

## EXAMINATION OF MORPHOLOGICAL AND MOLECULAR CHANGES IN TOMATO (*Solanum lycopersicum* L.) SEEDLINGS WITH THE APPLICATION OF TEBUCONAZOLE

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### ABSTRACT

This study aims to determine the effects of tebuconazole substance used at different doses on the quality of tomato seedlings, retrotransposon mobility caused by this substance in the plants and its genotoxic effects and to determine the optimum dose to be used in practice in seedling production. Tebuconazole applied different concentrations (25, 50, 75, 100, 125 and 150 ppm) were tested. According to the study results, the seedling length and stem length significantly according to the applied doses. The study found out that the stem, leaf and root dry matter contents and leaf chlorophyll content (SPAD values) also statistically significantly changed based on the applied doses. According to the data obtained, the lowest seedling length (12.68 cm) and stem length (4.75 cm) were obtained from a dose of 150 ppm. The highest dry matter content in seedlings was obtained at a dose of 50 ppm in stem (22%) and at 0 ppm (control) in leaves (25.01%) and root. In the study, the highest leaf chlorophyll content (SPAD values) was determined at doses of 150 ppm, 125 ppm and 75 ppm, respectively. This study revealed that various doses of tebuconazole had a positive effect on controlling the height of tomato seedlings and specific quality characteristics of seedlings. In addition, molecular analyzes showed that polymorphism ratios in plants that were applied the substance at different doses varied between 4.70% and 38.09% and the GTS (genomic template stability) value varied between 61.91% and 95.30%. Analyses indicated that the polymorphism ratio increased depending on the increase in dose whereas the GTS value decreased.

**Key words:** seedling, tomato, quality, tebuconazole, IRAP, genotoxic

### INTRODUCTION

Tomato is one of the major vegetables grown around the world. The use of high-quality seeds and the resulting high-quality seedling are essential in successful vegetable production [Demir et al. 2010]. Using high-quality seedlings for production is paramount for yield and product quality [Sönmez 2017]. A high-quality seedling should have a thick stem, dark

green leaves and a vibrant and robust root structure. Low-quality seedlings overgrow, the leaf area is reduced, the leaf chlorophyll content decreases and the colour of leaves gets lighter. To control the seedling height and improve seedling quality and appearance, mechanical stress factors [Garner and Björkman 1996], various stress factors, ecological factors [Melton and

Dufault 1991, Głowacka 2004] and various plant nutrients were tested. However, these practices were not sufficient in increasing the seedling quality. Numerous studies have been conducted on chemicals with growth-slowing or retarding properties (such as daminozid, uniconazole, chlormequat chloride and paclobutrazol) that are effective in controlling seedling height in different vegetable species. However, such chemicals cause are mainly sprayed on the leaf surfaces during the young seedling period and problems such as chlorosis, leaf blight or long-term pauses in subsequent growth and development periods of seedlings and delay in harvesting may occur due to improper doses. In addition, such substances can also alter the epigenetic construct that affects the activity of genomic elements. Retrotransposons are known to play key roles in host genome evolution by altering gene expression or inducing DNA rearrangement. Literature studies conducted with IRAP analysis under different stress conditions in order to investigate the level of retrotransposon mobility are available [Yigider et al. 2016]. Against environmental stresses, epigenetic mechanisms play a key role in responding to stress by regulating gene expression of the genome differently [Angers et al. 2010]. Changing environmental conditions, stress factors or particular chemicals can modify the epigenetic structure, affecting the activity of these genomic elements [Wessler 2006]. Transposons can insert into several different regions of the genome as a result of their transposition. Since they can alter gene function, structure and activity, they can also change the genome and chromosome structures [Bennetzen 2000, Federoff 2000, Wicker et al. 2007]. This leads to differences in band profiles obtained in IRAP analysis. These differences are expressed as polymorphism. One of the most frequently used substances, paclobutrazol allows controlling the seedling height and increasing the quality of seedlings [Yim et al. 1997, Fletcher et al. 2000, da Silva Wanderley et al. 2014, Sipioni et al. 2016]. It is widely used in commercial preparations where the active ingredient is paclobutrazol. Paclobutrazol is a chemical substance from the triazol group. Tebuconazole, which is another substance from the same group, is used in fungal diseases. The efficiency of tebuconazole has been reported in seedlings of other vegetable species, such as cucumber [Mohsin et al. 2019, Bulut et al. 2021], eggplant [Öz-

türk and Dursun 2020], and melon [Öztürk and Bulut 2020]. However in the literature review, there are limited studies of tebuconazole to control seedling size of vegetables and improve seedling quality.

This study aims to determine the effects of tebuconazole active substance used at different doses on the height and quality of tomato seedlings, retrotransposon mobility caused by this substance in the plants and its genotoxic effects.

## MATERIALS AND METHODS

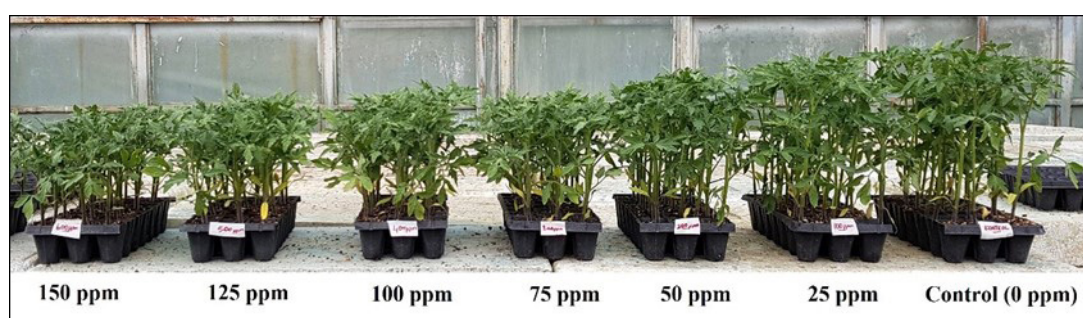
The study was conducted in March–May 2020 in the seedling greenhouse operated by Erzincan Horticultural Research Institute (latitude 39°43'34.4"N, longitude 39°28'11.2"E). During the experiment, the average day/night temperature in the greenhouse was approximately 25/12°C and the average humidity was measured at 70%. Kayra F1 (Anamas Tohum Company) variety of tomatoes was used in the experiment. Seedlings were grown in 128-compartment viols, each of which is 40×40 mm. A mixture of peat (Klasmann TS 1®) and perlite (Agrobit®) (60% peat, 40% perlite) was used as seedling growing medium. The seedlings were watered daily, but no nutrients were applied to the seedlings during the experiment. As the source of tebuconazole, a commercial preparation Folicur WP 25 containing 25% tebuconazole (developed by Bayer) was used. In this randomized-block-design study, seven different application doses (0, 25, 50, 75, 100, 125 and 150 ppm, respectively) were applied to plants. Approximately 300 ml of tebuconazole solution in spray form was applied to each group (25, 50, 75, 100, 125 and 150 ppm). Seedlings to which tebuconazole will be applied in different doses are given in Figure 1.

According to randomized block design, the study is planned with 3 repeats and 21 plants in each repeat. Tebuconazole solutions prepared at different doses in the laboratory were applied by spraying to the green parts of the plants 30 days after sowing, with two applications at 14-day intervals, and after the final application, the seedlings were grown under the greenhouse conditions until the sowing period (2 weeks later; Fig. 2). The necessary measurements, observation and analysis were made on the seedlings.

**Harvest and growth parameters.** Seedling height (cm), stem height (cm), number of leaf (number/plant),



**Fig. 1.** Tomato seedlings to which tebuconazole will be applied at different doses



**Fig. 2.** Tomato seedlings to which tebuconazole was applied at different doses and to be sowed

stem diameter (mm), leaf dry matter content (%), stem dry matter content (%) and root dry matter content (%) and leaf chlorophyll content (SPAD) were measured to determine the seedling development. Seedling height (cm) and stem height (cm) were measured with tape measure and stem diameter (mm) was measured with a digital calliper. Leaf chlorophyll content was measured using SPAD (Chlorophyll Meter SPAD-502Plus, Konica Minolta). Prior to the measurement of the root height, the roots were washed thoroughly without any root loss and measured in cm with the help of a tape measure. In order to determine the dry matter contents in seedlings, ten seedlings were taken randomly and leaves, stems and roots were dried outdoors for one week after the wet weight was determined. They were then dried at 105°C in an oven for 24 hours and weighed [AOAC 1980]. The wet and dry weights were determined using a scale with 0.01 g precision and dry matter content (%) was determined with the following formula [Kılıç et al. 1991]:

dry matter content (%) =  $\text{dry weight} \times 100 / \text{wet weight}$

SPSS 22.0 statistical program was used for statistical analysis of the data and the difference between

the means was determined by Duncan's test (5%). In addition, the relationships between harvest and growth parameters were determined by correlation analysis.

## MOLECULAR ANALYSIS

**DNA isolation.** DNA was isolated from plant samples for IRAP (Inter Retrotransposon Amplified Polymorphism) analysis. DNA isolation was accomplished with minor changes in the method expressed by Saghai-Maroo et al. [1984]. DNA concentrations were determined with ACTGene Spectrophotometer (ACTGene UVIS-99, NJ, USA) at A260 280-1 O.D. and the DNA of all samples was adjusted to 0.5 µg.

**IRAP marker analysis.** Six IRAP primers (Metabion International AG Lena-Christ-Strasse 44/I D82152 Martinsried, Deutschland) were used in the study. The name, sequence and melting temperatures of the primers are shown in Table 1.

The components and quantities required for the IRAP-PCR procedure for the evaluation of retrotransposon mobility were prepared with the values given in Table 2.

**Table 1.** Details of primers used in the IRAP-PCR analysis [Bulut et al. 2021]

Primer name	Sequence 5' → 3'	Melting temperature (°C)
SUKKULA	GATAGGGTCGCATCTTGGGCGTGAC	63.3
3LTR-5	TGTTTCCCATGCGACGTTCCCAACA	64.6
LTR 6150	CTGGTTCGGCCCATGTCTATGTATCCACACATGTA	64.4
NIKITA E2647-	ACCCCTCTAGGCGACATCC	58.7
5LTR1	TTGCCTCTAGGGCATATTTTCCAACA	58.4
LTR 6149-5	CTCGCTCGCCCACTACATCAACCGCGTTTATT	65.9

**Table 2.** Components of IRAP-PCR analysis [Bulut et al. 2021]

Component	Quantity (µl)
10 × PCR buffer	2
dNTPs (10 nM)	0.5
MgCl <sub>2</sub> (25 mM)	1.25
IRAP primer (5 mM)	1
Taq DNA polymerase	1
Ultra pure water	13.25
Genomic DNA	1
Total volume	20

The samples were subjected to the PCR protocol given in Table 3

**IRAP electrophoresis protocol.** The PCR products obtained were loaded with a gel loading solution in agarose gel and were run at 90 volts for 100 min. The bands formed as a result of electrophoresis were examined under a UV device at 256-nm UV light.

**IRAP analysis and calculation of genomic template stability (GTS).** Genomic mold stability (%) was calculated for each primer using the following formula of  $100(100 - a n)^{-1}$  by Atienzar [1999];  $a$  in the formula refers to the IRAP polymorphic profiles determined for each sample, and  $n$  refers to the total amount of DNA band obtained with the respective primary in the negative control group. The polymorphism observed in the IRAP profiles of the samples included the loss of a new band or the existing band that occurred compared to the negative control group. Total Lab TL120 was used to evaluate these bands.

## RESULTS

### Harvest and growth parameters

The study revealed that the application of tebuconazole at different doses had a statistically significant effect on all harvest and growth parameters of tomatoes.

**Seedling height (cm).** In the study, there was a decrease of 12.75%, 22.6%, 34.72%, 35.72%, 39.21% and 44.63% rate, respectively (25, 50, 75, 100, 125 and 150 ppm), in the seedling height compared to the 0 ppm (control group) application. The lowest and highest seedling height was detected in the at 150 and 0 ppm applications, respectively (Tab. 4).

**Stem height (cm) applications.** In the study, the highest stem height was determined to be in the (control) application and the lowest stem height was de-

**Table 3.** IRAP-PCR protocol [Bulut et al. 2021]

Cycle name	Temperature	Time (sec.)	Number of cycles
Initial denaturation	95°C	120	1
Denaturation	95°C	30	2
Primer binding	°C*	60	1
Elongation	72°C	120	1
Denaturation	95°C	30	41
Primer binding	35°C	60	1
Elongation	72°C	120	1
Last elongation	72°C	300	1
Termination	4°C	∞	1

\* Adhesion temperature of the relevant IRAP primer given in Table 1

**Table 4.** The effects of the application of tebuconazole on seedling growth parameters of tomato seedlings

Doses (ppm)	Seedling height (cm)***	Stem height (cm)***	Root height (cm)***	Stem diameter (mm)***	Leaf dry matter ratio (%)***	Stem dry matter ratio (%)***	Root dry matter ratio (%)***	Chlorophyll quantity (SPAD value)*
0	22.90a	7.25a	9.73b	3.90c	16.32a	16.05b	13.48a	48.63b
25	19.98b	6.22b	10.58a	4.00c	12.32c	15.79b	9.44c	50.25ba
50	17.72c	6.28b	9.40b	4.00c	12.17c	22.00a	8.87cd	49.88ba
75	14.95d	5.55c	8.65c	4.01c	14.71b	16.52b	8.69d	51.55a
100	14.72e	5.57c	8.27dc	4.30b	15.06b	17.36b	9.22cd	50.75ba
125	13.92f	5.50c	8.70c	4.42a	14.19b	16.22b	10.04b	51.85a
150	12.68g	4.75d	7.65d	4.40ba	14.18b	16.57b	9.36c	52.33a

The averages with various letters in each column have statistically significant differences at \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , respectively

terminated to be in the dose application of 150 ppm. In tebuconazole application, 14.2%, 13.38%, 23.45%, 23.17%, 24.13% and 34.48% suppression was detected in the stem height compared to the control application, respectively (Tab. 4).

**Root height (cm).** After the control group, the highest root height was determined at 25 ppm with an increase of 8.03%, and the lowest at a dose of 150 ppm with a decrease of 21.38%. In other applications, there was a decrease of 3.39%, 11.1%, 15% and 10.59% (50, 75, 100 and 125 ppm), respectively (Tab. 4).

**Stem diameter (mm).** In the study, the stem diameter showed an overall increase compared to the control application at the other all application of tebuconazole. Compared to the control application, tebuconazole applications provided an increase of 0.1 (25 and 50 ppm), 0.11 (75 ppm), 0.4 (100 ppm), 0.52 (125 ppm), and 0.5 mm (150 ppm) in stem diameter. The highest stem diameter was detected in the application of tebuconazole at 125 ppm (Tab. 4).

**Leaf dry matter content (%).** In the study, as a result of the application of tebuconazole, an overall decrease



**Table 5.** The correlation analysis between seedling characteristics of tomato seedlings to which application was made

	1	2	3	4	5	6	7	8
Seedling height (cm) (1)	1	–	–	–	–	–	–	–
Stem height (cm) (2)	0.939*	1	–	–	–	–	–	–
Stem diameter (mm) (3)	–0.808*	–0.767*	1	–	–	–	–	–
Root height (cm) (4)	0.804*	0.747*	–0.648*	1	–	–	–	–
Leaf dry matter ratio (%) (5)	0.068	0.121	0.030	–0.378	1	–	–	–
Stem dry matter ratio (%) (6)	–0.018	0.083	–0.128	0.013	–0.456**	1	–	–
Root dry matter ratio (%) (7)	0.584*	0.557*	–0.068	0.284	0.332	–0.132	1	–
SPAD (8)	–0.697*	–0.703*	0.537**	–0.468**	–0.073	–0.212	–0.430	1

\* Significant at the 1% level; \*\* significant at the 5% level

**Table 6.** Data from IRAP analysis

Primer name	Control (0 ppm)	25 ppm	50 ppm	75 ppm	100 ppm	125 ppm	150 ppm
3LTR5	3	...*	...	–252	–193	–252	–193
LTR 6150	2	...	...	...	...	+283	+390
Nikita	8	...	...	–1.156 –456	–1.156 –769 –456	+980 –1.156 –456	–1.399 –769 –519
Sukkula	1	...	...	...	...	...	...
LTR6149-5	5	–483	–200	–672	–672	–672	+356 –672
5LTR1	2	...	...	...	+354	+363	+378
Total number of bands	21	1	1	4	6	7	8
Polymorphism rate	–	4.70%	4.70%	19.04%	28.57%	33.33%	38.09%
GTS value	–	95.30%	95.30%	80.96%	71.43%	66.67%	61.91%

\* The presence of the same band compared to the control

was observed in the leaf dry matter content at all doses compared with the control group. The reduction rates were determined as 24.5%, 25.42%, 9.87%, 7.72%, 13.05% and 13.11% (25, 50, 75, 100, 125 and 150 ppm), respectively. While the control group had the highest leaf dry matter content, the highest dry matter content after the control group was obtained at doses of 100, 75 and 125 ppm, respectively (Tab. 4).

**Stem dry matter content (%).** While the stem dry matter content obtained from the control group in the study was 16.05%, the stem dry matter content increased at all doses, except 100 ppm, as a result of the

application of tebuconazole and the highest stem dry matter content was determined at 50 ppm application dose (Tab. 4).

**Root dry matter content (%).** In the study, the highest dry matter content in the root was determined in the control application (0 ppm). In the study, there was a decrease of 29.97%, 34.2%, 35.53%, 31.6%, 25.52% and 30.56% rate, compared to the control group (25, 50, 75, 100, 125 and 150 ppm), respectively (Tab. 4).

**Leaf chlorophyll content (SPAD value).** SPAD value increased in all other applications compared to the control group. There was ratios increase of 3.33%,

2.57%, 6%, 4.36%, 6.62% and 7.6% (25, 50, 75, 100, 125 and 150 ppm) in SPAD values after the application, respectively. The highest SPAD values were obtained from 150 ppm, 125 ppm and 75 ppm, respectively (Tab. 4).

**The correlation analysis between quality characteristics of seedlings.** The analysis indicated that the seedling height showed a positive correlation with stem height, root height and root dry matter content, and a significant negative correlation with stem diameter and the SPAD values. The stem length had

a statistically significant positive relationship with root height and dry matter content, and a negative relationship with stem diameter and the SPAD values. According to the analyses, a negative correlation was found between the stem diameter and root height. An inverse correlation was found between leaf dry matter content and stem dry matter content based on the correlation analyses (Tab. 5).

**IRAP analysis.** IRAP analysis was performed to determine retrotransposon mobility in the samples to which tebuconazole was applied and compared with

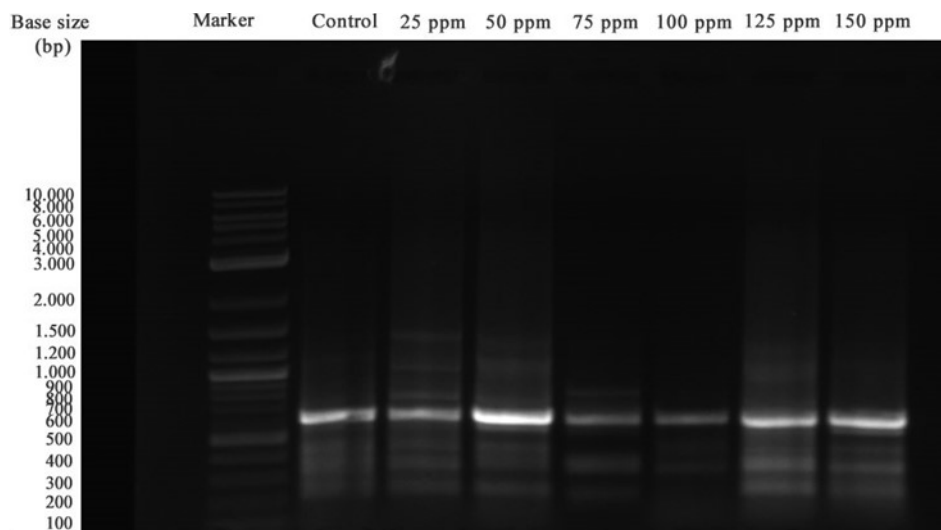


Fig. 3. Band image from Nikita primer

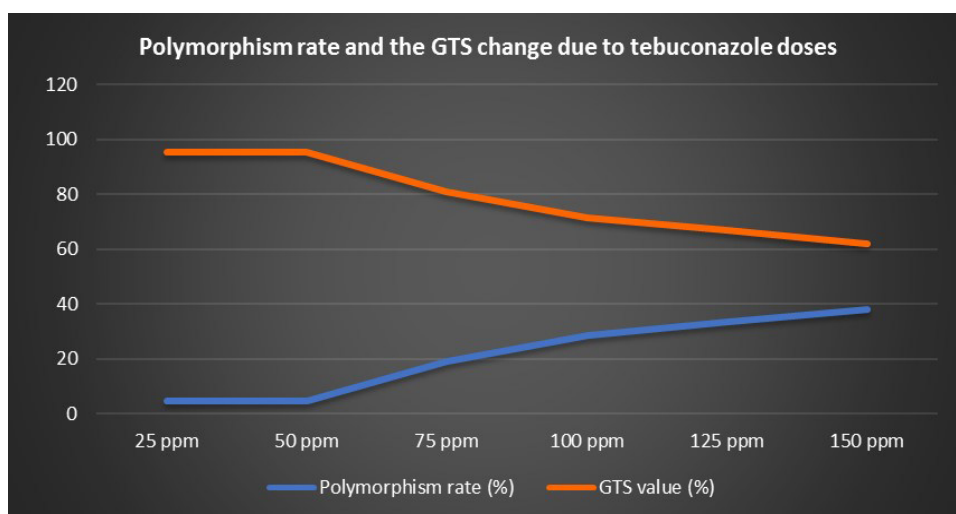


Fig. 4. Polymorphism rate and the GTS value

the control group. 113 bands were obtained from 6 IRAP primers used to determine the stress level caused by the fungicide of tebuconazole at the molecular level. The sizes of these bands ranged from 72 to 1,399 bp. The highest number of polymorphic bands was obtained from Nikita primer with 11 bands. Details of the data obtained from the IRAP analysis are given in Table 6.

Polymorphism was detected in all seedlings to which tebuconazole was applied. The resulting polymorphism varied between 4.70% and 38.09%. The lowest polymorphism was obtained as 4.70% in the samples to which tebuconazole was applied at 25 ppm and 50 ppm. Depending on the incremental dose, polymorphism value also increased in seedlings to which tebuconazole was applied. The highest polymorphism was determined as 38.09% in seedlings to which tebuconazole was applied at 150 ppm.

The GTS (genomic template stability) value, which represents the genomic stability, varied depending on the applied dose of tebuconazole. The GTS value varied between 61.91% and 95.30%. The highest GTS value (95.30%) was found in seedlings to which tebuconazole was applied at 25 and 50 ppm. The GTS value decreased to 61.91% depending on the increase in the dose of tebuconazole. Figure 3 shows the band image obtained from the Nikita primer.

The polymorphism rates and GTS values that vary depending on the dose of tebuconazole applied are given in Figure 4.

## DISCUSSION

As a result of the literature review, investigates the effects of the application of tebuconazole to tomatoes and other vegetable species on their seedling quality was limited studies [Mohsin et al. 2019, Bulut et al. 2021]. However, several studies investigating the effects of paclobutrazol and similar substances, which are included in triazol group similarly with tebuconazole, on vegetable seedling quality are available [Brigard et al. 2006, Çopur and Sarı 2012, Geboloğlu et al. 2015, Uçan 2019]. Geboloğlu et al. [2015] applied four different doses of paclobutrazole (50, 100, 200 and 500 ppm, respectively) to eggplant seedlings in 2 different seedling development stages and found that seedling height and stem height were significantly

suppressed as a result of this application. In another study, the researchers examined the characteristics of tomato seedlings after the application of paclobutrazole from the soil (1 ppm) and leaf spray (25 ppm) at the stage where 2 to 4 true leaves were formed in tomato seedlings. As a result of the measurements, the spray applications from soil and leaves suppressed the plant height by 20% and 16%, respectively [Berova and Zlatev 2000]. Similarly, in our study, the application of tebuconazole significantly suppressed the seedling height. The results in our study are similar to the results obtained by the researchers. This type of substances can inhibit gibberellic acid synthesis [Geboloğlu et al. 2015] and the decrease in height observed in our study is thought to originate from this fact. A similar study reported that the PP333 and CCC retarders applied to the eggplant during the period when they had 6 leaves decreased the stem height [Xue et al. 2008]. According to the control application in our study, the dose of 150 ppm suppressed the stem height at the maximum level. The resulting data and measurements indicated that the applications increased the stem diameter. The stem diameter in the control group was measured as 3.90 mm and the highest stem diameter (4.42 mm) was obtained from the dose of 125 ppm. Based on the control application, a 13.3% increase in stem diameter was determined. Several studies, where similar substances were applied, report an increase in plant stem diameter compared to the control application [Berova and Zlatev 2000, Zandstra et al. 2007, Teto et al. 2016]. One of the key characteristics for seedling quality is the dry matter content. Our applications showed that the leaf, stem and root dry matter contents varied statistically significantly between the applied doses. Compared to the control group, leaf and root dry matter contents decreased. In a similar study, the control applications showed decreases at 7% and 6% in dry weights of tomato seedlings [Berova and Zlatev 2000]. In our study, the stem dry matter content decreased at a dose of 25 ppm, but increased in all other doses, compared to the control group. Baninasab [2009] report a 6.11–16.45% increase in shoot dry weights of watermelon seedlings as a result from the application of paclobutrazol. Alterations in plant dry matter contents are thought to result from the impact of tebuconazole on the synthesis and transport of gibberellic acid in the plants and the resulting regression



in growth. As a result of the SPAD measurements on the leaves of the seedlings, this value showed a statistically significant increase in the seedlings to which the substance was applied compared to the control group. In similar studies, paclobutrazole, which was applied as leaf spray to the tomato seedlings, increased the leaf chlorophyll content [Berova and Zlatev 2000, de Moraes et al. 2005] and similar results were obtained in watermelon [Baninasab 2009], geranium [Zawadzinska and Dobrowolska 2004], potato [Tsegaw et al. 2005], liliium [Thakur et al. 2006] and lettuce [Akdemir 2018].

In our study, we determined that the stress caused depending on the applied dose of tebuconazole increased retrotransposon mobility and this mobility led to an increase in the polymorphism value. We also determined that this retrotransposon mobility caused a decrease in the GTS value, which represented the stability of the genome. The lowest polymorphism rates (4.70%) and the highest GTS (95.30%) values were obtained at doses of 25 and 50 ppm, respectively. The change in polymorphism and the GTS values was acceptable up to 75 ppm but higher doses resulted in significant changes in these values. The decrease in the GTS value is an indication that tebuconazole stress affects the stability of the genome (Tab. 6).

Nearly all of the different doses of tebuconazole prevented excessive and unnecessary elongation in seedling and stem heights during the growth of tomato seedlings. As a result, although the seedlings in the control application came to sowing-maturity, the duration to the sowing-maturing was observed to extend in seedlings to which the substance was applied. However, such chemicals (plant growth regulator, fungicide, herbicide, etc.) may cause stress in plants depending on the used dose [Sunar and Bulut 2019]. However, when the results of IRAP analysis and particular seed quality characteristics are evaluated together, the application of tebuconazole doses at 25, 50 and 75 ppm applications indicate that it will be beneficial in obtaining the desired seedling quality. As the applied dose increased, the genome stability (GTS) decreased but the rate of polymorphism increased. Therefore, application doses may be decreased in future studies and various variants may be tested by applying a higher number of doses or minimizing the number of applications to a single one.

## CONCLUSIONS

In conclusion, tebuconazole was determined to achieve height control in tomatoes and have positive effects on seedling quality. The data obtained in the study can be used in the practice.

## SOURCE OF RESEARCH FUNDING

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