine production will increase, especially in the Mediterranean region. The relatively warmer winters in this region show that we should consider the chilling requirements of the cultivars (Kaşka, 2001; Çalışkan et al., 2021a). Serious problems such as irregular flowering, bud drop, bareness on the branch, insufficient fruit set, and decrease in yield occur in stone fruit species such as peach/nectarine with the insufficient chilling requirement (Weinberger, 1950; Viti et al., 2013). Especially as a result of the fluctuations in winter temperatures in the Eastern Mediterranean Region of Türkiye, the chilling durations that take place over 700 hours over the last 40 years decreased to less than 400 hours in the last years. This fluctuation is a serious problem for early stone fruit species in the region.

Campoy et al. (2011) indicated that if global warming continues, the problems in meeting the chilling requirements of plants will continue to increase due to the reduction in the winter cold. However, in case of insufficient chilling duration in fruit species, adequate fruit set and yield can be obtained with applications (such as DNOC, mineral oils, hydrogen cyanamide, gibberellins, KNO_3, $CaNO_3$, thiourea) that allow the plant to come out of rest (Engin et al., 2004; Son and Küden, 2005; Zhuang et al., 2015; Imrak et al., 2016). Hydrogen cyanamide, which is the most effective of these applications, is banned by many countries as it is in Türkiye, for human health and environmental pollution. Therefore, it is necessary to explore alternate applications.

This study aims to determine the effects of Bud Feed application on fruit set, yield, and fruit quality in early 'Astoria', 'Maya' peach, and 'Garbaja' nectarine cultivars.

2. Material and Method

2.1. Plant materials and experiment design

This study was carried out in the research and application area of Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay, Türkiye, in the 2019-2020 season. In the study, 'Astoria' and 'Maya' peaches and 'Garbaja' nectarine (PSB Producción Vegetal, Spain) that grafted on the rootstock of Garnem (*Prunus dulcis* × *Prunus persica*) were used. The saplings were planted at 2.5 m × 3.0 m in rows and spacing in May 2017.

In cultivation, an open vase with four main branches was used with a wire support system. This system has 20-25 fruit branches on each main branch and the branches were cut regularly every year, 2–3 on buds after harvesting (Hoying et al., 2007). Technical and cultural processes such as fertilization, disease, and pest control of the research area were applied as standard. The soil pH of the study area was 7.81, and the soil structure has a sandy-clay structure with 39.5% sand, 25.3% clay, and 6.10% lime content. The total salt content of the soil was in the salt-free class with values of 0.035-0.041%.

2.2. Treatment

In the study, Bud Feed (Stoller Türkiye, İzmir) application containing 15% low biuret urea was applied as 6 L 100 L^{-1} in three replications and one plant in each replication. The application was carried out 35 days before the bud burst (January 10). In addition, 5 L 100 L^{-1} $CaNO_3$ (including calcium oxide 12% and total nitrogen 7%) was added to increase the effectiveness of the application (Çalışkan et al., 2021b). Control trees were sprayed with water. The period in which 50% of the bud burst was taken as the exit date from the rest (Küden and Kaşka, 1992).

2.3. Heat requirements

Heat requirements were investigated by adding the growing degree hours (GDH), and temperatures above 4.5°C were taken into account (Richardson et al., 1975). Temperatures above 25°C were not taken into account in this assessment. Growth temperature totals were calculated as GDH1 (up to 30 days after full bloom) and GDH2 (from full bloom to harvest).

2.4. Phenological stages

Budburst, first flowering (5% flowering), full flowering (70% flowering), and end of flowering (90% petal fall) were observed. In addition, flowering rate (%), first fruit set rate (%), and final fruit set rate (%) were evaluated according to Westwood (2009).

2.5. Fruit quality and Yield Parameters

Peach fruits were harvested as described by Kader (1999) when the cultivar-specific color and fruit size were formed and the amount of total soluble solids (TSS) exceeded 10%. Fruit quality analyzes were carried out on a total of 30 fruits with three replications and 10 fruits in each replication.

These analyzes included the fruit weight (g), fruit dimensions (diameter, length, and height; mm), seed weight (g), flesh/seed ratio, total soluble solids (TSS), pH, and titratable acid (%) measurements. Fruit skin and flesh color measurements were evaluated by colorimeter (CR-300, Minolta) as L, a*, b*, *C* (Chroma), and *h*° (hue) values. Fruit skin and flesh colors were measured in two opposite directions for each fruit (Caliskan et al., 2012). Yield characteristics such as yield per tree (kg tree^{-1}), yield per trunk cross-sectional area (kg cm^{-1}), and yield per hectare (t ha^{-1}) were investigated.

2.6. Statistical evaluation

The data on the effects of Bud Feed application on fruit set, yield, and fruit quality characteristics of each variety were compared with the T-test in the SAS package program (SAS Institute, Cary, NC, USA). In addition, the LSD multiple comparison test (P<0.05) was used to compare the cultivar averages.

3. Results and Discussion

3.1. Phenological observations and temperature data

The effects of Bud Feed application on the phenological stages of 'Astoria', 'Maya', and 'Garbaja' cultivars were presented in Figure 1. In 'Astoria', Bud Feed application provided 2-day earliness in bud bloom, budburst, and full bloom periods (February 10, 13, and 28, respectively), compared to control plants, while it provided 3-day earliness in the first flowering and end-bloom periods of the variety (21 February and 02 March, respectively). Harvest time for this cultivar with Bud Feed application was 2 days earlier than the control plants. While Bud Feed application was 2 days earlier (11 and 22 February, 8 May, respectively) for the first flowering and harvest date compared to control plants in the 'Maya' cultivar, this application resulted in 3 days earliness (29 February) in the full flowering of the cultivar (Figure 2).

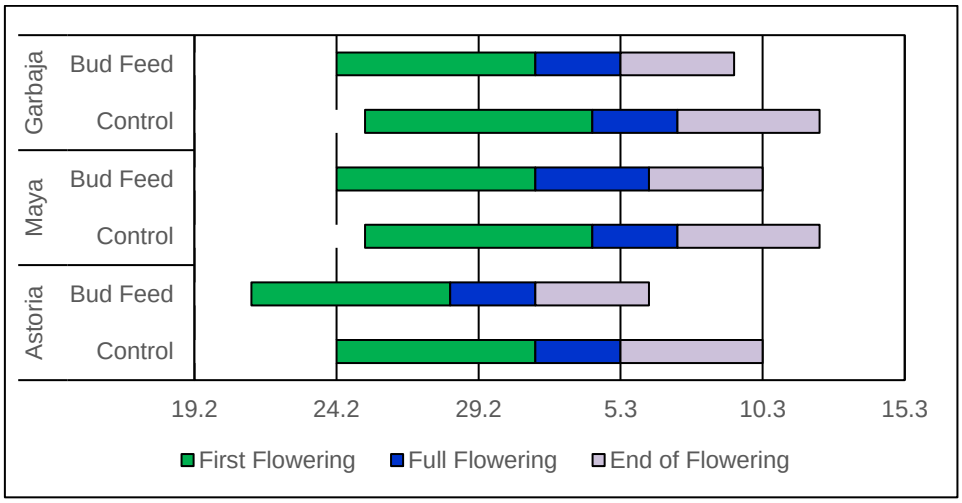

Figure 1. Effect of Bud Feed application on some phenological stages of 'Astoria', 'Maya', and 'Garbaja' cultivars.

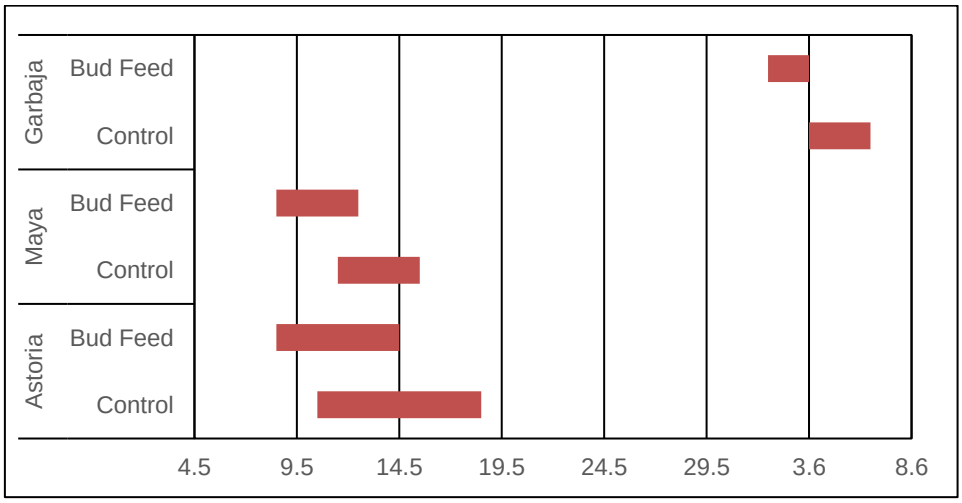

Figure 2. Effect of Bud Feed application on harvest times of 'Astoria', 'Maya', and 'Garbaja' cultivars.

The effect of Bud Feed application on phenology stages in 'Garbaja' revealed as earliness between 1 and 3 days compared to the control. These results were consistent with the results of other researchers (George et al., 1992; Tang et al., 2019). In addition, Eroğul et al. (2021) reported that Bud Feed + Sett (12% Cao+0.5% B) application 30 days before flowering in '0900 Ziraat' cherry cultivar was 7-8 days earlier in full blossoming compared to control plants. However, Campoy et al. (2011) indicated that bud break treatments such as hydrogen cyanamide and winter oil did not affect flowering time in the 'Early Maycrest' peach cultivar. These differences show that the results may differ depending on the fruit species, genotype and application time, and intensity of the applications. In addition, Viol et al. (2022) showed that long flowering periods are symptoms of insufficient chilling during the winter in peach grown in subtropical regions. Bud Feed applied peach-nectarines were harvested 2-3 days earlier than control plants. The data was in agreement with the results of George et al. (1993), who indicated that low chilling peaches applied to bud break were harvested 1-3 days earlier than control plants. Besides, Singh and Banyal (2020) reported that bud break applications in early peach cultivars showed earliness up to 13-14 days. This effect may be due to the early flowering of the trees, the rapid occurrence of phenological stages, and climatic conditions. In the 'Astoria' cultivar, chilling duration was 349 chill hour (CH) and 542 chill unit (CU) in control plants, while it was 337 CH and 518 CU in Bud Feed treated trees (Table 1). However, the chilling duration of 'Maya' and 'Garbaja' cultivars occurred as 354 CH and 552 CU occurred in control plants, whereas it occurred as 349 CH and 542 CU in Bud Feed applied plants. This result is because Bud Feed applied to peach-nectarine cultivars provides 1 to 2 days earlier bud bursting. Campoy et al. (2011) indicated that the correct application of dormancy-breaking agents is critical for the reveal the effect of the applications and preventing phytotoxicity.

The 3-day early maturation that occurs with Bud Feed application in all cultivars was because of the growing degree hours in the first 30 days (GDH1) after full bloom (Lopez and Dejong, 2007) and the growing degree hours from full bloom to harvest (GDH2) (Bolat and Ikinci, 2020) are higher. Indeed, the GDH1 values of 'Astoria', 'Maya', and 'Garbaja' cultivars applied in Bud Feed were 7.875, 7.528, and 8.395, respectively, whereas it was 7.632, 7.395, and 6.392, respectively in control plants of these cultivars (Table 2). Similarly, GDH2 values of 'Astoria', 'Maya', and 'Garbaja' cultivars (18.022, 17.973, and 25.246, respectively) applied Bud Feed was found to be higher than the GDH2 values of control plants (17.792, 17.963, and 23.021, respectively). These results were in agreement with Çalışkan et al. (2021c), who showed that the GDH1 and GHD2 values of the 'Madison' apricot cultivar were higher in trees treated with Bud Feed + $CaNO_3$, resulting in early fruit ripening.

3.2. Flowering and fruit set rates

The effects of Bud Feed application on fruit set and yield characteristics of 'Astoria', 'Maya' and 'Garbaja' cultivars were statistically significant (Table 3). Bud Feed application increased flowering rates (81.82%, 83.43%, and 77.78%, respectively) in 'Astoria', 'Maya', and 'Garbaja' cultivars compared to the control (66.44%, 80.40%, and 55.15%, respectively). According to the average of the cultivars, the flowering rate (81.92%) in the 'Maya' cultivar was higher than in the 'Astoria' (75.33 %) and 'Garbaja' (66.47%) cultivars.

The first fruit set rate in all cultivars was higher in trees treated with Bud Feed (15.75%, 6.25%, and 5.25%, respectively) than in the control plants. Similarly, bud feed application increased the final fruit set relative to control plants. According to the average data, the first fruit set and the final fruit set percentages were higher in 'Astoria' (11.00% and 8.38%, respectively) than in 'Maya' (5.44% and 3.48%, respectively) and 'Garbaja' (4.50 and 2.40, respectively) cultivars. Westwood (2009) reported

Table 1. Effect of Bud Feed and calcium nitrate application on the chilling duration of cultivars until bud break (2019-2020 season).

Months	Astoria			
	Bud Feed		Control	
	Chill hour	Chill unit	Chill hour	Chill unit
December	102	121	102	121
January	170	236	170	236
February	65	161	77	185
Total	337	518	349	542
Months	Maya and Garbaja			
	Bud Feed		Control	
	Chill hour	Chill unit	Chill hour	Chill unit
December	102	121	102	121
January	170	236	170	236
February	77	185	82	195
Total	349	542	354	552

Table 2. Effect of Bud Feed and calcium nitrate application on growing degree hours (GDH) of cultivars.

Months	Astoria			
	Control		Bud Feed	
	GDH1	GDH2	GDH1	GDH2
February	-	-	0.232	0.232
March	6.880	7.345	7.340	7.343
April	0.752	7.592	0.303	7.595
May	-	2.855	-	2.852
June	-	-	-	-
Total	7.632	17.792	7.875	18.022
Months	Maya			
	Control		Bud Feed	
	GDH1	GDH2	GDH1	GDH2
February	-	-	0.182	0.182
March	6.632	7.344	7.346	7.343
April	0.763	7.591	-	7.595
May	-	2.858	-	2.853
June	-	-	-	-
Total	7.395	17.793	7.528	17.963
Months	Garbaja			
	Control		Bud Feed	
	GDH1	GDH2	GDH1	GDH2
February	-	-	-	-
March	6.392	7.341	7.348	7.347
April	-	7.592	1.047	7.595
May	-	7.446	-	7.446
June	-	0.642	-	2.858
Total	6.392	23.021	8.395	25.246

Table 3. Effects of Bud Feed and calcium nitrate application on fruit set and yield characteristics of cultivars.

Cultivars	Application		Mean
	Bud feed	Control	
	Flowering (%)		
Astoria	81.82 a[(1)]	69.44 b	75.33 b[(2)]
Maya	83.43 a	80.40 b	81.92 a
Garbaja	77.78 a	55.15 b	66.47 c
	Initial fruit set (%)		
Astoria	15.75 a	6.25 b	11.00 a
Maya	6.25 a	4.63 b	5.44 b
Garbaja	5.25 a	3.75 b	4.50 b
	Final fruit set (%)		
Astoria	12.75 a	4.00 b	8.38 a
Maya	3.63 a	3.33 b	3.48 b
Garbaja	2.50 a	2.29 b	2.40 b
	Yield (kg tree^{-1})		
Astoria	33.95 a	22.50 b	28.23 a
Maya	15.73 a	9.28 b	12.51 b
Garbaja	10.35 a	4.95 b	7.65 b
	Yield (kg cm^{-2})		
Astoria	0.33 a	0.24 b	0.28 a
Maya	0.13 a	0.06 b	0.09 b
Garbaja	0.09 a	0.06 b	0.07 b
	Yield (t ha^{-1})		
Astoria	56.3 a	37.3 b	46.5 a
Maya	26.1 a	15.4 b	20.7 b
Garbaja	17.1 a	8.2 b	12.6 b

[(1)] Means followed by equal letters, in the rows, do not differ by Student's T, at 5% probability.
[(2)] Means followed by equal letters, in the columns, do not differ by LSD test, at 5% probability.

that fruit sets should be between 10-15% for adequate yield in peach. The 'Astoria' cultivar in this study was included in the specified rates. However, fruit set rates were low in 'Maya' and 'Garbaja' cultivars. This is due to chilling deficiency leading to poor-uneven bud break, poor foliage, and abnormal flowers, resulting in poor fruit set (Erez, 1987; Çalışkan et al., 2021a). The results from this study were consistent with the results that bud-break treatments increased flowering (Campoy et al., 2011) and fruit set formation (Mohamed and Sherif, 2015; Kotb et al., 2019). However, Chen and Beckman (2019) reported that the effects of bud break application can be changed to genotype, tree

Table 4. Effect of Bud Feed and calcium nitrate application on fruit quality characteristics of cultivars.

Cultivars	Application		Mean
	Bud Feed	Control	
	Fruit weight (g)		
Astoria	171.23 a[(1)]	154.97 b	163.10 a[(2)]
Maya	143.38 a	133.58 b	138.48 b
Garbaja	163.68 a	158.87b	161.28 a
	Fruit diameter (mm)		
Astoria	76.1 a	67.5 b	71.8 c
Maya	68.6 b	89.7 a	79.2 b
Garbaja	94.3 a	92.2 a	93.2 a
	Fruit length (mm)		
Astoria	75.7 a	68.3 b	71.9 c
Maya	65.8 b	87.5 a	76.6 b
Garbaja	95.0 a	94.7 b	94.9 a
	Fruit height (mm)		
Astoria	73.0 a	63.7 b	68.4 b
Maya	63.8 a	79.5 b	71.6 b
Garbaja	87.9 b	89.1 a	88.5 a
	Fruit firmness (kg-force)		
Astoria	5.83 b	8.13 a	6.98 a
Maya	7.02 b	7.03 a	7.03 a
Garbaja	4.97 a	4.73 b	4.85 b
	Seed weight (g)		
Astoria	10.72 a	10.56 b	10.64 b
Maya	10.82 a	10.04 b	10.43 b
Garbaja	12.36 a	12.01 b	12.19 a
	Flesh/seed ratio (%)		
Astoria	15.03 a	13.80 b	14.42 a
Maya	12.25 b	12.31 a	12.28 b
Garbaja	12.25	12.25	12.25 b
	Total soluble solids (%)		
Astoria	10.17 a	9.83 b	10.00 b
Maya	10.17 b	10.43 a	10.30 b
Garbaja	11.87 a	10.55 b	11.21 a
	pH		
Astoria	3.32 a	3.21 b	3.27 a
Maya	3.33 a	3.27 b	3.30 a
Garbaja	3.05 b	3.11 a	3.08 b
	Acidity (%)		
Astoria	1.19 b	1.23 a	1.21 b
Maya	0.93 b	1.00 a	0.97 c
Garbaja	1.68 a	1.35 b	1.52 a

[(1)] Means followed by equal letters, in the rows, do not differ by Student's T, at 5% probability.
[(2)] Means followed by equal letters, in the columns, do not differ by LSD test, at 5% probability.

age, shoot type, and application time. In addition, nitrogen compounds, including amino acids, were constituted at low levels in the buds during the dormancy stage and reached maximum levels just before bud opening (Imrak et al., 2016). An increase in the amino acids contents, such as proline and arginine, and of growth-promoting hormones, such as auxins and gibberellins, occurred in the buds after the bud break applications (Seif El-Yazal et al., 2014). This can be associated with an increase in flowering and fruit set of bud break applications.

3.3. Fruit quality characteristics

The results of the effects of Bud Feed application on fruit quality in 'Astoria', 'Maya', and 'Garbaja' cultivars were presented in Table 4. Fruit weight values of 'Astoria', 'Maya', and 'Garbaja' cultivars were higher in Bud Feed application (171.23 g, 143.38 g, 163.68 g, respectively) than in plants of these cultivars in control. The 'Astoria' and 'Garbaja' cultivars had the highest average fruit weight (163.10 g and 161.28 g, respectively). Bud Feed applied 'Garbaja' and 'Astoria' cultivars had the highest fruit diameter (94.3 and 76.1 mm, respectively) and fruit length (95.0 and 75.7 mm, respectively).

The highest fruit height in the 'Astoria' (73.0 mm) and 'Maya' (63.8 mm) was found in Bud Feed treated plants. According to the average data, fruit diameter, fruit length, and fruit height were highest in the 'Garbaja' cultivar (93.2, 94.9, and 88.5 mm, respectively). Fruit size in peaches is one of the most important quality parameters and the increase in fruit size positively affects the commercial value of the fruit. The genetic capacity of the cultivar, fruit thinning, and cultural applications affect the fruit size of the peach (Crisosto and Costa, 2008).

Increasing fruit size, especially in varieties suitable for early cultivation, is one of the critical factors in cultivation. In this study, positive Bud Feed on fruit quality characteristics of peach cultivars will create useful results in practice. Bound and Jones (2004) reported that since fruit from early-blooming flowers has a faster initial growth rate than fruit from later flowers, progress in flowering may result in larger fruit. Similarly, Eroğul et al. (2021) showed that the fruit weight of the control trees in the '0900 Ziraat' cherry cultivar was 8.63 g, while it increased to 10.69 g in Bud Feed + 12% Cao + 0.5% B application. These results were similar to those obtained by Ferreira et al. (2022), who indicated that bud break applications in peach have the effect of increasing fruit weight and size. Besides, our results were in agreement with the results of the different fruit species such as apple (Chauhan et al., 2018), fig (Gaaliche et al., 2017), and apricot (Çalışkan et al., 2021b).

Flesh firmness was highest in 'Astoria' and 'Maya' cultivars in control plants (8.13 kg-force and 7.03 kg-force, respectively), whereas it was highest for 'Garbaja' (4.97 kg-force) in Bud Feed applied plants. Comparing the average fruit firmness, 'Maya' and 'Astoria' cultivars had firmer fruits (7.03 kg-force and 6.98 kg-force, respectively). Bud Feed application increased the seed weight (10.72 g, 10.82 g, and 12.36 g, respectively) in all cultivars compared to the control. The 'Garbaja' cultivar had the highest average seed weight (12.19 g). The highest flesh/seed ratio was found in Bud Feed application (15.03%) in the 'Astoria' cultivar, while it was the highest in control (12.31%) for the 'Maya' cultivar. The effect of bud feed application on flesh/seed ratio in the 'Garbaja' was statistically insignificant. 'Astoria' had the highest average flesh/seed ratio (14.42%). Similarly, Mohamed and Sherif (2015) indicated that the application of hydrogen cyanamide in the 'Florda Prince' peach cultivar decreased the fruit firmness. Our data was compatible with the results of Çalıskan et al. (2021b), that Bud Feed application reduced the fruit firmness of apricot cultivars.

While the highest TSS content was measured in 'Astoria' and 'Garbaja' cultivars (10.17% and 11.87%, respectively) applied Bud Feed, the highest TSS content in 'Maya' cultivar was measured in control fruits (10.43%). The highest average TSS content was in the 'Garbaja' cultivar (11.21%). These results were similar to the findings of Mohamed & Sherif (2015) and Kotb et al. (2019) that bud break applications increased TSS content in peach.

The highest pH values were detected in 'Astoria' and 'Maya' (3.32 and 3.33, respectively) treated with Bud Feed (Table 4). However, the highest pH in the Garbaja cultivar (3.11) was found in the control fruits. According to the average pH values, 'Maya' and 'Astoria' cultivars had the highest pH values (3.30 and 3.08, respectively). Bud Feed applications were reduced titratable acidity in 'Astoria' and 'Maya'' cultivars (1.23% and 1.00%, respectively). According to the cultivar average, titratable acidity was the highest in 'Garbaja' (1.52%), whereas it was lowest in 'Maya' (0.97%). These results showed that the effect of the bud feed application on the acid content of the fruit varied depending on the cultivar. Ferreira et al. (2022) displayed that nitrogen fertilizer and calcium nitrate treatments in the 'Douradão' peach cultivar increased the acid content of the fruit. Bettiol Neto et al. (2014) reported that the effects of bud break treatments on fruit quality may be due to the uniformity and density of bud growth, flowering, and fruiting, and decreasing the harvest period. In addition, Leonel et al. (2014) showed that the chemical content of the fruit, such as soluble solids, pH, and titratable acidity is probably associated with the shortening of the harvest time, which can have a positive or negative effect, based on the climatic conditions in the fruit development stages.

Bud Feed application in peach-nectarine cultivars affected fruit skin and flesh color at different levels depending on the cultivar (Table 5). Bud Feed application increased the fruit skin color brightness (L) for 'Maya' and 'Garbaja' cultivars (47.83 and 73.49, respectively) compared to control (45.73 and 66.31, respectively). However, the L value for the 'Astoria' cultivar was higher in the control (56.68) than in the Bud Feed application (55.00). In the comparison of the averages of the cultivars, the 'Garbaja' cultivar had a brighter fruit skin color (69.90). The highest a* value showing red (positive value)-green color (negative value) in 'Astoria' was obtained from the Bud Feed application (20.67), while the highest a* value in 'Maya' and 'Garbaja' were obtained from the control (31.19 and 32.68, respectively). The mean a* value of 'Maya'' and 'Garbaja' cultivars (31.03 and 28.89, respectively) was higher than 'Astoria' (19.20). Positive b* value showing yellow color was highest in 'Maya' and 'Garbaja' cultivars applied Bud Feed (25.32 and 18.91, respectively) compared to control. The average b* value was higher in the 'Astoria' cultivar (31.98). The *C* value indicating the intensity of the color (lower values indicate the intensity of the color) was more intense in 'Maya' and 'Garbaja' cultivars (39.96 and 32.55, respectively) applied Bud Feed than in control. However, the most intense fruit skin color was detected in the control plants (39.50) in the 'Astoria'. According to the average C values, the 'Garbaja' (32.90) had a more intense fruit skin color. Fruit skin color *h*° value was higher (68.00 and 39.60) in Bud Feed application for 'Garbaja' and 'Maya' cultivars. It was higher in the control (39.58) for 'Astoria'. The average *h*° value of the cultivars was the highest in 'Garbaja' (62.43).

The brightest flesh color (L value) of 'Astoria' and 'Garbaja' cultivars was determined in the Bud Feed application (79.07 and 72.68, respectively). In the 'Maya' cultivar, the brightest fruit flesh color value was in control (79.16). The mean L value in the fruit

Table 5. Effect of Bud Feed application on fruit skin and flesh color characteristics of cultivars.

Cultivars	Application		Mean
	Bud Feed	Control	
	Fruit skin color		
	L		
Astoria	55.00 b[(1)]	56.68 a	55.84 b[(2)]
Maya	47.83 a	45.73 b	46.78 c
Garbaja	73.49 a	66.31 b	69.90 a
	a*		
Astoria	20.67 a	17.72 b	19.20 b
Maya	29.87 b	32.19 a	31.03 a
Garbaja	25.09 b	32.68 a	28.89 a
	b*		
Astoria	31.43 b	32.53 a	31.98 a
Maya	25.32 a	24.02 b	24.67 b
Garbaja	18.91 b	20.72 a	19.82 c
	C		
Astoria	39.58 a	39.50 b	39.54 a
Maya	39.96 b	40.77 a	40.37 a
Garbaja	32.55 b	39.25 a	35.90 b
	h°		
Astoria	55.35 b	60.38 a	57.87 b
Maya	39.60 a	35.82 b	37.71 c
Garbaja	68.00 a	56.85 b	62.43 a
	Fruit flesh color		
	L		
Astoria	79.07 a	77.12 b	78.10 a
Maya	78.91 b	79.16 a	79.04 a
Garbaja	72.68 a	71.01 b	71.85 b
	a*		
Astoria	-7.19 b	-6.03 a	-6.61 b
Maya	-7.56 b	-6.38 a	-6.97 b
Garbaja	-7.31 b	-7.23 a	-7.27 a
	b*		
Astoria	51.30 a	50.84 b	51.07 a
Maya	50.96 a	50.70 b	50.83 a
Garbaja	43.40 a	41.79 b	42.60 b
	C		
Astoria	51.82 a	51.26 b	51.54 a
Maya	51.53 a	51.11 b	51.32 a
Garbaja	47.39 a	43.02 b	45.21 b
	h°		
Astoria	97.99 a	96.67 b	97.33 a
Maya	98.45 a	96.05 b	97.25 a
Garbaja	94.20 a	93.75 b	93.98 b

[(1)] Means followed by equal letters, in the rows, do not differ by Student's T, at 5% probability.
[(2)] Means followed by equal letters, in the columns, do not differ by LSD test, at 5% probability. LSD, least significant difference.

flesh of 'Astoria' and 'Maya' cultivars (78.10 and 79.04, respectively) was higher than 'Garbaja' (71.85). The negative a* value representing green color was the lowest in the control (-6.03, -6.38, and -7.23, respectively) in all cultivars (Table 5). Similarly, according to the cultivar averages, 'Astoria' and 'Maya' cultivars had the lowest flesh color a* value (-6.61 and -6.97, respectively). Fruit flesh color was more yellow (b*) in 'Astoria' and 'Maya', and 'Garbaja' cultivars treated with Bud Feed (51.30, 50.96, and 43.40, respectively). 'Astoria' and 'Maya' cultivars had the highest flesh color b* value. However, in all cultivars, the flesh color density was the highest in the control fruits with low C and *h*° values. Also, the 'Garbaja' had the most intense fruit flesh color.

In peach-nectarines, fruit skin color characteristics are used to determine fruit maturity and harvest time. In addition, fruit color in peaches-nectarines is one of the important characteristics that affect consumer preferences (Crisosto and Costa, 2008). In this study, Bud Feed application had positive effects on the formation of red color on the fruit skin, but this differed depending on the cultivar. These findings were similar to the results obtained by Çalışkan et al. (2021b), who showed that the Bud Feed application in 'Mikado' and 'Mogador' cultivars increased the orange color intensity as well as the red color formation in the fruit peel. Raffo et al. (2014) reported that optimal color intensity in the 'New Star' cherry cultivar treated bud break applications for harvest one week was earlier

than the control plants. However, Eroğul et al. (2021) indicated that Bud Feed and Erger applications did not adversely affect the fruit skin color characteristics of the '0900 Ziraat' cherry cultivar.

3.4. Yield variables

The highest yield per tree, yield per trunk cross-section, and yield per hectare had the cultivars 'Astoria' (33.95 kg tree^{-1}, 0.33 kg cm^{-1}, and 56.3 t ha^{-1}, respectively), 'Maya' (15.73 kg tree^{-1}, 0.13 kg cm^{-1}, and 26.1 t ha^{-1}, respectively), and 'Garbaja' (10.35 kg tree^{-1}, 0.09 kg cm^{-1}, and 17.1 t ha^{-1}, respectively) applied Bud Feed (Table 3). Average data showed that the 'Astoria' cultivar was the highest yield per tree, yield per trunk cross-section, and yield per hectare (28.23 kg tree^{-1}, 0.28 kg cm^{-1}, and 46.5 t ha^{-1}, respectively). Similar to these results, Ferreira et al. (2022) showed that nitrogen fertilizer and calcium nitrate applications for bud break in the 'Douradão' peach cultivar increased the yield by 30.92% compared to control plants. In addition, these results were consistent with the findings that bud break applications increased fruit yield in peach (George et al., 1992), pistachio (Ghrab and Ben Mimoun, 2014), fig (Theron et al., 2011; Gaaliche et al., 2017), cherry (Sheard et al., 2009), kiwifruit (Veloso et al., 2003). The high yield in plants applied with the bud break may be due to an increase in flowering and higher fruit set percentages.

4. Conclusion

The accumulation of chilling hours is essential for the breaking of the dormancy stage in the buds of stone fruits such as peach-nectarine, apricot, and sweet cherry. However, due to the increasing effect of global warming in recent years, especially in the Mediterranean region of Türkiye, there has been a decrease in winter cold and this can cause significant decreases in yield. Thus, there is a need for studies on bud break applications that can prevent yield and quality loss in early cultivars. Low biuret urea (Bud Feed) and calcium nitrate application increase fruit set, yield, and fruit quality in early ripening 'Astoria' and 'Maya' peach and 'Garbaja' nectarine cultivars. This application provides earliness of 2 days in 'Astoria' and 'Garbaja' cultivars and 3 days in 'Maya' cultivar at harvest time. The harmful effects of these compounds on the environment are much less than chemical applications may also encourage their widespread use.

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RESEARCH PAPER

Banker Boxes, A Novel Release Method, Improve The Biological Control of *Planococcus citri* by *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* in Pomegranate

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Abstract

A study was conducted to determine the efficacy of a predator, *Cryptolaemus montrouzieri* (Muls.) (Coleoptera: Coccinellidae) and a parasitoid, *Leptomastix dactylopii* (How.) (Hymenoptera: Encyrtidae), in biological control of citrus mealybug *Planococcus citri* Risso (Hemiptera: Pseudococcidae), a major pest in pomegranate orchards in Antalya. When the pests were observed in 2013, 10 predators and 20 parasitoids were concurrently released per tree in plots using the standard procedures. In 2014 and 2015, despite the partial control (74%) in 2013, a different method release method was used; a modified banker box. In this method, beneficial insects were established in cardboard boxes that included potato tubers infested with prey pest before release. These boxes were then placed in the orchard before pest seen. The total number of beneficials in three boxes was equal to the number of beneficials released in 2013. The banker box method gave promising results, with control of nearly 90% in both 2014 and 2015. These findings demonstrated that the banker box application increased the effectiveness of the release of beneficials and it suggested that this method should be considered for biological control of citrus mealybug in pomegranate.

1. Introduction

Pomegranate (*Punica granatum* L.) is a fruit species with a significant cultural history. Although it is mostly a tropical and subtropical fruit, it can also grow to a more limited in hot and temperate climates. Pomegranate cultivation is more than 300 kha with 3 Mt of production in the world. The top countries for the pomegranate cultivation are India, Iran, China, United States, Israel, Egypt and Spain (Melgarejo-Sánchez et al., 2015).

In pomegranate cultivation is affected by a wide range of insect pests. Citrus mealybug [*Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)], Mediterranean fruit fly [*Ceratitis capitata* Wied. (Diptera: Tephritidae)], carob moth [*Ectomyelois ceratonia* (Zell.) (Lepidoptera: Pyralidae)] and honeydew moth [*Cryptoblabes gnidiella* Mill. (Lepidoptera: Pyralidae)] are important pests particularly during the ripening period. The pomegranate aphid [*Aphis punicae* Passerini (Hemiptera: Aphididae)] and ash whitefly [*Siphoninus phillyreae* (Haliday) (Hemiptera: Aleyrodidae) are important pests during the growth and flowering. Leopard moth [*Zeuzera pyrina* L. (Lepidoptera: Cossidae) also causes significant damages to pomegranate tree trunk and branches (Öztürk and Ulusoy, 2009; Öztop et al., 2010; Öztop et al., 2016; Karaca et al., 2018).

The hosts of the polyphagous citrus mealybug are mostly the citrus, but the pest harmful in other fruit species, some vegetables and ornamental plants including avocado, banana, begonia, bougainvillea, eggplant, mango, melon, mulberry, oleander, olive, peanut, pomegranate, pumpkin, vine and watermelon. Immature stages and adult females feed on fruits by sucking sap, and contribute to the development of sooty mold,

severely reducing the marketable quality of the fruit. Also, their honeydew secretions support the development of other pests such as the Honeydew moth.

To control of citrus mealybug, chemical methods are generally adopted. However, chemical control of citrus mealybug is difficult because at some stages they feed between the leaves and the fruit and are inaccessible to contact insecticides. Also, resistance to some commercial insecticides has been reported (Franco et al., 2004; Franco et al., 2009; Venkatesan et al., 2016).

A potentially better option is biological control using of mass-reared and commercially available predators, *Cryptolaemus montrouzieri* (Muls.) (Coleoptera: Coccinellidae), and parasitoids, *Leptomastix dactylopii* (How.) (Hymenoptera: Encyrtidae) and *Anagyrus pseudococci*. In some cultivated plants, including cotton, citrus and guava, biological control of various mealybugs has been successfully implemented using *C. montrouzieri* (Panis and Brun, 1971; Khan et al., 2012; Kütük et al., 2014; Omkar and Kumar, 2016). The parasitoid, *L. dactylopii,* also has advantages for mealybug control including faster colonization and ability to find the host in places where the predator could not reach. The use of both beneficials in mealybug control increases the success. However, *C. montrouzieri* and *L. dactylopii* do not enter quiescence or diapause under cold temperatures (Roy and Migeon, 2010), thus they are unable to establish in the cooler temperate regions of western Europe, northern Mediterranean areas and California (except coastal areas) (Olivero et al., 2003; Hoy, 2008; Zappalà, 2010; Maes et al., 2015). Also, neither *C. montrouzieri* nor *L. dactylopii* can overwinter under climatic conditions in Turkey, so they must be released every growing season (Kütük et al., 2014).

Banker plants are hosts for the pest that can also support alternative prey or hosts for the natural enemies and are mostly used sustainable management of arthropod pests in greenhouse vegetable production (Jacobson and Croft, 1998; Schoen, 2000; Huang et al., 2011; Payton Miller and Rebek, 2018). They allow the beneficial insect to establish early, even when there is no prey or hosts in the target agroecosystem, thus frequent or high-rate of release of beneficial insects is not required (Payton Miller and Rebek, 2018).

Scientific evaluation of the use of beneficials in pomegranate plants has been quite limited. Therefore, the present study aimed to determine the potential effectiveness of *C. montrouzieri* and *L. dactylopii* in biological control of the citrus mealybug using banker boxes, a novel release method in pomegranate orchards.

2. Materials and Methods

2.1. *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* rearing

Leptomastix dactylopii were mass-reared in wooden cages 55 × 35 × 45 cm (LWH) with the top covered with glass and the rear side covered by netting for ventilation. A single layer of potato tubers with sprouts infested with citrus mealybugs were placed in these cages and *L. dactylopii* adults collected from a stock culture with a vacuum pump were released into the cages. For *C. montrouzieri* mass-rearing, potato tubers infested with the citrus mealybug were likewise placed into these cages then, *C. montrouzieri* adults were released to the rear the predators. Rearing was conducted in a controlled environment room at 25 ± 1°C, 65 ± 10% RH and 16:8 h L:D photoperiod.

2.2. Experimental design

Two treatments were examined; release of both *C. montrouzieri and L. dactylopii* and a control with no beneficials released. The experiments were set-up in a randomized complete block design with four replicates plots 0.1 ha that included 66 trees. A buffer of three rows was allocated between the plots to minimize any potential dispersal of beneficials between treated and untreated plots. The 2013, 2014 and 2015 experiments were set-up in an orchard of 13-year-old *P. granatum* cv. Hicaz trees in Başköy Village in Antalya Province, Turkey. The daily temperature data was provided by the National Meteorological Service Station located approximately 12 km away from the orchard.

2.3. *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* release

Adults were released directly into the orchard in 2013 following standard practice when 5% infected fruit detected. Since the proportion of infested fruits was higher than expected at the end of the 2013 growing season, a banker box release method (described below) was used in 2014 and 2015. In both methods, 10 predators and 20 parasitoids per tree were concurrently released.

The standard practice for releasing *C. montrouzieri* release involved placing 1 × 1 × 8 cm release boxes on tree trunks (1 box/tree). To release *L. dactylopii* the netting from box that contained parasitoid adults was removed and the adults were released while walking quickly around the middle are of treated plots.

For *C. montrouzieri* and *L. dactylopii* banker box release, 20 × 20 × 20 cm cardboard boxes with 10 × 10 cm windows covered with netting on four sides were used. Circular holes (1 cm) were made above each window to allow the exit of beneficials. The top of the box had a window covered transparent hard plastic sheet that permitted observation of the box contents (Figure 1). The box included two to three potato pieces (according to the size of the potato tuber) infested with mainly third instar of citrus mealybug to support *L. dactylopii* and third instar or adult females to support *C. montrouzieri*. The

number of parasitoids or predators adequate for the release was collected and placed in a banker box 2 days before the planned release date. For release, boxes were hung on a branch of the tree, without direct contact with that, or any other, branch using metal wire. Adhesive gel was applied to the wire to prevent any mealybug instars spreading to the tree. Three boxes were installed in each treated plot. The release rates and dates for the three years are given in Table 1.

2.4. Sampling

Cryptolaemus montrouzieri adult population was determined at monthly intervals by a limb-tap method (Steiner, 1962). One hundred taps were made to limbs of arbitrarily selected trees. The *Cryptolaemus montrouzieri* adults collected in Steiner funnel were counted and left in the plot. *Leptomastix dactylopii* population was determined by randomly collecting five mealybug-infested fruits from each plot for examination in the laboratory. Non-target organisms were removed and the fruit placed in boxes to trap emerging adults. These boxes were checked three times per week for 3 weeks and the number of *L. dactylopii* was recorded. The proportion of mealybug-infested fruit was determined at the harvest from nine trees in the center of each plots and all fruit from these trees were assessed.

2.5. Statistical analysis

Data were processed using Jamovi version 1.6.9.0 (The Jamovi Project, 2021) for analysis and visualization. General linear models were fitted and the populations of *C. montrouzieri* and *L. dactylopii* in released plots were plotted for each of the three seasons with 95% confidence intervals, and used to compare the efficacy of different release methods. Percentage infestation data at harvest were arcsine transformed and mean separations were done on the fruit infestation rates using a paired t-test at P = 0.05. Biological efficacy was calculated using Abbott's formula (Abbott, 1925).

3. Results

During the first-year experiments conducted with the standard release method, the mean daily temperatures in June, July and August were 24.3,

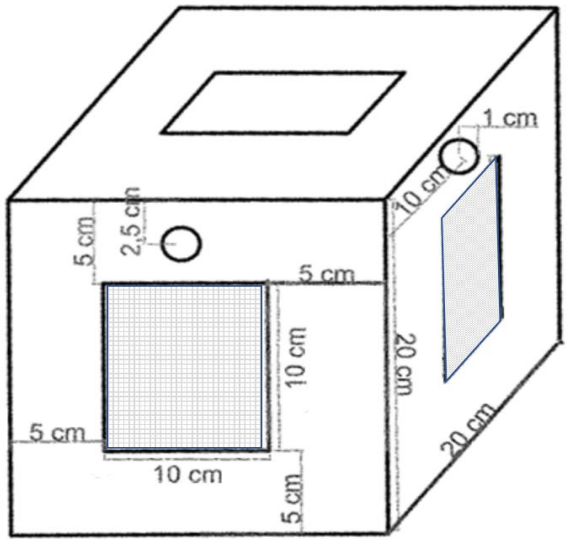

Figure 1. Banker box.

Table 1. Release rate and date of *Cryptolaemus montrouzieri* (*Cm*) and *Leptomastix dactylopii* (*Ld*) against *Planococcus citri* on pomegranate.

Year	Release method	Release rate	Release Date
2013	Standard	10 *Cm* adults and 20 *Ld* adults/tree*	9 July 2013
2014	Banker box	220 *Cm* adults/box and 440 *Ld* adults/box (3 boxes for each beneficial per treated plot)	10 June 2014
2015	Banker box	as above	18 June 2015

* The number of beneficial released per plot is the same for all years.

27.8 and 28.9ºC, respectively. Similar temperatures were occurred in the years when the banker box release method was applied. These temperatures were 24.3, 26.6 and 28.0ºC for 2014, and 22.8, 27.6 and 28.3ºC for 2015.

Although buffers were allocated between the treated and control plots, small number of predator and parasitoids were observed in the untreated plots, but the numbers can be ignored. In treated plots, the *C. montrouzieri* populations increased from the first to the last observation days in all years (Figure 2). The pattern of increase of *C. montrouzieri* was distinctly different between 2013 and the subsequent two years when the banker boxes were used. There was a clear lag in population increase in 2013; in August and September, the predator population was significantly lower than subsequent years. However, by the end of the growing season the populations in the three years were similar. The highest predator numbers were 11.3, 12.3 and 14.3 adults per sampling in October 2013, 2014 and 2015, respectively (Figure 2).

For *L. dactylopii* the response was similar by not quite as distinct, although a lag in its population development was clearly evident in the 2013 data (Figure 2). There were 2.25, 3.25, and 3.75 parasitoids per five infested fruits in August 2013, 2014 and 2015, respectively. With a similar difference in September at 3.25, 6.75 and 5.25 parasitoids, respectively. At the final assessment, the parasitoid populations were 6.75, 7.75 and 7.75 parasitoids, respectively. So, as with *C. montrouzieri*, the end of seasons populations in three years were similar.

The proportion of infested fruit were 4.1 and 15.5% in treated and control plots in 2013 (P = 0.001), indicating 73.6% control efficacy. With the banker box release in 2014 and 2015, proportion of infested fruit were 1.9 and 2.0%, respectively. Compared to 16.9 and 18.2% in control plots, giving 88.8 and 89.0% control efficacies, respectively (Table 2).

Figure 3 shows the clear benefit of banker box release in terms of high beneficial numbers and lower damage to fruit. With banker box release there was a clear decline in damaged fruit with increasing numbers (particularly in 2014), whereas this was not evident with release the standard practice (2013). Low numbers of beneficials and highly variable response is consistently with the lag evident in their population increase (Figure 3).

4. Discussion

This study aimed to determine the efficacy of the release of beneficials (10 predators and 20

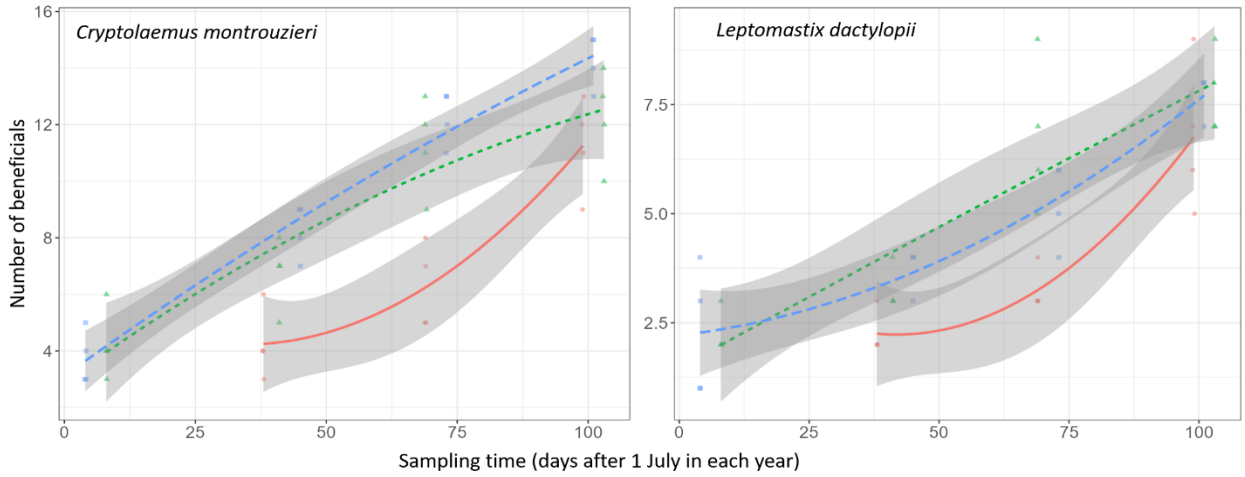

Figure 2. Numbers of *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* throughout the growing season having been released by standard practice in 2013 and with banker boxes in 2014 and 2015 (Lines are quadratic general linear models fits surrounded by 95% confidence intervals).

Table 2. Mean proportion of fruit with beneficials and mealybug (%, mean ± SE) at harvest and consequent control efficacies (corrected with Abbott's formula).

Year	Fruit infestation (%) in beneficials released plots	Fruit infestation (%) in control plots	Efficacy (%)
2013	4.1 ± 0.47 a	15.5 ± 1.02 b	73.6 ± 3.14
2014	1.9 ± 0.13 a	16.9 ± 0.79 b	88.8 ± 0.37
2015	2.0 ± 0.17 a	18.2 ± 0.49 b	89.0 ± 1.16

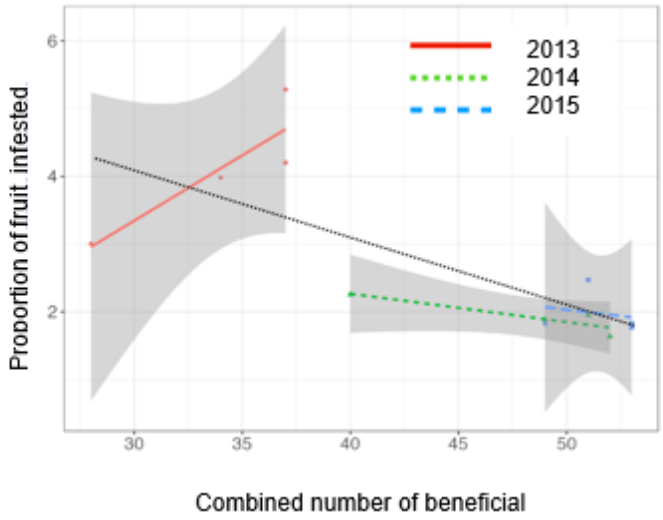

Figure 3. Proportion of fruit infested (damaged) with mealybug versus the combined number of beneficials (*Cryptolaemus montrouzieri* and *Leptomastix dactylopii*) that had been released by standard practice in 2013 and with banker boxes in 2014 and 2015 (The colored lines are simple general linear models fits surrounded by 95% confidence intervals within each year. The dotted line is the linear regression across all years emphasizing the increased number of beneficials and reduced fruit damage with banker box release in 2014 and 2015).

parasitoids) against pomegranate mealybug. While 73.6% control efficacy was obtained with release by standard practice, efficacy increased to 88.8 and 89.0% in 2014 and 2015, respectively, when banker boxes were used. Similar success has been reported using *C. montrouzieri* alone or combined with a parasitoids, such as *L. dactylopii*, in control of various mealybug species in croton (Copland et al., 1985; Afifi et al., 2010), citrus (Katsoyannos, 1996; Olivero et al., 2003; Moore and Hattingh, 2004; Rahmouni and Chermiti, 2013; Erkilic et al., 2015), grape (Mani and Thontadarya, 1988; Mani and Krishnamoorthy, 2008) and tobacco (Gautam et al., 1988). However, there appears to be no published study on the use of these beneficials in combination in pomegranate. Mani and Krishnamoorthy (2000) found that *L. dactylopii* and *Coccidoxenoides perminutus* Girault (Hymenoptera: Encyrtidae) effectively suppressed mealybug populations in August within a month of parasitoid activity in southern India. They reported high efficacy with parasitoids but this might not be the same in Antalya under its cooler conditions. In the warmer climate of their study, parasitoids are able to survive through winter and increase quickly in the spring (Krishnamoorthy, 1990).

Although somewhat promising results were obtained in in the first year of the present study using the standard release practices, 4% fruit infestation would still lead to unacceptable economic losses. Also, with this release method there is insufficient time for the populations of the beneficials to increase given the threshold number of pests were observed in July. In October, despite the increase in the beneficials inhibiting the mealybugs, the damage to the fruit already caused by the pest would lead to unacceptable quality reduction. It has been reported that *C. montrouzieri* provide efficacy from 6 weeks to 3 months (Srinivasan and Sundara Babu, 1989; Afifi et al., 2010). For citrus, the recommendation is to release *C. montrouzieri* and *L. dactylopii* when 8% tree or fruit are found to be infested in May or June in Türkiye (Erkilic et al., 2015). This means biocontrol agents do have enough time to increase in population in citrus. However, the flowering is extended in pomegranates and fruit set occurs about a month later than in citrus (Öz Atasever et al., 2011; Albrigo et al., 2019). Thus, mealybugs appear in fruits towards the end of June. This delay in fruit set means the rate of increase in the population of beneficial is insufficient. Also, in some plants such as in citrus, it is easy to detect nymphs and overwintering females on the trunks and their eggs in the cottony ovisacs. However, overwintering mealybugs in pomegranate occur mostly under the bark and difficult to detect, so it is not easy to determine as suitable release date.

To prevent mealybug damage, it is necessary to suppress the pest population in August, when the pest population is the highest and leads to sooty mold development. One strategy to solve this problem would be to use repeated releases (Erkilic et al., 2015) or higher release rates as suggested for croton (50 adult per tree) (Afifi et al., 2010). However, the additional costs involved are unlikely to be acceptable to growers. Thus, the banker box system, which is based on potato tubers infested with pests, similar to the banker plant system used for the control of pests especially in greenhouse vegetable cultivation (Osborne et al., 2005; Payton Miller and Rebek, 2018), was developed to obtain intensive beneficial population in the orchards.

Banker boxes were deployed in the second and third years of the present study achieving higher numbers of *L. dactylopii* in August and September

than with release by standard practice in the first year. Similar results were also obtained for *C. montrouzieri*. Although the similar beneficial populations were seen at the end of season with both release methods, *L. dactylopii* and *C. montrouzieri* populations increased faster to a higher number with banker box release in the middle of season avoiding the lag seen with release by standard practice. The efficacy of the banker box release method was nearly 90% in 2014 and 2015.

This study has demonstrated for the first time that a banker box method would be beneficial for the suppression of the citrus mealybug populations overcoming some of the challenges of using biological control in pomegranates. Further studies are needed to optimize release rates.

5. Conclusions

The citrus mealybug is an important insect pest in pomegranate growing areas especially in Mediterranean countries. Despite using exotic natural enemies, *C. montrouzieri* and *L. dactylopii* in citrus growing, there are no studies on the efficacy of released beneficials in pomegranate. Standard release of *C. montrouzieri* and *L. dactylopii* increased control efficacy. However, based on the result of this study, the novel banker box release method is likely to increase the control efficacy of these beneficials but being able to establish their population about one month earlier than the standard approach.

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RESEARCH PAPER

Relationships of Shading-Induced Reductions in Yield and Morphological Traits with Mineral Nutrition of Apple Trees

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Abstract

Protective nets are commonly used in orchards to prevent hail damage and sunburns. However, these nets partially prevent sunlight exposure of the trees. Sunlight directly influences plant physiology. In present study, the effects of reduced sunlight on mineral nutrition of trees were investigated. Experimental orchard had protective nets with different shading ratios (0, 32, 42 and 56%) for 7 years. In 8, 9 and 10th year of the orchard, to reveal relationships of protective nets and mineral nutrition, apple trees were sampled from part of leaves, bud, and flower and subjected to mineral analyses. Leaf nutrients were all influenced by light intensity and increasing N, K, Fe, Cu, Mn and B levels were observed with increasing shading ratios. In fruit buds, shading treatments all had more Ca, Fe and Cu concentrations. In flower samples, only P and Mg were significant and the lowest values were obtained from the greatest shading ratio. Nutrient ratios were assessed for each sample group and only the leaf nutrient ratios were significant. It was observed when the common ratios (N:K and K:Mg) were assessed that the greatest N:K ratio was obtained from the control treatment and the other treatments were placed into the same group; the lowest K:Mg ratio was obtained from the control treatment and the other treatments were placed into the same group. It couldn't be detected relationships between decreasing yield, morphological traits and reduced sunlight with nutrient contents based on concentrations under experimental conditions.

1. Introduction

Plant productivity largely depends on the absorption of light energy by green tissues and the conversion of this energy into biomass through photosynthesis. Previous studies conducted with several plant species revealed that there was a linear relationship between light intake and dry matter production. For high yield and fruit quality, orchards should take sufficient quantity of light and the light should be well distributed within the tree canopy (Wünsche et al., 1996). Sunlight is composed of photons of different wavelengths and the solar energy they conveyed is dependent of their frequencies (Taiz and Zeiger, 2006). Majority of the energy supplied by the plants is within the visible spectrum (400-740 nm) (Raven and Johnson, 1999). In apple trees, since light directly or indirectly influence photosynthesis, flower bud formation and fruit quality, it is quite a significant parameter for fruit yield and quality. Limited light before or after flowering may reduce fruit set, size and quality (Rom, 1991).

Protective or shade nets are used in orchards to prevent hail damages and sunburns. Shade nets alter or reduce direct sunlight quantities through absorption or reflection of the light by the net. In this way, potential energy used by the plant is reduced,

then probably plant energy balance is distorted and the type of growth is influenced (Stampar et al., 2001). In practice, various cover nets with different density and color are used in orchards. Previous research revealed that net color and density play a critical role in fruit quality and tree growth. Density of the cover system significantly influences solar radiation reaching to plant, thus play an important role in fruit quality traits like red coloration, fruit size, soluble solids content and starch conversion ratio (Stampar et al., 2001; Shahak et al., 2004). Better spur development is observed in trees receiving high sunlight as compared to low sunlight conditions and fruit quality increased in high sunlight conditions. Differences in size and quality of the fruits collected from different sections of the tree canopy are mostly related to total light ratio passing through the canopy. In practice, light is the most significant factor designating economic performance of the orchard (Tustin, 2005).

Shading may result in reductions in flower bud formation and fruit set. Leite et al. (2002) conducted a study for five years on apple trees and reported about 19% less flower bud formation in shade net-covered trees than in uncovered trees. Similarly, Middleton and McWaters (2002) indicated that there was no need for chemical thinning in 'Hi Early' and 'Red Delicious' apple cultivars under shade net, but there was a need for chemical treatments twice in adjacent uncovered trees (Smit, 2007). Shading is also effective in nutrition of trees. Light designates auxin synthesis. Auxin plays an important role in calcium (Ca) transfer. Montanaro et al. (2006) reported that light intensity increased Ca concentration of xylem sap in kiwi fruit. Rosati et al. (1999) reported that peach leaves in sun-exposed outer sections of the tree had greater N contents than the leaves in shaded sections and increased N contents were related to photosynthesis capacity. Zhao and Oosterhuis (1998) conducted a study on cotton and reported that shading increased NO_3-N, P, K, S, Ca and Mg concentrations of petioles and such a case was related to reduced carbohydrate accumulation.

In this study, the effects of protective nets with different shading ratios on nutrient uptake of 'Granny Smith' apple cultivars grafted on M9 rootstocks were investigated through leaf, fruit bud and flower analyses.

2. Material and Method

Experiments were conducted at experimental plots of Fruit Research Institute (Isparta-Türkiye) with 'Granny Smith' apple cultivar grafted on M9 rootstock in randomized blocks design with 6 replicates and 3 trees in each replicate. Shading treatments were initiated about 50 days after full bloom (10th of June) and continued until the end of October. 3 different net materials providing 32, 42 and 56% shading were used for 10 years (2003-2013) beginning from plantation of the trees. However, effects of shading on nutrient uptake were assessed with nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) manganese (Mn), zinc (Zn) and boron (B) contents of fruit bud, flower and leaf samples collected during the last 3 years. Fruit buds were sampled at dormant season (in March) from the youngest shoots. Flowers together with pedicle were sampled from flower bouquet formed over the youngest shoots and from the flowers at balloon stage (in April). Leaf sampling with petiole was performed 85-90 days after full bloom (in July) from the mid-sections of the shoots of the same year within the tree canopy.

Samples were brought to laboratory and immediately washed through tap water, then washed through 0.1 N HCl and finally washed through deionized water and roughly dried out with drying papers. Samples were then placed into paper bags and dried in an oven at 65-70°C until a constant mass (for about 48 hours) (Kacar and İnal, 2010). Dried samples were ground and prepared for N, P, K, Ca, Mg, Fe, Cu, Mn, Zn and B analyses. Nitrogen concentration was determined through Kjeldahl method. Dry-ashing method was carried out for P, K, Ca, Mg, Fe, Cu, Mn, Zn and B (Ryan et al., 2001) and they detected in ICP-AES. NIST-brand reference apple leaf (1515) was used to check the accuracy of leaf analyses.

Experimental data were subjected to one-way ANOVA with the use of "JMP© 8.0" (SAS Institute, Inc.). Significant means were compared with the use of LSD (Least Square Difference) test at $P<0.05$ and $P<0.01$ significance levels.

3. Results and Discussion

In first 7 years of this study, it was revealed the "shade net systems or anti-hail nets" commonly used to prevent hail and sunlight damages generated on fruit peel affected yield and fruit quality traits. Present findings revealed that shading delayed harvest time and reduced yields. A linear decrease was observed in yield with increasing shading ratios. Such a decrease was not distinctive in the initial years of the study, but got more distinctive in subsequent years. Lower fruit weight, width and length values were observed in shading treatments. Shade nets are generally used in 'Granny Smith' apples to prevent sunburns and cheek redness and significant linear decreases were observed in these parameters with increasing shading ratios. Parallel to sunburns, soluble solids content values also decreased. In addition, fruit color parameters were influenced by shading treatments. While b* and L* values increased, a* values decreased with increasing shading ratios (unpublished data). When the leaf nutrient contents were assessed based on shading treatments, it was observed that the effects of shading on the mineral

nutrition of the trees were found to be significant for all nutrients. The N, K, Fe, Cu, Mn and B nutrition of the trees linearly increased with increasing shading ratios and the greatest values were obtained from the greatest shading ratio. While the greatest Ca and the lowest Zn values were obtained from the 42% shading treatment, these nutrients were placed into the same statistical group. P and Mg nutrition of the trees were similar and the greatest values were obtained from the control treatment and the 56% shading treatment (Table 1). Nutrient accumulation in fruit buds was similar for some nutrients, but exhibited differences for some others. The greatest P values were obtained from 32% shading treatment and control treatment. As compared to control treatment without shading, significantly greater Ca, Fe and Cu values were obtained from the shading treatments. On the other hand, changes in the other nutrients were not found to be significant (Table 2). In flower tissues, changes only in P and Mg were found to be significant and the lowest values were obtained from the greatest shading ratios (Table 3).

Significant effects of sunlight intensity on sunburn (Piskolczi et al., 2004), redness (Reay, 1999), yield (Tustin, 2005; Robinson, 2007), flower bud formation (Dennis, 2000), soluble solid concentration (Amarante et al., 2011), fruit size and harvest time (Smit, 2007) were reported in previous studies. Decreased fruit bud formation and resultant decreasing yields in subsequent years were found to be remarkable. Therefore, it was thought that such negative effects of shade nets might directly or indirectly be related to mineral nutrition. In the experimental plot provided with shade nets with different shading ratios for long years, shading provided for additional 3 years and treatments were compared in terms of nutrient accumulation in fruit bud, flower and leaf tissues.

Zhao and Oosterhuis (1998) conducted a study on cotton and reported that shading treatments increased concentrations of some nutrients in petioles, but related such increases to carbohydrate accumulation. Cui et al. (2014) reported that shading reduced dry matter accumulation, N and P absorptions in maize. N and P absorptions increased to some extent with different treatments, but decrease in dry matter was greater than the increase in N and P absorption. Gao et al. (2020) conducted a study on maize and indicated that decreasing yields were resulted from the negative effects of shading on dry matter and N uptake and transport.

It was indicated in previous studies that even at sufficient level of a nutrient in leaf, there may be deficiency symptoms of that nutrient based on

Table 1. Effects of shading levels on leaf nutrient contents of apple trees grafted on M9 rootstock cv. 'Granny Smith' (average of three subsequent years).

Shading levels (%)	N (% DW)	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)
0	2.32 ± 0.070 b	0.20 ± 0.006 a	1.27 ± 0.064 c	1.14 ± 0.041 b	0.37 ± 0.014 ab
32	2.37 ± 0.039 b	0.18 ± 0.005 b	1.44 ± 0.038b	1.12 ± 0.021 b	0.35 ± 0.008 b
42	2.39 ± 0.042 b	0.21 ± 0.014 b	1.47 ± 0.040 b	1.32 ± 0.041 a	0.35 ± 0.009 b
56	2.50 ± 0.042 a	0.18 ± 0.002 a	1.61 ± 0.038 a	1.18 ± 0.037 b	0.38 ± 0.011 a
P value	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05
Shading levels (%)	Fe (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)	B (mg kg^{-1} DW)
0	94 ± 3.98 b	8.8 ± 0.35 c	26.0 ± 1.37 b	21.5 ± 3.12 a	32.5 ± 0.72 c
32	98 ± 4.56 b	9.8 ± 0.38 b	25.6 ± 1.94 b	17.2 ± 1.48 b	34.2 ± 1.09 c
42	124 ± 6.32 a	11.0 ± 0.26 a	27.2 ± 1.22 ab	21.9 ± 2.99 a	37.4 ± 0.65 b
56	118 ± 5.91 a	11.2 ± 0.29 a	29.4 ± 1.47 a	22.4 ± 3.68 a	39.5 ± 0.89 a
P value	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01

NS: non-significant. ±: standard error of mean. DW: dry weight

Table 2. Effects of shading levels on fruit bud nutrient contents of apple trees grafted on M9 rootstock cv. 'Granny Smith' (average of two years).

Shading levels (%)	N (% DW)	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)
0	1.72 ± 0.090	0.30 ± 0.014 ab	0.55 ± 0.026	2.04 ± 0.102 b	0.13 ± 0.004
32	1.89 ± 0.053	0.31 ± 0.008 a	0.58 ± 0.013	2.46 ± 0.092 a	0.14 ± 0.005
42	1.82 ± 0.077	0.28 ± 0.005 c	0.55 ± 0.022	2.31 ± 0.065 a	0.13 ± 0.003
56	2.00 ± 0.070	0.29 ± 0.006 bc	0.59 ± 0.016	2.38 ± 0.083 a	0.14 ± 0.004
P value	NS	P<0.05	NS	P<0.01	NS
Shading levels (%)	Fe (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)	B (mg kg^{-1} DW)
0	58 ± 4.19 b	77 ± 11.7 b	17.6 ± 1.26	63 ± 5.54	26.8 ± 1.93
32	76 ± 5.49 a	105 ± 9.05 ab	19.6 ± 0.93	75 ± 4.80	29.3 ± 1.13
42	72 ± 3.35 a	113 ± 10.56 a	19.4 ± 1.07	78 ± 6.21	28.7 ± 1.11
56	79 ± 4.40 a	130 ± 9.05 a	19.1 ± 0.93	73 ± 3.23	28.8 ± 1.21
P value	P<0.01	P<0.01	NS	NS	NS

NS: non-significant, ±: standard error of mean, DW: dry weight

Table 3. Effects of shading levels on flower nutrient contents of apple trees grafted on M9 rootstock cv. 'Granny Smith' (average of two years).

Shading levels (%)	N (% DW)	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)
0	3.37 ± 0.16	0.43 ± 0.008 a	1.88 ± 0.022	0.44 ± 0.013	0.24 ± 0.005 a
32	3.54 ± 0.12	0.42 ± 0.004 ab	1.85 ± 0.021	0.42 ± 0.008	0.24 ± 0.003 a
42	3.26 ± 0.13	0.42 ± 0.005 ab	1.87 ± 0.043	0.43 ± 0.009	0.24 ± 0.003 a
56	3.44 ± 0.13	0.41 ± 0.006 b	1.81 ± 0.030	0.45 ± 0.010	0.23 ± 0.004 b
P value	NS	P<0.05	NS	NS	P<0.05
Shading levels (%)	Fe (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)	B (mg kg^{-1} DW)
0	91 ± 5.67	52 ± 3.39	36 ± 6.69	45 ± 3.08	58 ± 2.80
32	83 ± 4.16	53 ± 3.99	38 ± 7.56	46 ± 3.09	56 ± 2.84
42	92 ± 5.67	57 ± 4.57	33 ± 5.71	44 ± 2.74	62 ± 2.81
56	86 ± 6.76	60 ± 8.00	35 ± 7.30	39 ± 3.38	56 ± 3.82
P value	NS	NS	NS	NS	NS

NS: non-significant, ±: standard error of mean, DW: dry weight

Table 4. Effects of shading levels on leaf nutrient ratios of apple trees grafted on M9 rootstock cv. 'Granny Smith' (average of three years).

Shading levels (%)	C:B	Ca:Fe	K:Mg	Mg:B	Mg:Fe	N:B
0	362 ± 16 a	128 ± 7 a	3.69 ± 0.16 b	114 ± 6.0 a	40 ± 2.0 a	731 ± 16 a
32	334 ± 11 a	119 ± 6 ab	4.22 ± 0.15 a	103 ± 3.9 b	36 ± 1.4 ab	703 ± 23 a
42	355 ± 14 a	110 ± 6 bc	4.23 ± 0.19 a	96 ± 3.8 c	30 ± 1.5 c	641 ± 14 b
56	305 ± 14 b	103 ± 4 c	4.32 ± 0.13 a	97 ± 4.2 bc	33 ± 1.4 bc	638 ± 17 b
P value	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01
Shading levels (%)	N:Fe	N:K	P:B	P:Ca	P:Fe	P:Mn
0	262 ± 14 a	1.82 ± 0.08 a	62 ± 2.2 a	0.18 ± 0.007 a	22.2 ± 1.39 a	80 ± 3.5 a
32	251 ± 10 a	1.66 ± 0.06 b	55 ± 2.2 b	0.17 ± 0.004 ab	19.7 ± 1.12 ab	78 ± 5.5 a
42	202 ± 12 b	1.65 ± 0.06 b	57 ± 3.8 ab	0.16 ± 0.008 b	17.9 ± 1.56 bc	79 ± 4.9 a
56	220 ± 10 b	1.56 ± 0.04 b	46 ± 1.3 c	0.15 ± 0.004 b	15.8 ± 0.78 c	63 ± 2.6 b
P value	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01

±: standard error of mean

relative quantities of the other elements (Bergmann, 1992; Stiles, 1994; Hoying et al., 2004; Uçgun et al., 2013; Rietra et al., 2017). Therefore, relative quantities of all nutrients in leaf, flower bud and flower were assessed based on treatments and significant outcomes were observed only for leaf nutrient concentrations. Explainable results, in other words, linear increase of decreases with increasing shading ratios, are provided in Table 4. In leaves, shading-dependent N:B, N:Fe and P:Ca ratios were similar. These ratios were similar and greater in control and 32% shading treatments and different and lower in 42 and 56% shading treatments. Ca:B and P:Mn ratios were also similar. These ratios were significant and lower only in the greatest shading treatments and the other treatments were placed into the same statistical group. K:Mg and N:K ratios were different only in the control treatments and similar in all shading treatments. The lowest K:Mg and the greatest N:K ratios were observed in control treatment. Ca:Fe, P:Fe and P:B ratios linearly decreased with increasing shading ratios. Mg:B and Mg:Fe ratios were similar and decreased with increasing shading ratios, but both ratios were greater in 56% shading treatment than in 42% shading treatment. In terms of nutrient ratios, all ratios, except for K:Mg, were found to be more favorable for tree nutrition.

4. Conclusions

The damage directly generated by sunlight on fruit peels could totally be prevented with increasing shading ratios of the protective nets used especially against sunburn in orchards. However, apart from yield and sunburn, there is an inverse relationship between the other quality traits and shading ratios of protective nets. Since plant physiology is directly related to light intensity, the decrease especially in bud formation and yield with decreasing light intensity was also thought to be related to mineral nutrition of the trees. In other words, nutritional disorders were expected through reduced nutrient uptake with increasing shading ratios or possible imbalances between the nutrients. Present data revealed that the case was different from the expectations since increases were observed in uptake of several nutrients with increasing shading ratios. The nutrient ratios also revealed that there was a more balanced nutrition in shading treatments. In plant analyses, identified values generally express as concentrations in dry matter. But there is a linear negative relationship between light intensity and dry matter accumulation. Therefore, when expression of nutrients based on concentration in dry matter, proportional increase realizes in nutrient concentrations with decreasing

dry matter quantities. This case complicates the assessment of the on-going relationships between shading and nutrition of the trees. Such cases even may lead to erroneous outcomes. Therefore, in similar studies, it is thought that nutrient accumulations should be determined instead of nutrient concentrations or resultant values should be assessed based on different criteria like leaf area to get more reliable outcomes.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Investigation of *In vitro* Propagation Possibilities of Endemic *Campanula phitosiana* Yıldırım & Şentürk

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Abstract

Campanula phitosiana Yıldırım & Şentürk, is a local endemic to the Aydın Mountain range, which distribute across in Western Anatolia (both İzmir and Aydın). This species belongs to the Mediterranean basin floristic region. According to the International Union for Conservation of Nature's criteria, *C. phitosiana* is classified as "Critically Endangered" (CR). The aim of the study is to develop *in vitro* regeneration protocol for critically endangered endemic *Campanula phitosiana*. To investigate the efficient medium and plant growth regulator combinations for callus initiation and shoot proliferation, petiole and leaves were used as explant and explants cultivated on MS medium including NAA (1-Naphthylacetic acid) (0.3 mg L^{-1}), TDZ (Thidiazuron) (0.5, 1.0, 2.0, and 3.0 mg L^{-1}), BA (6-Benzylaminopurine) (0.5, 1.0, 2.0, and 3.0 mg L^{-1}) and Gibberellic Acid (GA_3) (0.5, 1.0, 2.0, and 3.0 mg L^{-1}). Explants subcultured 3 times. Experiments were conducted according to completely randomized design repeated with 5 replicates and each replicates including 5 explants. As a result of the experiment, callus initiation and shoot proliferation were investigated. Efficient callus initiation was observed petiole explants as 100% rate. Shoot proliferation was observed on MS medium including 0.3 mg L^{-1} NAA + 2.0 mg L^{-1} GA_3.

1. Introduction

The genus *Campanula* is known as one of the crowded family in Campanulaceae family with 420 species. This genus separated to 6 subgenus such as Megalocalyx, Damboldt, Rapunculus (Fourr) Charadze, Roucela (Dumort.) Damboldt, Brachycodonia (Fed.) Damboldt, Sicyodon (Feer) Damboldt and Campunala (Özdöl et al., 2022). Additionally, 138 of this species naturally grown in Türkiye (Fedorov and Kovanda, 1976; Contandriopoulos, 1984; Lammers, 2007; Alçıtepe et al., 2011; Yıldırım, 2018; Yıldırım and Özdöl, 2019; Özdöl et al., 2022). These species are distributed across eastern Mediterranean region, Balkans, Caucasia, and Türkiye. A total of 140 taxa, 68 of them are endemic to Türkiye. These species are known as annual, biennial, and perennial shrubs which has high ornamental potential due to shape of its flowers and compact plant structures. Campanula species has been used in ornamental plant industry and landscape architecture as outdoor, indoor pot plant (Scariot et al., 2008). Most of the campanula species is grown naturally in Türkiye and Yıldırım et al. (2019) defined the Türkiye as a key point of the Campanula species. *C. phitosiana* is one of the endemic species and it is classified as critically endangered according to the International Union for Conservation of Nature's (IUCN) criteria. This species distributed in a limited area (0.76 km^2) in Western Anatolia especially in İzmir and Aydın province. *Campanula phitosiana* is known as chasmophyte and flowers of this species blooms in June and July. Leaves of this species is tomentose, rosette leaves are lyrate shape, petiolate or sessile, inflorescence is racemose,

flowers are sub-secund or secund, flower colour is pale purplish blue (Yıldırım and Özdol, 2019). This species has a great potential for the landscape design of rocky gardens, and it is one of the important endemic genetic resources for Türkiye. To protect and propagate the critically endangered species, tissue culture techniques are very significant methods. Development of efficient regeneration protocol is the first step for *in vitro* conservation of the genetic resources. There have been too many reports about *in vitro* propagation of different *Campanula* species such as *C. isophylla* (Brandt, 1994), *C. glomerata* (Tanaka et al., 1999), *Campanula glomerata* 'Acualis' (Joung et al., 2002), *Campanula carpatica* Jacq. (Frello et al., 2002), *Campanula rotundifolia* (Mørk et al., 2005), *Campanula punctata* (Shim et al., 2005), *Campanula punctata* var. Rubriflora Makino (Sivanesan et al., 2007), *C. sabatia* (Airo et al., 2009), *C. polymorpha* Witas (Paunescu, 2010), hybrid Campanula (Röper et al., 2015), *Campanula incurva* (Grigoriadou et al., 2014), *Campanula rotundifolia* (Maria, 2014) *C. sclerophylla* (Kolomiets et al., 2016). However, there is no report about the *Campanula phitosiana* Yıldırım & Şentürk.

In this study, developing efficient *in vitro* regeneration protocol for critically endangered *Campanula phitosiana* Yıldırım & Şentürk were investigated. Plants were collected from flora of İzmir, Tire, Dallık province. Leaf and petiole were cultivated on MS medium including NAA with two different cytokinin (TDZ and BA).

2. Material and Method

2.1. Plant material

Campanula phitosiana Yıldırım & Şentürk plants were collected from the natural flora of İzmir, Tire, Dallık province in Türkiye, in June 2020. Fifteen *C. phitosiana* genotypes were cultivated in greenhouse in Research and Application Centre of Botanical Garden and Herbarium of Ege University. One of the plants was sampled for Herbarium coded with AD3761. Plants were cultivated in 10 cm diameter pots containing sand / turf (1:1; v/v). Plants were irrigated once a week in the greenhouse. Leaf and petioles were used as explant and explants were chosen healthy donor plants.

2.2. Method

In vitro experiments were set up in the tissue culture laboratories of Research and Application Centre of Botanical Garden and Herbarium of Ege University. All materials used in the tissue culture studies (Pince and lancet) were sterilized with autoclave. Medium used in the *in vitro* regeneration studies and distilled water used in the sterilization were autoclaved (121℃, 1.05 ATM pressure for 15 minutes).

2.3. Surface sterilization

Randomly selected leaves and petioles of the healthy donor plants were washed under tap water during 30 min. Explants were immersed in 70% EtOH in the sterile laminar flow cabinet for 1-2 minutes and rinsed with sterilised distilled water. Then explants were soaked into 30% Domestos (NaOCl, 4.5% v/v) for 20 minutes and washed four or five times with sterile distilled water in the laminar flow cabinet.

2.4. Explant preparation and regeneration medium

Sterile petiole and leaves were used as explant. Fresh leaves were selected and cut into two pieces due to the limited explant number. Petioles were cut into equal parts and each petiole explant was 0.5 cm length. To obtain organogenic callus explants were cultured on MS medium including 30.0 g L^{-1} sucrose, 4.0 g L^{-1} gelrite and 0.3 mg L^{-1} NAA and different concentrations of TDZ and BA (0.5, 1.0, 2.0, and 3.0 mg L^{-1}) as plant growth regulator. Medium pH was adjusted to 5.6-5.8 with 1 N HCl and 1 N KOH. Callus were subcultured to the MS medium containing 30.0 g L^{-1} sucrose, 4.0 g L^{-1} gelrite and 0.3 mg L^{-1} NAA and GA_3 (0.3, 0.5, 1.0, and 2.0 mg L^{-1}) to induce shoot proliferation. Shoots and shoot like structures were transferred to the hormone free MS medium containig 30.0 g L^{-1} sucrose, 4.0 g L^{-1} gelrite to root formation.

2.5. Experimental design and statistical analyses

In vitro regeneration experiments were conducted as completely randomised design. Each concentration included 5 replicates (5 petri dishes) each petri dishes contains 5 explants. Observations was carried out each 4 weeks and data were analysed with JMP 8 programme. Experiment was repeated two times. Percentage values were arcsine transformed. LSD test was performed to separate the means at the 0.05 level of probability.

3. Result and Discussion

There was no regeneration for both explant type in control group. Each explant from different genotypes showed different *in vitro* regeneration capacity in all regeneration medium except leaf explants cultured on MS medium including 0.3 mg L^{-1} NAA + 3.0 mg L^{-1} TDZ. Efficient callus regeneration was obtained from the petiole explants cultured on MS medium containing 0.3 mg L^{-1} NAA + 1.0 mg L^{-1} BA, 0.3 mg L^{-1} NAA + 3.0 mg L^{-1} BA, 0.3 mg L^{-1} NAA + 1.0 mg L^{-1} TDZ, 0.3 mg L^{-1} NAA + 2.0 mg L^{-1} TDZ as 100% (Table 1, Figure 1). Petiole was determined as efficient explant type for

Table 1. Regeneration results of the *C. phitosiana* leaf and petiole explants.

Plant growth regulator concentration	Leaf	Petiol
0.3 mg L^{-1} NAA + 0.5 mg L^{-1}BA	13.33 cd (17.71)	86.66 a (76.92)
0.3 mg L^{-1} NAA + 1.0 mg L^{-1} BA	46.66 ac (43.07)	100.00 a (90.00)
0.3 mg L^{-1} NAA + 2.0 mg L^{-1} BA	60.00 a (56.15)	80.00 a (68.07)
0.3 mg L^{-1} NAA + 3.0 mg L^{-1} BA	20.00 bd (26.57)	100.00 a (90.00)
0.3 mg L^{-1} NAA + 0.5 mg L^{-1} TDZ	46.66 ac (43.07)	66.00 a (60.00)
0.3 mg L^{-1} NAA + 1.0 mg L^{-1} TDZ	33.33 ac (30.00)	100.00 a (90.00)
0.3 mg L^{-1} NAA + 2.0 mg L^{-1} TDZ	60.00 ab (51.14)	100.00 a (90.00)
0.3 mg L^{-1} NAA + 3.0 mg L^{-1} TDZ	0.00 e (0.00)	20.00 b (16.92)

LSDleaf = 27.62, LSDpetiole= 32.31 (p>0.05). All percentage values, indicated in parentheses, were arcsine transformed.
Different letters show significant differences.

Table 2. Shoot formation results of the *C. phitosiana* leaf and petiole explants.

Plant growth regulator concentration	Leaf	Petiol
0.3 mg L^{-1} NAA + 0.5 mg L^{-1} GA_3	0.00 b (0.00)	0.00 b (0.00)
0.3 mg L^{-1} NAA + 1.0 mg L^{-1} GA_3	0.00 b (0.00)	0.00 b (0.00)
0.3 mg L^{-1} NAA + 2.0 mg L^{-1} GA_3	100.00 a (90.00)	100.00 a (90.00)
0.3 mg L^{-1} NAA + 3.0 mg L^{-1} GA_3	0.00 b (0.00)	0.00 b (0.00)

LSDleaf = 0, LSDpetiole=0 (p>0.05). All percentage values, indicated in parentheses, were arcsine transformed.
Different letters show significant differences.

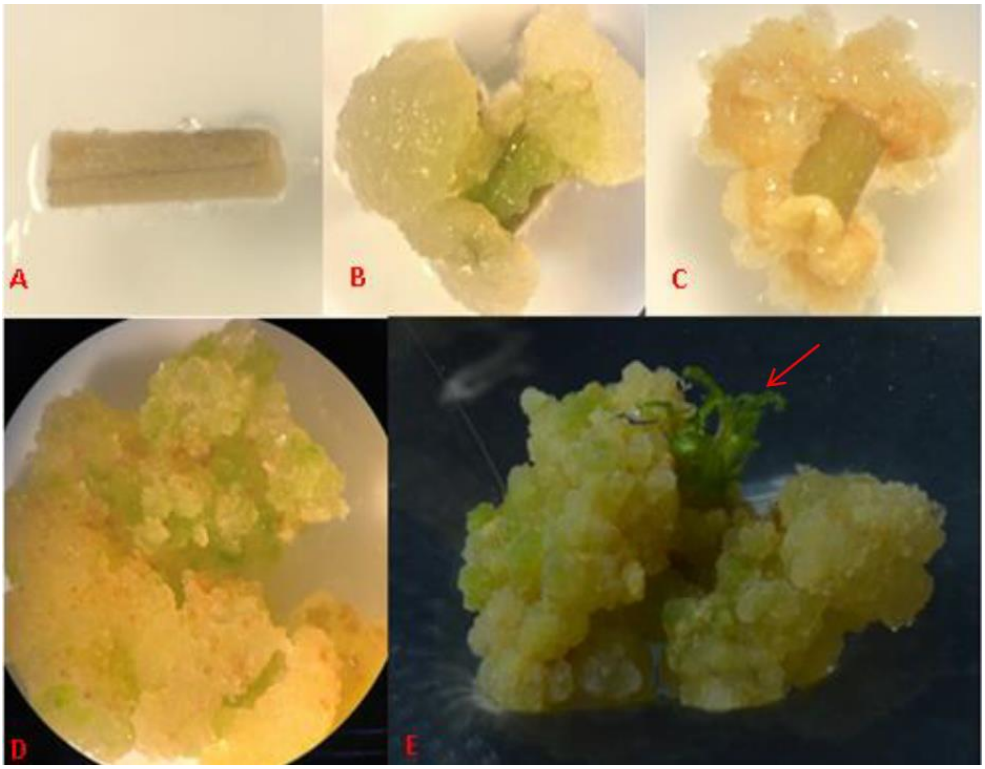

Figure 1. *In vitro* shoot regeneration from the petiole explants of *C. phitosiana* (A: First week of the culture, B-C: Callus at 4th week of the experiments, D: Shoot like formations, E: Shoots from petiole explants at 12th week).

Campanula phitosiana Yıldırım & Şentürk. Highest callus regeneration was obtained from leaf explants on the MS medium containing 0.3 mg L^{-1} NAA+ 2.0 mg L^{-1} BA and 0.3 mg L^{-1} NAA+ 2.0 mg L^{-1} TDZ as 60% (Table 1, Figure 2). These results showed that explant type was one of the important factors for *in vitro* regeneration of *Campanula phitosiana.* To transform the callus to the shoot, callus was cultured on MS medium containing 0.3 mg L^{-1} NAA and 0.5, 1.0, 2.0, and 3.0 mg L^{-1} GA_3. Shoot formation was obtained only from the MS medium supplied with 0.3 mg L^{-1} NAA + 2.0 mg L^{-1} GA_3 for both leaf and petiole explants (Table 2). There was no shoot formation on the other shoot proliferation medium. Shoots and shoot like structure were cultured on hormone free MS medium for root induction but root formation was not observed.

Protection and propagation of the genetic resource via *in vitro* technique has been developed for different species. Microporpagation provides the clonal multiplication of the rare endemic and endangered plants. In this study, *Campanula phitosiana* endemic to İzmir/Türkiye was efficiently propagated. There has been many reports on micropropagation of Campanula species but microporpagation of this endangered *Campanula*

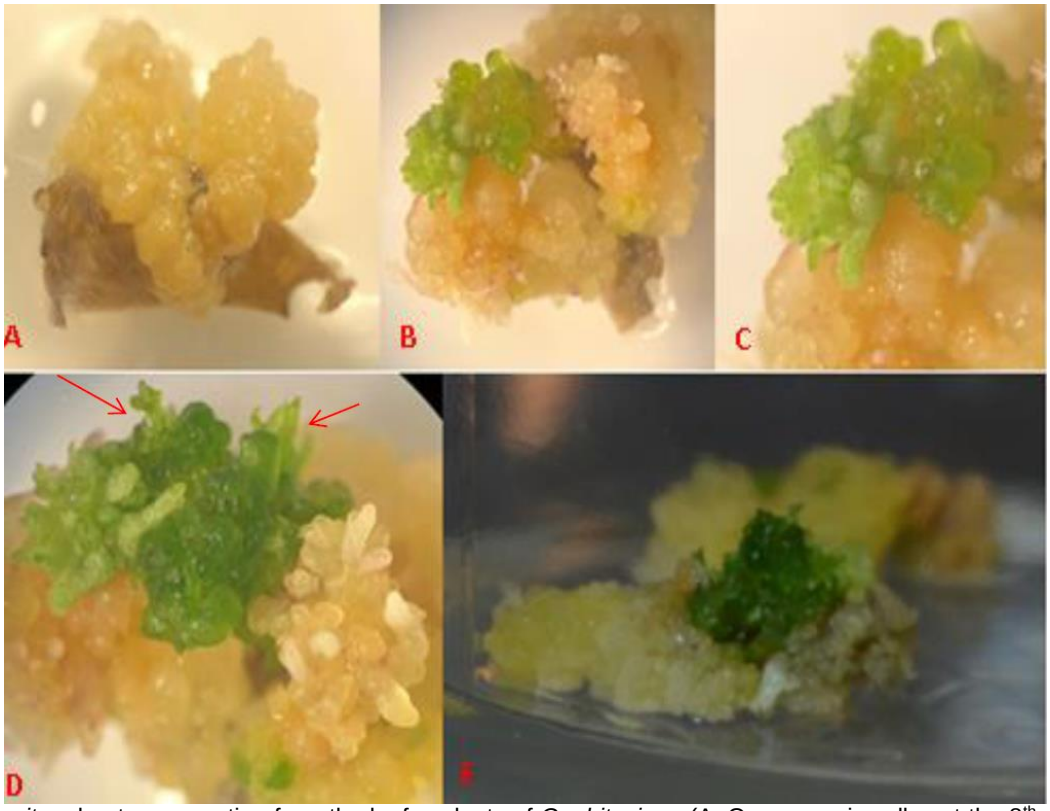

Figure 2. *In vitro* shoot regeneration from the leaf explants of *C. phitosiana* (A: Organogenic callus at the 8th week of the culture, B: Callus colours turned to the green at 10th week, C: Shoot like formations, D-E: Shoots from leaf explants at 12th week).

phitosiana was not performed. In this study, petiole explant showed efficient regeneration capacity according to leaf explants. On the other hand, leaf (Joung et al., 2002), cotyledon (Frello et al., 2002), buds (Maria, 2014), leaf (Tanaka et al., 1999) and petiole (Sivanesan et al., 2007; Sivanesan et al., 2011), ovule (Röper et al., 2015), hypocotil (Sriskandarajah et al., 2001; Mørk et al., 2005), nodes (Paunescu, 2010), shoots (Brandt, 1994), seedlings (Grigoriadou et al., 2014), seeds (Seglie et al., 2012) were used as explant for different Campanula species. Plant growth regulator is an important factor to manuplate the cell and tissue to obtain efficient regeneration. In this study, BA and TDZ were used as cytokinin and NAA used as auxin. Efficient callus regeneration was observed on MS medium 0.3 mg L^{-1} NAA + 1.0 mg L^{-1} BA, 0.3 mg L^{-1} NAA + 3.0 mg L^{-1} BA, 0.3 mg L^{-1} NAA + 1.00 mg L^{-1} TDZ, 0.3 mg L^{-1} NAA + 2.0 mg L^{-1} TDZ. Joung et al. (2002) carried out micropropagation of *Campanula glomerata* 'Acualis' on MS medium and they cultured the leaves obtained from MS medium including 1.0 mg L^{-1} BA + 0.01 mg L^{-1} NAA with different cytokinins such as BA and 2iP. They reported that BA was the efficient cytokinin for the clonal uniform propagation. On the other hand, our result presented that there was no significant differences between BA and TDZ. Frello et al., (2002) cultured cotyledon explant of the two different genotype of *Campanula carpatica* on MS medium including 0.50 mg L^{-1} NAA + 0.25 mg L^{-1} 2.4-D + 0.75 mg L^{-1} BA. They reported that there was a significant difference between genotypes. In our study, genotypes were randomly selected and there were no differences between the genotypes. Kolomiets et al., (2016) cultured the *C. sclerophylla* species on MS medium including 3.0 mg L^{-1} BAP+ 1.0 mg L^{-1} IAA to preserve the genetic resource via slow growth preservation. BA was determined as efficient cytokinin for *in vitro* regeneration of different Campanula species (Brandt, 1994; Airo et al., 2009; Paunescu, 2010; Stamenković et al., 2012; Maria, 2014). Sriskandarajah et al., (2001) cultured the *Campanula* sp. on MS medium including 10.0 mg L^{-1} TDZ + 0.25 mg L^{-1} NAA for the regeneration before transformation experiments. According to our observations, TDZ can be used as alternative cytokinin for the *in vitro* micropropagation of *Campanula phitosiana*. Efficient shoot proliferation was obtained from MS medium including 0.3 mg L^{-1} NAA + 2.0 mg L^{-1} GA_3. Sivanesan et al., (2011) germinated the somatic embryos on MS medium including 1.0 mg L^{-1} GA_3. Sevindik et al. (2017) cultured the shoots including nodes of *Origanum sipyleum* on MS medium BA+GA_3 combinations for multiplication. Ullah et al. (2011) reported that addition of GA_3 to the medium promotes the shoot elongation and plant growth (Roest and Bokelmann, 1976; Muller and Lipschutz, 1984). In our study, addition of the GA_3 to the medium induce the shoot formation from callus. To obtain root formation shoots and shoot like formations was transferred to the hormone free MS medium but root formation was not occurred.

4. Conclusion

Protection and sustainability of the genetic recourse is very important issue. *In vitro* techniques provide important advantages to clonal and mess production of the endemic genetic resources. Micropropagation is one of the important techniques to multiplicate the plants by using different parts as explant. *Campanula phitosiana* is native to İzmir and Aydın Mountains and it is defined as critically endangered plant according to red list. Development of efficient micropropagation protocol for this critically endemic species is promising due to sustainability of this species. In a conclusion, to optimize the regeneration protocol, MS medium combined with NAA as auxin, BA and TDZ as cytokinin. Efficient callus regeneration was obtained from the petiole explants. NAA and GA_3 was the efficient combination for the shoot induction. This is the first study that shows the efficient organogenic callus obtention for the *C. phitosiana.* This protocol could be very important for *in vitro* propagation, cryopreservation, synthetic seed production and transformation experiments for *C. phitosiana.*

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RESEARCH PAPER

HORTICULTURAL STUDIES

Effects of Some Plant Growth Regulators on Quality of Potted Sunflower

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Thidiazuron

Abstract

This study was carried out to evaluate the effects of different plant growth regulators on the ornamental sunflower plant. The seedlings belonging to *Helianthus annuus* L. cv. Sunsantion F1 were planted in plastic pots (2 L) containing peat:perlite (1:1, v/v) medium at four true leaf stage. Spray treatments of aminoethoxyvinil glycine (AVG; 250 and 500 ppm), promalin (5 and 10 ppm), thidiazuron (TDZ; 5-10 µM), ethephon (100 ppm) were applied to seedlings 20 days after transfering to the pots, and treatments were repeated 10 days after the first application. Flower life (days), flower diameter (cm), stem diameter (cm) and plant height (cm) were determined. According to statistical analysis, AVG at 250 ppm significantly increased the flower life from 9.17 d in control plants to 11.08 d whereas there were no significant effects on flower diameter and plant height. Both concentrations of Promalin and TDZ increased stem diameter significantly. However, TDZ increased the flower life from 9.17 d (control plants) to 10.36 at 10 µM and 10.33 at 5 µM TDZ. Ethephon application prevented flower bud opening and caused the leaves to be yellowing. These results may suggest AVG and TDZ pre-treatments increase the quality and shelf-life of the potted sunflower plant.

1. Introduction

The sunflower (*Helianthus annuus* L.) is a member of Asteraceae family and *Helianthus* genus. The term Helianthus is derived from the Greek words 'Helios' and 'anthos', meaning sun and flower, respectively. It has consumed as medicine and food since ancient times. In the following years, with the industrial importance of sunflower oil worldwide, it became a very important oilseed plant (Naik et al., 2017). In the past 2-3 years, the problems in the food supply chain due to the COVID-19 pandemic and the Russia-Ukraine war have turned the sunflower plant into a strategic product. Today, sunflowers are also widely used as potted ornamental plants and cut flowers or garden plants for decoration purposes due to their attractive flowers in landscape areas (Elisheba and Sudhagar, 2021).

The main quality parameters for potted plants are attractive flowers, flower longevity, shape, size, and visual appearance, but each species or cultivar may have unique characteristics contributing to defining quality (Ferrante et al., 2015). Different practices can undoubtedly increase the quality of ornamental plants. However, among the various management practices adopted for manipulating growth and flowering in ornamental plants, perhaps no other management practice is as popular as using chemicals to manipulate plant growth (Sethy et al., 2016). Numerous chemicals known as Plant Growth Regulators (PGRs) are available to control plant growth in various commercial formulations (Bañón Arias et al., 2013).

Plant growth regulators can be classified into six groups including gibberellins, auxins, cytokinins, ethylene generators, growth inhibitors and growth retardants (Sajjad et al., 2017). Among gibberellins, Promalin is a common commercial PGR consisting of gibberellins A4+A7 and 6-Benzyladenine. It has long been used in many ornamental plant studies to deal with apical dominance and promote lateral bud development and branching in Algerian ivy (Al-Juboory and Williams, 1990), to reduce the incidence of leaf senescence after cold storages in potted Asiflorum lily (Funnel and Heins, 1998), to reduce disorders related with postharvest cold storage in potted *Leucospermum* (Hoffmann et al., 2015), and to increase flower development in cyclamen (Alshakhaly and Qrunfleh, 2019). Previous studies have shown that gibberellins supplemented with cytokinins work better than the use of gibberellins alone. However, the use of cytokinins or cytokinin analogues alone affects plant growth and development in different ways. Çelikel et al. (2021) reported that shoot elongation and stem enlargement of potted rose plants are regulated by TDZ applications. Additionally, TDZ spray treatments extended flower longevity in *Euphorbia fulgens* (Jiang et al., 2009), delayed leaf senescence in chrysanthemum, alstroemeria, and tulip (Ferrante et al., 2002, Ferrante et al., 2003), and increased shoot formation in nandina (Keever and Morrison, 2003).

While auxins, cytokinins, and gibberellins promote growth and development in plants, ethylene, ethylene-releasing compounds, growth inhibitors and growth retardants also play an important role in increasing the quality characteristics of ornamental plants such as plant size and compactness by suppressing growth (Marosz and Matysiak, 2005). Ethephon releases the natural plant hormone ethylene and increases its production. Ethylene release caused by Ethephon application reduces apical dominance and encourages the development of lateral shoots (Haver et al., 2002). Etephon also enables the plant to become compact by suppressing the height (Demir and Çelikel, 2018), but it is accelerated the ethylene related senescence (Wang et al., 2020). On the other hand, plants experience stress due to environmental and cultivation conditions such as temperature, poor light, low humidity, or watering during post-production period (Wagstaff et al., 2010). As a results of this stress, ethylene production is triggered, and the visual quality of the plant deteriorates (Morgan, 2011). Plants exposed to ethylene can usually no longer be sold (Olsen et al., 2015). Therefore, ethylene inhibitors are used to reduce ethylene-induced quality losses in ornamental plant. Aminoethoxyvinylglycine (AVG) is one of the ethylene inhibitors that blocks activity of ACC synthase which is a key enzyme involved in ethylene biosynthesis (Saltveit, 2005). There are much more studies on AVG treatments used for preventing ethylene production and maintaining quality parameters of fruits such as plum (Kim et al., 2021), peach (Bregoli et al., 2022), apple (Yildiz et al., 2012). There are limited studies about the use of AVG in ornamental plants such as pelargonium, ruscus, rose (Elad and Volpin, 1988) and chrysanthemum (Zheng et al., 2004).

The quality of ornamental plants is generally evaluated by flower longevity (Olsen et al., 2015) and other visual parameters which maintained by producer using PGRs. It is essential to assess the efficiency of PGRs because the effects of PGRs in plants depend on various factors such as type of PGRs, application method, application frequency, concentration, time, the plant species even cultivar, as well as the environmental conditions in which the plants were grown (Sajjad et al., 2017). Therefore, it was aimed to determine the effect of some growth regulators applied as a pre-application in sunflower (*Helianthus annuus* L.) in this study.

2. Material and Method

2.1. Plant material and cultivation

The study was conducted at a polyethylene greenhouse in the application area of the Agriculture Faculty in Ondokuz Mayıs University, Samsun, Türkiye, during the summer of 2022. The minimum, maximum, and average temperature values in the greenhouse were measured at hour intervals using a data logger throughout the growing season (Figure 1). Ornamental sunflower (*Helianthus annuus* L. cv. Sunsantion F_1) was used as plant material in the study. The seedlings were obtained from a local ornamental production company in vials at four true leaf periods. They were planted in plastic pots (2 L) containing peat: perlite (1:1 v/v) medium. The irrigation was performed with approximately 250 mL of tap water per pot daily. No fertilization and chemicals for pest and disease control were used.

2.2. PGRs treatments

Spray treatments of 250-500 ppm AVG (Retain,15% aminoethoxyvinil glycine), 5-10 ppm Promalin (1.9% Gibberellins A4+A7, 1.9% 6-Benzyladenine), 5-10 µM TDZ (Thidiazuron), 100 ppm Ethephon (2-Chloroethylphosphonic acid) and distilled water (as a control) were made before flowering time in plants. The whole plant was sprayed with PGRs (30 ml plant^{-1}) until thoroughly washed. The first chemical applications were applied to seedlings 20 days after transfer (DAT) to the pots. Then applications were repeated at 30 DAT.

2.3. Evaluated parameters

Flower longevity (the day from blooming to wilting), flower diameter (cm), stem diameter (cm)

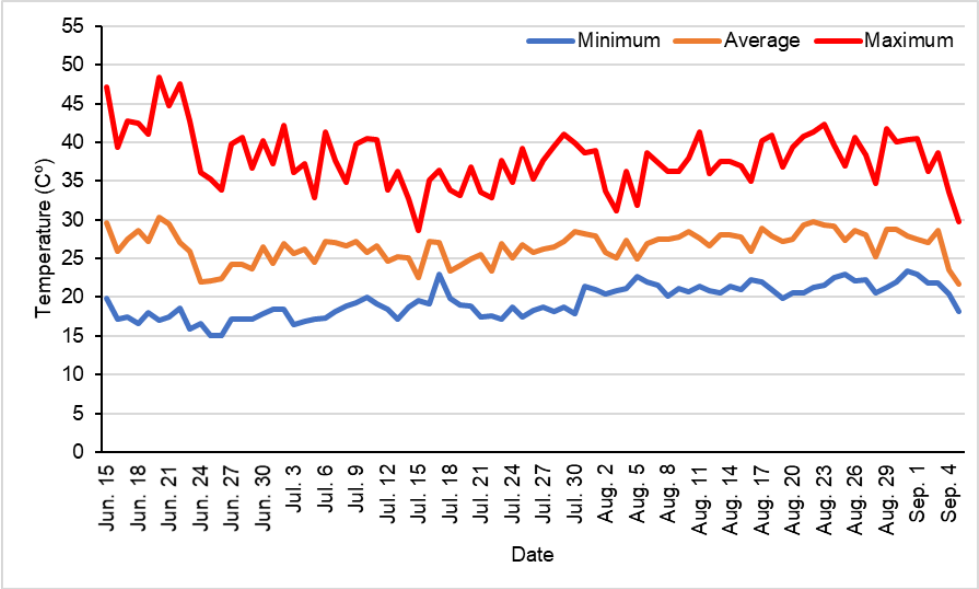

Figure 1. Temperature values in the greenhouse.

Table 1. Effects of some treatments on different quality parameters of potted sunflowers.

Treatments	Flower longevity (day)**	Flower diameter (cm)	Stem diameter (cm)*	Plant height (cm)
Control	9.17 b	10.43	8.22 b	26.43
Promalin 5 ppm	10.28 ab	9.36	9.17 a	25.37
Promalin 10 ppm	9.97 ab	9.46	9.03 a	25.95
TDZ 5 µM	10.33 ab	9.90	8.77 ab	25.23
TDZ 10 µM	10.36 ab	9.76	9.11 a	26.06
AVG 250 ppm	11.08 a	9.52	8.86 ab	24.09
AVG 500 ppm	9.11 b	10.08	8.77 ab	25.79
Ethephon 100 ppm	-	-	7.84 b	22.87

** and *, level of significance are represented by at $p<0.01$ and $p<0.05$, respectively.

and plant height (cm) were determined. Flower and stem diameters were measured with a digital caliper at full flowering and maturation stages, respectively. Plant height was measured with a ruler from the soil surface to the tip of the plant.

2.4. Experimental design and statistical analysis

The research was established according to a completely randomized design with ten replications. Four different chemicals, seven treatments, and a control group were used for applications. Each replication had a single seedling and 10 replications for each treatment were evaluated. Variance analysis was performed using SPSS statistical software version 18.0 and differences between threatments compared with Duncan multiple comparison test.

3. Results and Discussion

3.1. Effects of promalin

Effects of Promalin (GA + BA) treatments at 5 and 10 ppm on different quality parameters of sunflowers are given in Table 1. Both concentrations increased the flower life from 9.17 d (control plants) to 9.97 at 10 ppm and 10.28 at 5 ppm Promalin. There were no significant effects on flower diameter and plant height. However, both doses of Promalin increased stem diameter significantly ($p<0.05$), about 1 cm. Promalin consists of GA and cytokinin BA. It is well known that cytokinins increase stem thickness (Werner et al., 2001; Çelikel et al., 2021). Axillary bud growth was observed at 45 DAT at 5 and 10 ppm treatments of Promalin (Figure 2). Plants develop single flower heads when the ratio of auxin to cytokinin does not change. Therefore, axillary bud growth may suggest that Promalin treatments altered endogenous hormone translocation and increased cytokinin levels led to axillary bud initiation (Nagarathna et al., 2010).

3.2. Effects of TDZ (Thidiazuron)

Effects of TDZ (Thidiazuron) treatments at 5 and 10 µM on different quality parameters of sunflowers are given in Table 1. Both concentrations increased the flower life from 9.17 d (control plants) to 10.36 at 10 µM and 10.33 at 5 µM TDZ. There were no

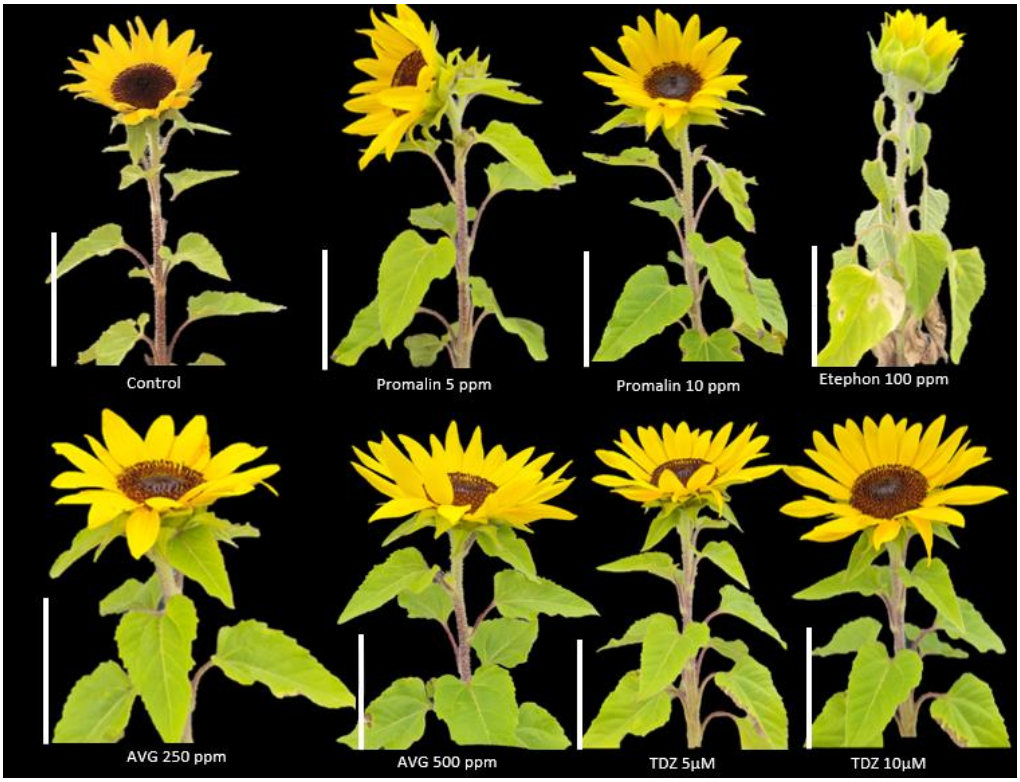

Figure 2. Effects of some treatments on visual quality of potted sunflowers 45 days after the seedlings were transferred into the pots (Scale bars: 10 cm).

significant effects on flower diameter and plant height. However, both concentrations of TDZ increased stem diameter. Thidiazuron at 10 µM significantly ($p<0.05$) increased the stem diameter about 1 cm. Thidiazuron as a cytokinin is known to increase stem diameter (Çelikel et al., 2021) and longevity (Ferrante et al., 2002; Çelikel et al., 2019).

3.3. Effects of AVG (Aminoethoxyvinil Glycine) and Ethephon

Effects of AVG treatments at 250 and 500 ppm on different quality parameters of sunflowers are given in Table 1. AVG at 250 ppm ($p<0.01$) significantly increased the flower life from 9.17 d (control plants) to 11.08 d. Sunflower from Asteraceae family is known not to be sensitive to ethylene (Reid, 1989). However, prolonged exposure of sunflowers to low concentrations of ethylene results in the abscission of ligules (Reid, 2004). In this study, ethephon (released ethylene) spray at an early stage had a negative effect on flower opening and quality (Figure 2) and it prevented flower bud opening and caused the leaves to be yellowing. Therefore, flower longevity and flower diameter were not measured. In addition, ethylene inhibitor AVG (Saltveit, 2005) at a lower concentration significantly increased the flower longevity in this experiment, while a higher concentration of 500 ppm AVG had no significant effect. Kılıç et al. (2020) reported that ethylene action inhibitor silver thiosulfate (STS) extended the vase life of sunflower cv. 'Sunrich Orange'. However, another ethylene inhibitor amino-oxyacetic acid (AOA) treatment did not lengthen the vase life of sunflowers (Mensuali-Sodi and Ferrante, 2005). Additionally, Redman et al. (2002) indicated that STS had no effect on the vase life of *Helianthus maximilianii*, while exogenous ethylene application significantly decreased vase life compared to the control group. Gast (1995) reported that the longevity of sunflower cultivars varied from 4 to 13 days. More research study with different cultivars is needed on this issue. There were no significant effects of AVG in this study on flower diameter and plant height. However, both doses of AVG slightly increased stem diameter. On the other hand, stem diameter and plant height exposed to ethephon were the lowest among treatments (Table 1).

4. Conclusion

The results of the study on the post production performance of potted sunflower may suggest that ethylene inhibitor AVG at 250 ppm as spray treatment before flowering maintains the quality and shelf life of flowers. In addition, 10 µM TDZ treatments also extend flower longevity compared to the 5 µM TDZ and control that may suggest higher concentrations of TDZ should be evaluated. Ethephon which releases the natural plant hormone ethylene negatively affected flower opening. Therefore, exposure to ethylene or stress factors which increase endogenous ethylene production should be avoided during plant growth.

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