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Evaluation with physiological, cytogenetic, biochemical and anatomical aspects of dose-dependent inhibitory activity of jasmonic acid, a lipid-derived growth regulator, in onion root tip cells

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Abstract: The effects of different doses of exogenous jasmonic acid (JA), were investigated on physiological parameters such as germination percentage, root length, root number and fresh weight, cytogenetic parameters such as mitotic index (MI), micronucleus (MN), frequency and chromosome aberration (CA), biochemical parameters as such superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and proline (PR) contents of *Allium cepa* L., known as onion. In addition, the changes in the root anatomical structures of the bulbs were examined under the microscope by taking cross-sections. Onion bulbs were divided into four groups as one control (C) and three treatments. The bulbs of the C group were kept in cuvettes containing tap water for 7 days and the bulbs of the treatment groups were kept in cuvettes containing 100 µM, 250 µM and 500 µM JA. JA administration caused a decrease in all physiological parameters examined, an increase in the frequency of MN and CA, and a reduce in MI compared to group C. In addition, the mentioned application caused a dose-dependent increase in CAT and SOD activities and MDA and PR contents compared to group C. Moreover, 500 µM JA, the highest application dose, caused quite significant damages such as giant cell nucleus, unclear vascular tissue, binuclealar cell and accumulation of some substances in the cells of the epidermis/cortex in root anatomical structure of the bulbs. In summary, it was concluded that JA is a chemical with inhibitory functions and the *Allium cepa* test is a useful bioindicator for monitoring these effects.

Key words: antioxidant activity; germination of bulb; jasmonic acid; membrane damage; mitotic division; toxicity test

INTRODUCTION

Developmental stage of plants is rather a complex process. However, it is regulated and very well-coordinated through the action of small active molecules like phyto-hormones. Plants respond to their natural environment by synthesizing phyto-hormones that regulate growth and development in organs such as roots, buds and leaves. In addition, phytohormones can be transported from the place where they are synthesized to other regions to mediate the molecular, biochemical and physiological reactions that occur in plants grown in normal or stressful environments (Rademacher, 2015; Per et al., 2018). Their effects vary depending on the plant's development stage, target tissues, concentration, water and nutrient availability, and environmental conditions. At

the same time, phytohormones are widely used in agriculture to alleviate the negative effects of various stresses on plant growth (Ferguson & Grafton-Cardwell, 2014; Rademacher, 2015).

Jasmonic acid (JA) has conjugates such as jasmonoyl-isoleucine (JA-Ile) and methyl jasmonate (MeJA). JA and its conjugates, which have a wide distribution in the plant kingdom and act as natural plant growth regulators, are called jasmonates (Engelberth et al., 2001; Ghasemi Pirbalouti et al., 2014). The type and concentration of jasmonates in plants vary depending on cell and tissue type, environmental conditions and developmental stages of plants (Creelman & Mullet, 1997). The highest levels of jasmonates found in algae, fungi, mosses, ferns, and many plant species were found in developing reproductive organs such as seeds, fruit and flowers and in plants exposed to stress (Parthier, 1990; Krupina & Dathe, 1991). Jasmonate biosynthesis is a receptor-mediated process resulting from linolenic acid release and membrane damage (Meyer et al., 1984; Creelman & Mullet, 1997; George et al., 2008).

In recent years, an important part of phytohormone research has been the role of jasmonates in plant growth and development (Huang et al., 2017). Jasmonates, which are lipid-derived growth regulators, have been found to have a dual effect, that is, both inhibitory and stimulating, in plants when applied externally (Parthier, 1990). Jasmonates play vital role in different developmental and physiological processes such as the seed germination (Cavusoglu & Kabar, 2006), pollen germination (Muradoglu et al., 2010), seedling growth (Cavusoglu et al., 2007), ripening of fruits (Han et al., 2019), senescence induction (Shan et al., 2011), embryogenesis (Reinbothe et al., 1994), potato tuberization (Sarkar et al., 2006), abscission (Marasek-Ciolakowska et al., 2020), carotenoid biosynthesis (Luo et al., 2020), chlorophyll formation (Ueda & Saniewski, 2006), photosynthesis (Kurowska et al., 2020), respiration (Chen et al., 2004), synthesis of protein (Kumari & Sudhakar, 2003) and root, stem and leaf anatomies of plants (Kilic et al., 2008).

On the other hand, the role of jasmonates in the adaptation of plants to abiotic stresses such as temperature (Sharma & Laxmi, 2016), ultraviolet (Liu et al., 2012), drought (Wasternack, 2014), ozone (Rao et al., 2000), heavy metal (Dar et al., 2015), osmotic (Santino et al., 2013) and salinity (Kazan, 2015) has been highly determined (Ahmad et al., 2016; Wasternack & Strnad, 2016). In addition, plants are known to produce jasmonates to help defend against various biotic stresses, particularly pathogen and insect attacks (O'Brien & Benkova, 2013).

Allium test, which is a fast, inexpensive and sensitive method, associated two aims: toxicity and mutagenicity (Tedesco & Laughinghouse, 2012). The pace at which chromosomes break down is correlated with mutagenicity, and root growth inhibition is used to gauge toxicity. Comparable to test systems that use human lymphocytes or algae, the *Allium* test's sensitivity is comparable. Similar results to those of the *Allium* test were obtained from other tests conducted using a range of biological entities. Because of this, the aforementioned test is a trustworthy scanning test (Fiskesjo, 1985; Chaparro et al., 2010). It has been confirmed in international cooperative works under US Environmental Protection Agency (USEPA), World Health Organization (WHO) and United Nations Environmental Program (UNEP) as an effective test for genetic monitoring of environmental Pollutant (Grant, 1999).

This study focused on determining the effects of exogenous JA concentrations of 100, 250 and 500 μM on some physiological, cytogenetic, biochemical and anatomical parameters of onion (*Allium cepa* L).

MATERIALS AND METHODS

Study plant and dosages of jasmonic acid

Bulbs of *Allium cepa* L., popularly referred to as onion, were utilized as test plant. Bulbs were bought from a greengrocery in Isparta-Turkiye and JA (CAS number: 77026-92-7) was acquired from Sigma-Aldrich Company in the United Kingdom and utilized in the studies. The determined JA concentrations were based on the found EC_{50} dose. 250 μM was determined to be the EC_{50}

dosage. Choosed dosages were 500 µM, which is twice the EC_{50} dose, and 100 µM, the lowest.

Process of bulb germination and growth

A selection of roughly equivalent-sized, healthy and plump bulbs was made. Bulbs divided into four separate groups. Figure 1 and Figure 2 depict the groups and the experimental setup.

Twenty bulbs from each identified group were put into sterile plastic 1.7-liter containers with a perforated cover with the root sections inside and the remaining parts outside. The bulbs were then allowed to germinate for 168 hours (7 days) in the dark in an incubator that was kept at 20 °C. First group control bulbs were kept in tap water medium until the end of the study; second group bulbs were kept in 100 µM JA medium; third group bulbs were kept in 250 µM JA medium; and fourth group bulbs were germinated in 500 µM JA treated medium.

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The hairy roots of the germinated bulbs in the control and treatment groups were counted at the conclusion of the 168-hour application period. Root lengths were measured in millimeters (mm) using a ruler fitted with millimetric scales. Precision balance was used to determine fresh weights in grams and Eq. 1 was utilized to express the germination percentages in percentage. The germination threshold was determined to be the radicle's 10 mm protrusion from the testa. In this investigation, every trial was set up in triplicate to enable statistical analysis of the collected data.

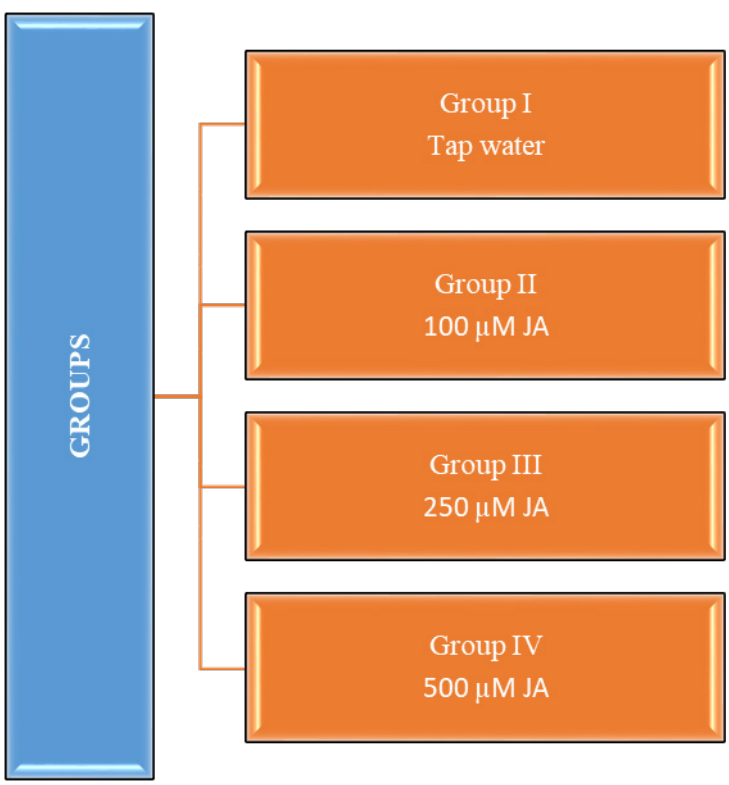

Figure 1. Experimental groups. JA: jasmonic acid

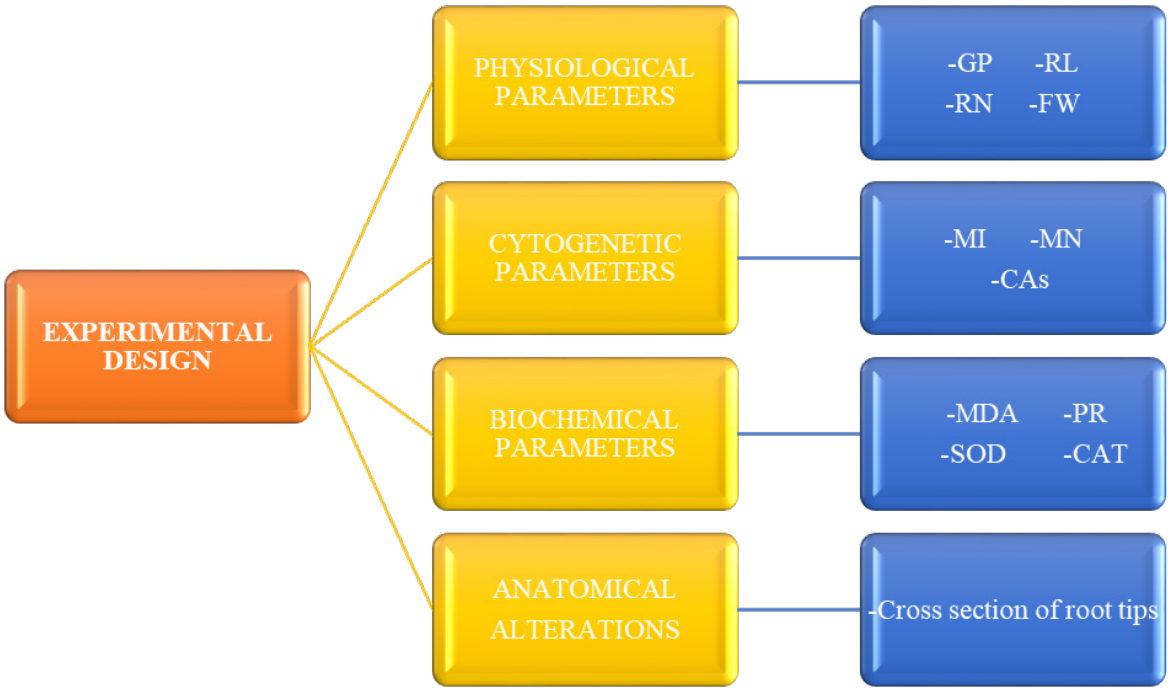

Figure 2. Experimental stages of the study. GP: germination percentage, RL: root length, RN: root number, FW: fresh weight, MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, PR: proline, MI: mitotic index, MN: micronucleus, CAs: chromosomal abnormalities

Germination (%) = [number of germinated bulbs / total number of bulbs] × 100 (1)

Method for ascertaining cytogenetic variations

Materials cut to a length of 1-2 cm from the onion root tips were immersed in saturated paradichlorobenzene for 4 hours, fixed in a mixture of 3 parts ethyl alcohol and 1 part acetic acid solution, and then stored in 70 % ethyl alcohol in order to identify chromosomal damage. In order to prepare the root tips permanently, they were first hydrolyzed in 1 N HCl at 60 °C for 17 minutes, then stained for 1 to 1.5 hours with Feulgen, crushed in 45 % acetic acid on a slide, covered with a coverslip, balm applied around the coverslip, and photographed at 100X magnification using a microscope (Sharma & Gupta, 1982). 30.000 cells were counted for each root tip from the prepared preparations in order to calculate the mitotic index (MI), and Eq. 2 was used to determine the percentage of cells entering mitosis. In order to calculate chromosomal abnormalities (CAs), 2.000 dividing cells were counted.

MI (%) = [number of cells undergoing mitosis / total number of cells] × 100 (2)

Method for ascertaining antioxidant capacity

50 mM cold sodium phosphate buffer (pH 7.8) was used to homogenize 0.2 g of root sample. After passing through coarse filter paper, the homogenate was centrifuged for 20 minutes at 10.000 rpm. The activity of the enzymes catalase (CAT) and superoxide dismutase (SOD) were measured spectrophotometrically using the supernatant.

Nitroblue tetrazolium chloride (NBT) photochemical reduction at 560 nm was used to measure SOD activity. 1.5 mL of 0.05 M sodium phosphate buffer (pH 7.8), 750 µM NBT, 130 mM L-methionine, 0.1 mM EDTA-Na_2, 20 µM riboflavin, 4 % polyvinylpyrrolidone, supernatant, and deionized water were used for the reaction. The reaction mixture was incubated for 10 min-

utes under 15-W fluorescent light after riboflavin was added last in the dark (Beauchamp & Fridovich, 1971). U mg^{-1} FW was used to express SOD activity (Zou et al., 2012).

CAT activity was determined by monitoring the absorbance drop at 240 nm. In 200 mM pH 7.8 sodium phosphate buffer, 0.1 M H_2O_2, supernatant, and deionized water were incubated for two minutes at 37 °C. The reaction was then stopped with 1 M HCl. The amount of enzyme needed to break down one µmol of H_2O_2 was defined as one unit of enzyme activity. The expression for CAT activity was OD240nm min g^{-1} FW (Beers & Sizer, 1952).

Method for ascertaining membrane damage

The expression for lipid peroxidation is the concentration of malondialdehyde (MDA). After homogenizing a 0.5 g sample of onion roots with 10 ml of 5 % trichloroacetic acid (TCA), the homogenate was centrifuged for 15 minutes at 12.000 rpm and 24 °C. One milliliter of the clear portion of the centrifuged sample was extracted, and to it was added four milliliters of 20 % TCA diluted in 0.5 % thiobarbituric acid (TBA). The mixture was rapidly chilled in an ice bath after being maintained at 96 °C for twenty-five minutes and centrifuged at 10.000 rpm for five minutes. Next, the absorbance from the clear portion was measured at 532 nm, and the MDA concentration was computed using the extinction coefficient of 155 M^{-1} cm^{-1} and represented as µmol (Unyayar et al., 2006).

Method for ascertaining the amount of free proline

A 0.5 g fresh root sample was homogenized using 10 mL of 3 % sulfosalicylic acid. After that, Whatman filter paper was used to filter the root samples. A volume of 2 milliliters was extracted, followed by an equal volume of acid anhydrin and glacial acetic acid. The combination was stored for one hour in a water bath at 100 °C and for five minutes in an ice bath. To create two phases, 5 mL of toluene was added to the reaction mixture, vortexed for 15 to 20 seconds, and then allowed to settle. Using a micropipette, the upper phase was obtained, and the absorbance values were measured in the spectrophotometer at 520 nm in comparison to the pure toluene control. The outcomes of the L-proline standard were contrasted with the examples' results. With the use of Eq. 3, the amount of free proline was determined, and µg g^{-1} is represented as fresh weight (Bates et al., 1973).

[(µg proline / mL × mL toluene) / 115.5 µg / µmole] / [(g sample) /5] = µmoles proline/g of fresh weight material (3)

Method for ascertaining anatomical disparities

To get rid of the residues on the onion roots' surface, the root tips were cleaned with purified water. After cutting cross-sections from the tips of the roots using a sharp razor blade and dyeing them with 2% methylene blue, the stained samples from each group were inspected under a 500x magnification research microscope (Cavusoglu, 2022; Cavusoglu et al., 2022).

Assessment of the acquired information

Using "the SPSS 23 analytical software" for Windows, statistical analyses of the collected data were performed, and the variations in the outcomes were shown as "mean ± standard deviation". It was examined using a one-way ANOVA and the Duncan test at the $p < 0.05$ significant level.

RESULTS AND DISCUSSION

Influence of JA on the physiological parameters

Figure 3 shows the effects of externally applied JA on the physiological parameters of *Allium cepa* bulbs. At the end of the 7th day, while the germination percentage of the control group (Group I) was 100 ± 0.0 %, this value was 80 ± 0.0 %, 62 ± 2.8 % and 44 ± 2.8 % at 100 µM, 250 µM and 500 µM JA levels, respectively. That is, with the increase in JA concentrations, the percentage of germination was inhibited by 20 % at

100 µM, 38 % at 250 µM and 56 % at 500 µM compared to control (Group I). It was reported that JA inhibited the germination of non-dormant seeds (Bialecka & Kepczynski, 2003; Kumari & Sudhakar, 2003) and stimulated the germination of dormant seeds (Bogatek et al., 2002; Yıldız et

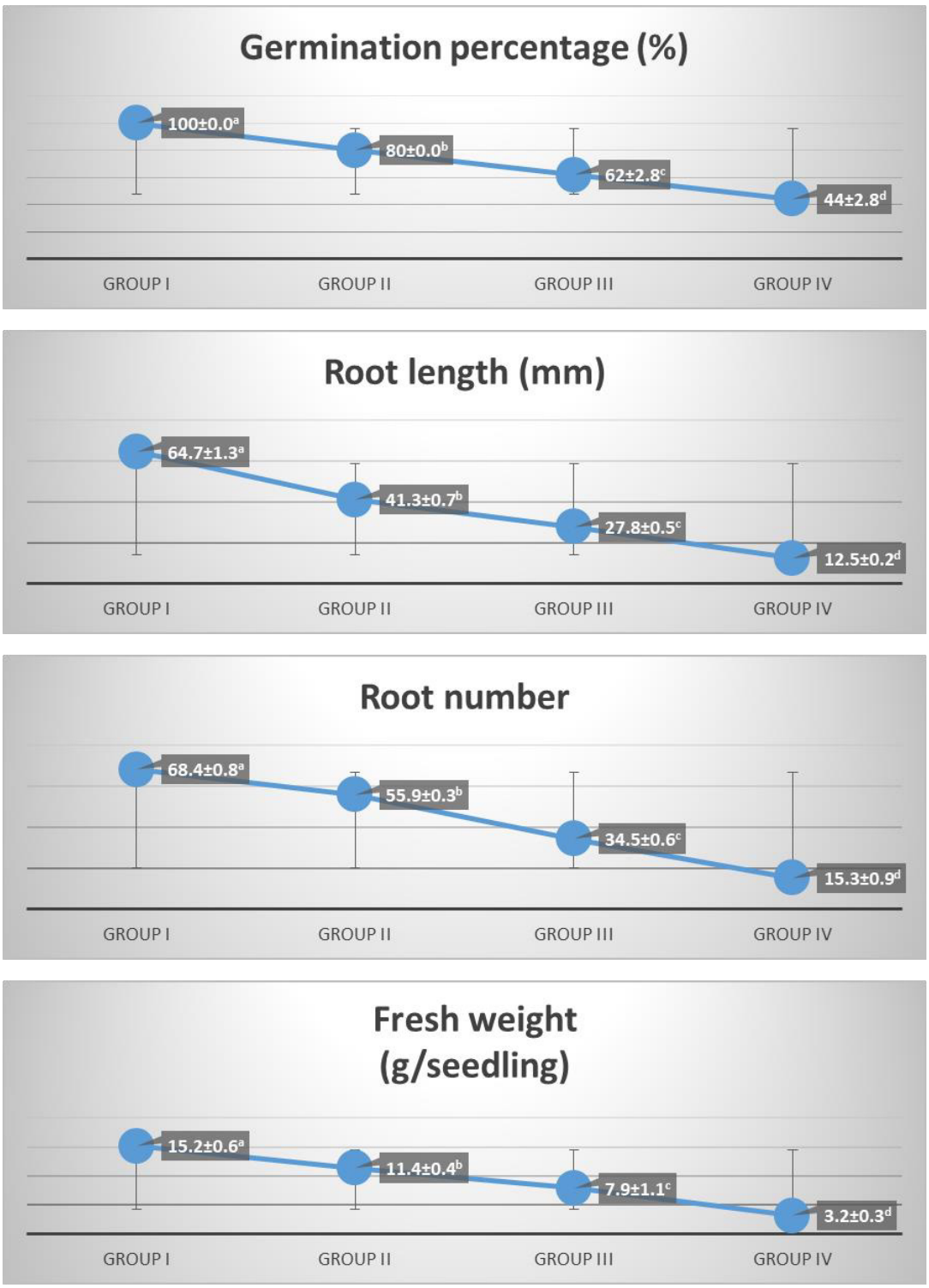

Figure 3. Effects of different doses of JA on certain physiological parameters of *Allium cepa* L. Tap water (Group I), 100 µM JA (Group II), 250 µM JA (Group III) and 500 µM JA (Group IV). The error bars indicate the standard deviation (± SD)

al., 2008). On the other hand, it was determined that there were dual influences of JA on the seedling growth and germination, low concentration of JA promoted the germination and seedling growth whereas its high concentration inhibited the germination and seedling growth (Bin et al., 2001; Cavusoglu & Kabar, 2006). The findings of the present study are consistent with all of these results.

Similarly, increases in JA concentrations led to a very serious inhibition on root length, root number and fresh weight of onion bulbs. Maximum root elongation was measured as 64.7 ± 1.3 mm in Group C (Group I) and minimum root elongation was measured as 12.5 ± 0.2 mm in Group IV treated with 500 µM JA. Root lengths decreased by 23.4 mm in Group II, 36.9 mm in Group III and 52.2 mm in Group IV, compared to Group C. While the root number of Group I bulbs, known as the control group, was 68.4 ± 0.8, this value was 55.9 ± 0.3 at 100 µM, 34.5 ± 0.6 at 250 µM and 15.3 ± 0.9 at 500 µM JA concentration. As can be seen, the highest dose of JA application (500 µM) reduced the number of roots 4.5 times compared to the control. While the mean fresh weight of Group I bulbs grown in tap water medium was 15.2 ± 0.6 g, it was calculated as 11.4 ± 0.4 g in Group II, 7.9 ± 1.1 g in Group III and 3.2 ± 0.3 g in Group IV. As understood from the results, it was determined that Group C (Group I) bulbs had maximum fresh weight value and Group IV bulbs treated with 500 µM JA dose had minimum fresh weight values. The mentioned reduces were significant at $p < 0.05$ level (Figure 3).

These results were supported by Mosblech et al. (2011) in Arabidopsis, Cavusoglu et al. (2007) in barley, Norastehnia et al. (2007) in maize and Tsai et al. (1997) in rice. Harashima and Schnittger (2010) discovered that root growth of plants is associated with the elongation level in the differentiation phase and the increase in cell number. Also, a healthy mitotic division is key to strong root growth (Andrade et al., 2010). The inhibitory effect of JA on root number and root elongation may be due to decreased cell division (Swiatek et al., 2002). This statement is also supported by the fact that it had reduced the mitotic activity in root tip meristematic cells in the present study (Figure 4). In addition, the decrease in fresh weight and water content of bulbs grown in JA medium may be associated with insufficient water intake of the roots (Figure 3).

Influence of JA on the cytogenetic parameters

Figure 4 shows that the toxic effect of exogenous JA on cytogenetic parameters such as mitotic index (MI), micronucleus (MN) frequency and chromosome aberrations (CAs) increases in direct proportion to the increase in application doses. MI was used as a parameter of mitotic activity and it is an acceptable measure of cytotoxicity for all living organisms in studies of environmental biomonitoring (Smaka-Kincl et al., 1996; Fernandes et al., 2007). MI was calculated as 9.6 ± 1.0 % in Group I, the control group, 8.1 ± 0.7 % in Group II, 6.4 ± 0.3 % in Group III and 2.8 ± 0.2 % in Group IV, the treatment groups. MI in the treatment groups decreased by approximately 16 %, 33 % and 71 %, respectively, compared to the control group. These findings show that an increase in JA concentration adversely affects MI.

Similarly, increases in exogenous JA concentration also promoted MN formation in root tip cells of bulbs. While MN formation was not observed in Group I (control), MN formation was observed to increase in JA applied groups and this value was 10.4 ± 0.4 % in Group II, 23.9 ± 1.7 % in Group III and 52.8 ± 2.3 % in Group IV (Figure 4).

It has also been demonstrated by current research findings that externally applied JA increases the number of CA in root tip meristem cells. As a result of microscopic examination, CA was not observed in the root tip cells of Group I bulbs, while the number of these abnormalities reached 11.6 ± 0.8 % at 100 µM, 37.5 ± 1.4 % at 250 µM and 58.7 ± 1.6 % at 500 µM JA concentration (Figure 4). Both promotive and inhibitive impacts of JA on cell division have been reported. The inhibitory action of JA has been demonstrated with the findings obtained by Mahfouz et al. (2014) and Norastehnia et al. (2007) while its promotive action has been showed with the findings obtained by Advanci et al. (2010). As a re-

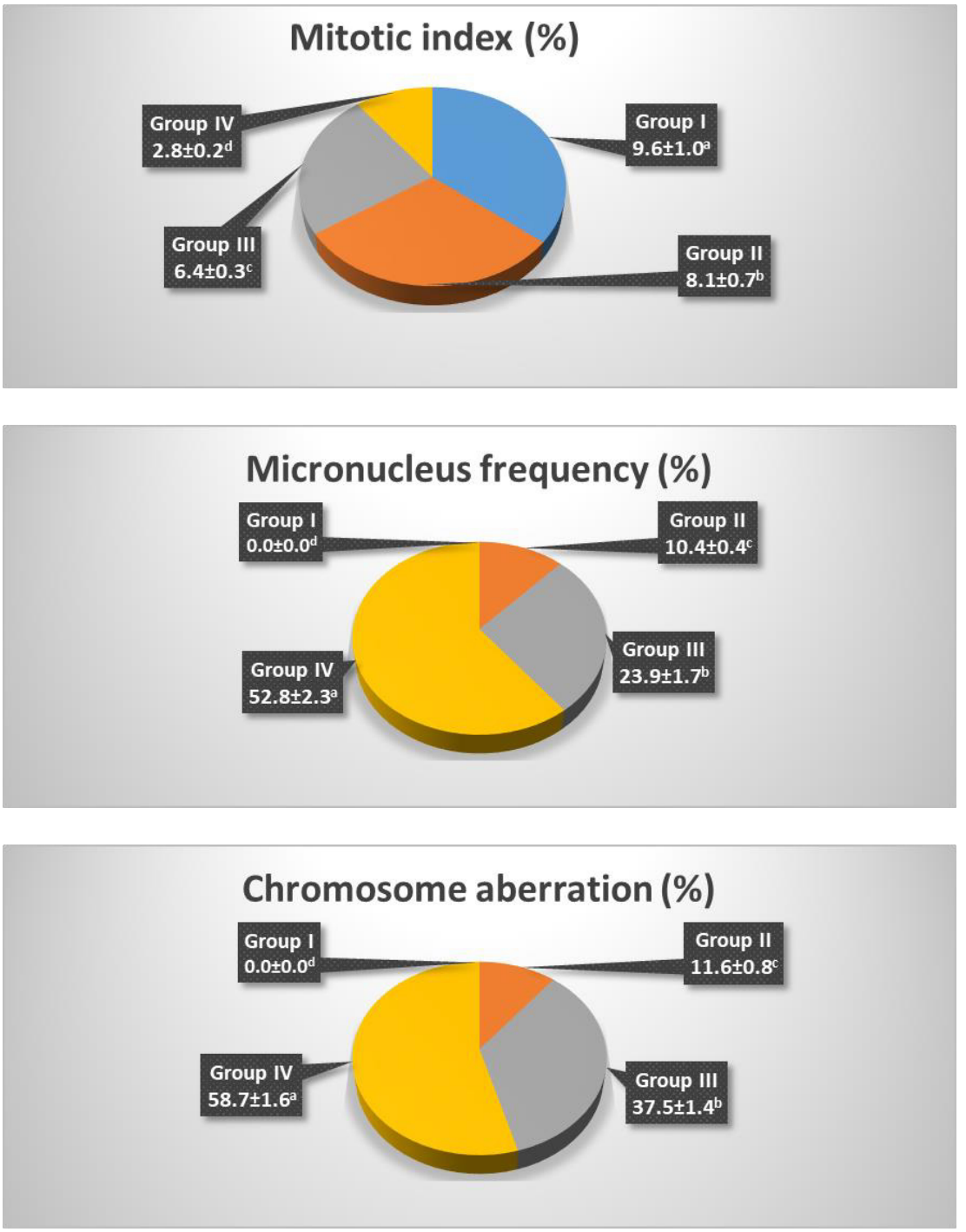

Figure 4. Effects of different doses of JA on certain cytogenetic parameters of *Allium cepa* L. Tap water (Group I), 100 µM JA (Group II), 250 µM JA (Group III) and 500 µM JA (Group IV). The ± symbol indicate the standard deviation (SD)

sult, it can be said that exogenous JA applied at low doses generally increases the number of cell divisions in plants, but decreases it at high doses.

Normal and abnormal mitotic stages observed as a result of microscopic examination of meristem cells of bulb roots are shown in Figures 5

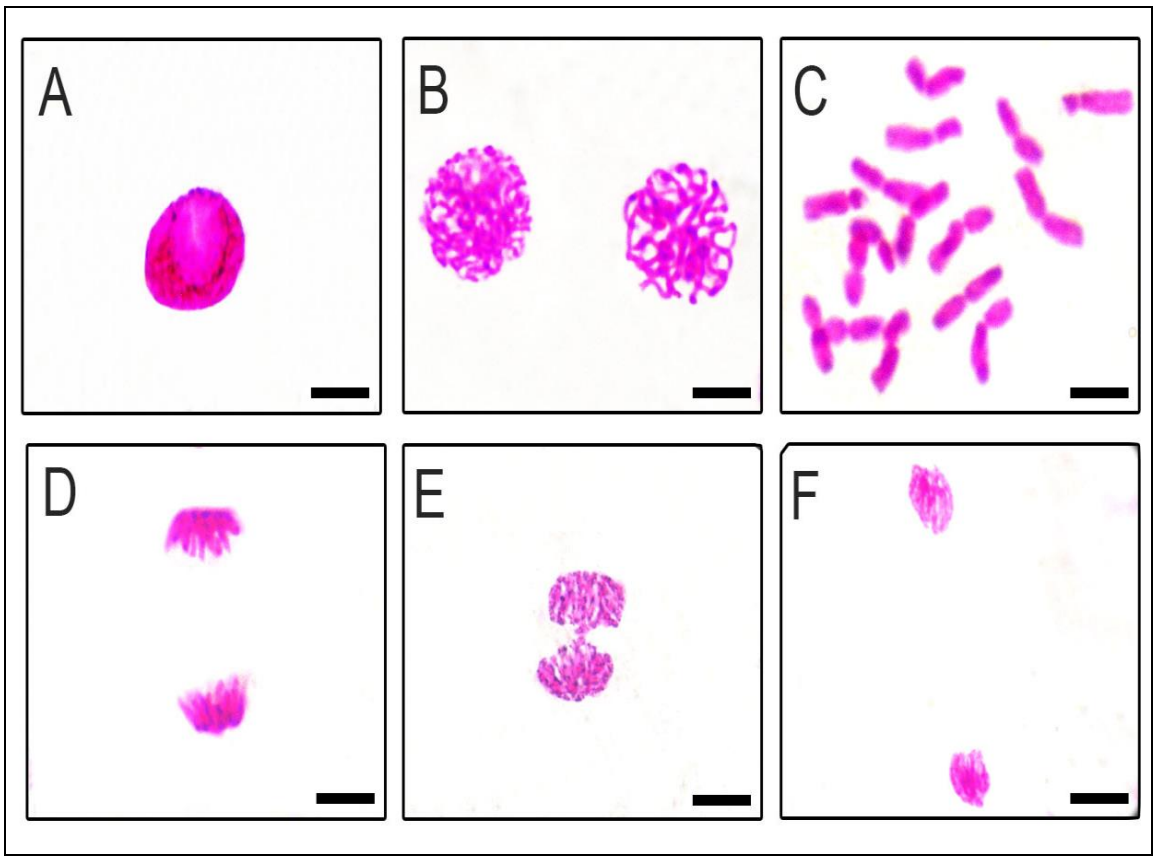

Figure 5. Photomicrograph showing of normal mitosis stages in root-tip meristem cells of *Allium cepa* L. A interphase, B prophases, C metaphase 2n=16 chromosome, D anaphase, E early telophase, F late telophase. Scale bar (10 μm)

and 6. Common and notable abnormalities were micronucleus (Figure 6a), nucleus distribution (Figure 6b), fragments (Figure 6c), chromosome stickiness (Figure 6d), forward chromosome (Figure 6e), bridge formations (Figure 6f), lobated nuclei (Figure 6g) and anaphase/telophase with fault polarization (Figure 6h, ı). Some growth promoters may specially cause cell distortions, CAs and mitotic irregularities even non-stress medium (Unal et al., 2002). There is limited literature data regarding to the affects of JA on CAs in stressless media. Mahfuz et al. (2014) reported that chromosomal abnormalities such as chromosome stickiness, chromosome breaks, bridge formations, micronuclei, c-metaphase and polyploidy cells were observed as a result of microscopic examination of onion root tip meristem cells exposed to high JA concentrations. The present study findings were similar to results of these researchers.

Chromosomal or chromosomal breaks that remain in the anaphase stage and cannot combine with both nuclei in the telophase stage lead to the formation of MN (Fenech, 2000; Norppa & Falck, 2003). Nucleus distribution are morphologically like to MN, with the only exception that it combineted with the nucleus (Fenech & Crott, 2002). Formation of MN and nucleus distribution might be concluded in genetic material loss (Ruan et al., 1992). Observed chromosome fragments show clastogenic action while chromosome stickiness could be a consequence of inter-chromosomal linkages coupled with excessive formation of nucleoproteins (Leme & Marin-Morales, 2009). Forward chromosome derives from unevenly size or irregular shape nuclei in daughter cells with inequal chromosomes (El-Ghamery et al., 2003). Due to the stickiness of the chromosomes and the separation of the defected chromosomes, bridge formations occur in anaphase/telophase

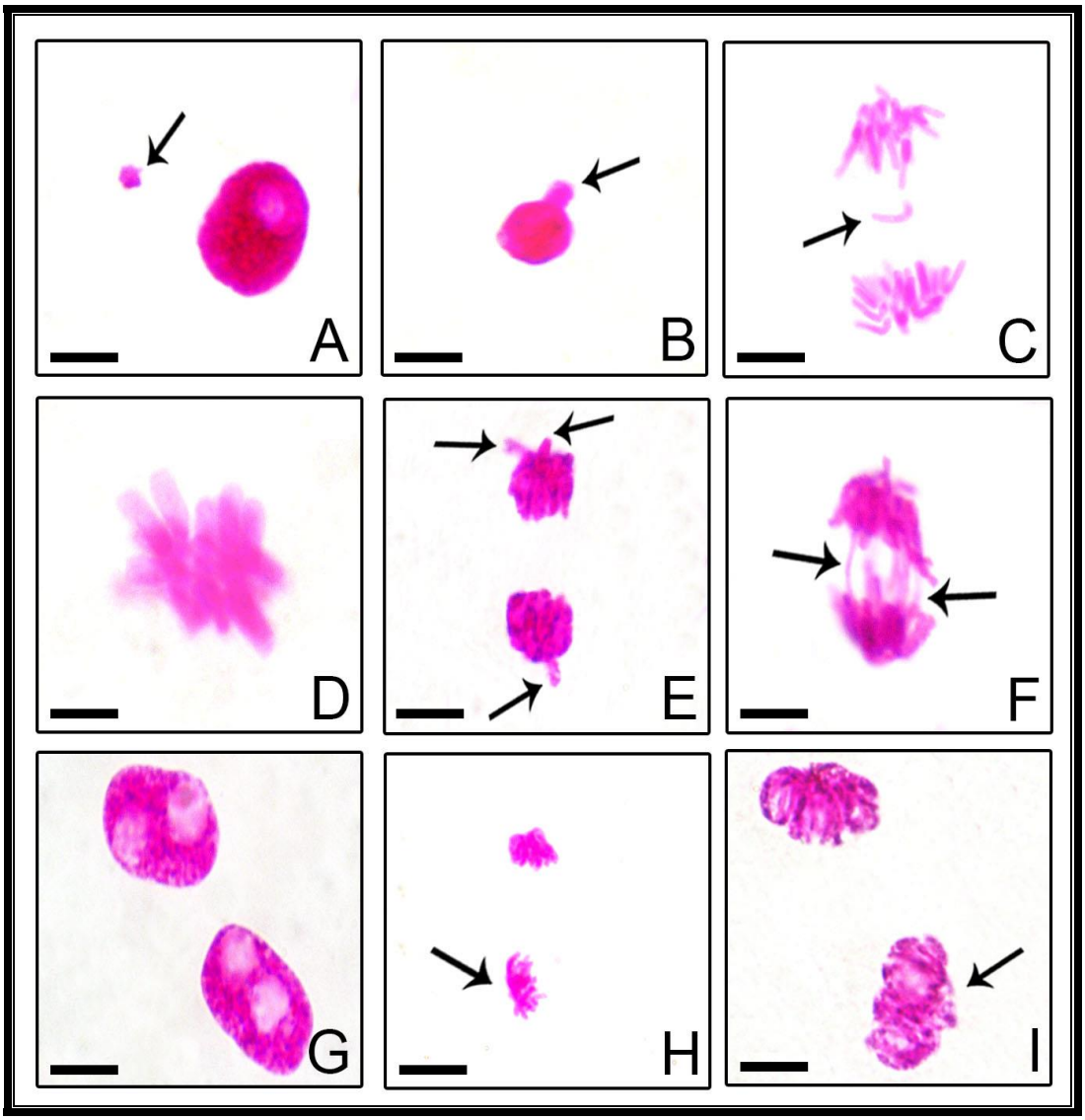

Figure 6. Types of chromosomal aberration. A micronucles=arrow, B nucleus distribution=arrow, C anaphase with fragment=arrow, D chromosome stickiness, E forward chromosomes=arrows, F bridge formations=arrows, G lobated nuclei, H anaphase with fault polarization, I telophase with fault polarization. Scale bar (10 μm)

and structural mutations occur (Turkoglu, 2007). During the S phase of mitosis, the suppressive effect of a nuclear poison 214 on DNA synthesis causes the formation of lobed nuclei as a nuclear deformation (Sutan et al., 2014). Spindle disorders lead to anaphase/telophase with fault polarization, which is highly correlated with the incidence of the aforementioned abnormalities other than vacuole nuclei (Kalcheva et al., 2009). The fact that exogenous JA is a genotoxic agent for *Allium cepa* bulbs is evident from the increase in MN formation and CAs.

Influence of JA on the biochemical parameters

In the current study, oxidative stress caused by JA application was determined by CAT, SOD, MDA and PR analyzes. The dose-dependent effects of exogenous JA on CAT, SOD, MDA and PR content in root tip cells of onion bulbs are

shown in Figure 7. In parallel with the increase in JA concentrations, both SOD and CAT activities increased significantly. SOD activity was measured at the lowest level with 80 ± 2.3 U/mg FW

Figure 7. Effects of different doses of JA on certain biochemical parameters of *Allium cepa* L. Tap water (Group I), 100 µM JA (Group II), 250 µM JA (Group III) and 500 µM JA (Group IV). The ± symbol indicate the standard deviation (SD)

in the roots of Group I (control) seedlings grown in tap water medium, and at the highest level with 178 ± 5.2 U/mg FW in the roots of Group IV seedlings grown in 500 μM JA medium. Moreover, SOD content increased 1.2-fold in Group II, 1.79-fold in Group III and 2.23-fold in Group IV when compared to control seedlings. CAT activity was calculated as 0.7 ± 0.1 OD240nm/min. g FW in the roots of Group I seedlings, the control group and this value was 1.0 ± 0.3 OD240nm/min. g FW in Group II, 1.5 ± 0.4 OD240nm/min. g FW in Group III and 2.1 ± 0.7 OD240nm/min. g FW in Group IV seedlings, the treatment groups. Extremely high intrinsic levels of these two enzymes indicated that JA administration triggered excessive ROS production in *Allium cepa* bulbs (Figure 7).

Reactive oxygen species (ROS), cytotoxic substances with highly deleterious effects, act as intermediate signaling molecules that regulate the expression of genes associated with antioxidant defense systems. Plants contain antioxidant mechanisms to mitigate the damage caused by ROS (Neill et al., 2002; Vranova et al., 2002; Srivastava & Singh, 2020) and antioxidant enzymes are an important part of these mechanisms. Among the most important antioxidant enzymes are SOD and CAT. CAT is a tetrameric homoprotein while SOD is a metalloenzyme. These two antioxidant enzymes are found in microbodies called glyoxisomes and peroxisomes in the cell (Soares et al., 2010). Soares et al. (2010) determined that JA caused a significant decrease in SOD activity in *Ricinus communis*, at 24 and 48 h after treatment. In addition, in this study, CAT activity showed the same value as the control after fluctuating in the 1–12 hour portion of JA administration. Chong et al. (2005) detected that CAT activity increased after one day and it dramatically decreased after three and six days of 50 μM JA treatment in *Morinda elliptica* (Hook.f.) Ridl. The same researchers determined that 100 μM JA did not cause a change in enzyme activity until the 3rd day, but decreased the enzyme activity on the 6th day. Jung (2004) revealed that SOD activity in *Arabidopsis thaliana* (L.) Heynh. exposed to external JA application decreased on the 3rd day and increased 2 times on the 7th day. According to these findings, it can be said that antioxidant enzyme activity vary depending on application time and concentration of JA used.

Similarly, increasing doses of exogenous JA led to significant increases in MDA and PR content. While the mean MDA content was 9.3 ± 1.4 μmol/g FW in the roots of the control group, the MDA content in the JA test groups increased in a dose-dependent manner. The MDA levels of Group II, III and IV were determined as 12.6 ± 2.4 μmol/g FW, 17.1 ± 2.9 μmol/g FW and 23.4 ± 3.6 μmol/g FW, respectively. While the free PR content in the roots of Group I (control) seedlings grown in tap water medium was at the lowest level with 19.4 ± 1.7 μmol/g FW, this value reached the highest level with 62.6 ± 3.4 μmol/g FW in the roots of Group IV seedlings grown in 500 μM JA medium. Moreover, exogenous JA administration increased the free PR content 1.8-fold at 100 μM, 2.5-fold at 250 μM and 3.2-fold at 500 μM compared to control (Figure 7).

Lipid peroxidation is an important metabolic process that leads to oxidative degradation of lipids, polyunsaturated fatty acids, ROS and MDA production (Odjegba & Adeniran, 2015). Cell membrane damage causes an increase in MDA content (Fedina & Benderliev, 2000). MDA is both an oxidative product of membrane lipids and a biological indicator of oxidative stress level (Janero, 1990). ROS damage the peroxidation of various biological molecules such as RNA, DNA, lipids and proteins (Shah et al., 2001; Dinakar et al., 2010). Free oxygen groups can cause mutations in nucleic acids and changes in chromosomes by affecting DNA (Yarsan, 2014). It has been done little research relating to the effects of exogeneous JA on the lipid peroxidation and free PR accumulation during germination under especially normal conditions until now. The lipid peroxidation were reported to be induced by JA application in several plant species (Bachmann et al., 2002; Hung & Kao, 1998; Kumari et al., 2015) and the present research findings are consistent with these reports. Kumari and Sudhakar (2003) determinated that JA treatment increased the free PR content in the root of groundnut seedlings and

also free PR accumulation under JA treatments could be due to an increased synthesis or to an inhibition in the oxidation of PR.

Influence of JA on the anatomical parameters

As a result of microscopic examinations, the changes and damages caused by externally ap-

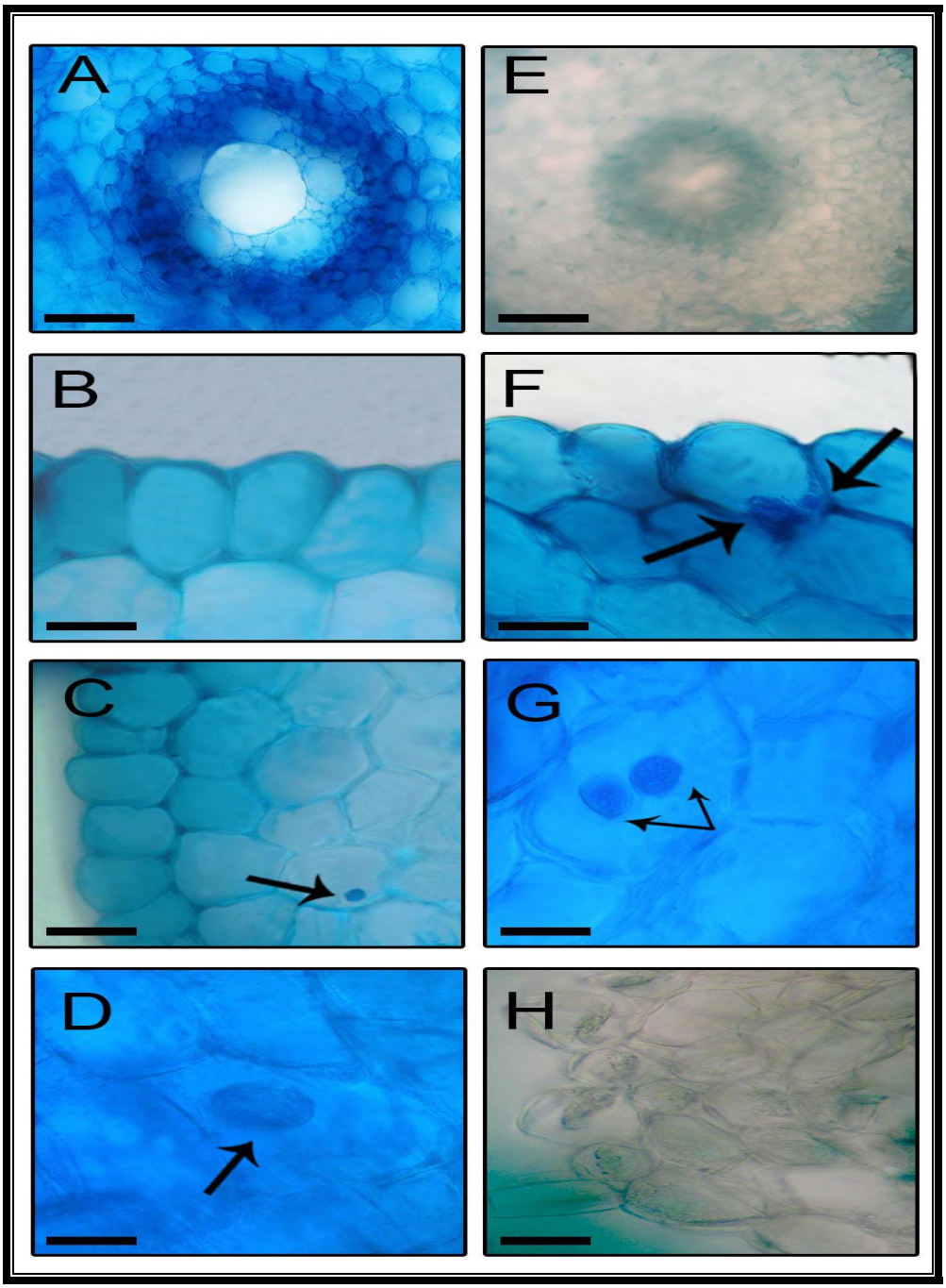

Figure 8. The root anatomic alterations induced by JA. A clear vascular tissue, B normal apperance of epidermis cells, C normal view of the cells nuclei=arrow, D giant cell nucleus=arrow, E unclear vascular tissue, F accumulation of some substances in the cells of the epidermis=arrows, G binuclealar cell=arrows, H accumulation of some substances in the cells of the cortex=arrow. Scale bar (10 μm)

plied JA in the root anatomical structure of onion bulbs are shown in Figure 8. While no change or damage was observed in the root anatomical structure of the bulbs of the control group (Group I), damage and changes such as giant cell nuclei, binuclealar cells, indeterminate vascular tissue and substance deposition in the epidermis and cortex cells were observed in the roots of the JA treated groups. Accumulation of the applied chemical substance in the root epidermis cells prevents it from reaching the inner tissues and thus the plant can gain resistance and tolerance (Bahmani et al., 2015). Therefore, it is an anatomical adaptation to chemical exposure rather than damage that JA causes accumulation of certain chemicals in the epidermis and cortex cells of the root (Figure 8f, h).

The present study showed that low doses of JA usually cause anatomical changes, while high doses of JA cause severe anatomical damage. In some cases, the highly toxic effect causes anatomical damage rather than anatomical change. The fact that cell deformations such as binuclealar cell and giant cell nucleus (Figure 8d, g) observed in microscopic examinations are quite intense at 500 µM JA dose confirms this hypothesis. Giant cell nuclei are a marker of damaged mitosis, as are chromosomal abnormalities. Indistinct vascular tissue formations (Figure 8e) develop in bulb roots due to decreased water intake.

Although there is no study on the effects of exogenous JA on the root anatomy of onion bulbs, there is only one study on the effects of JA on the root anatomy of radish seedlings. In this study, Kilic et al. (2008) reported that 100 µM JA application decreased the root diameter of *Raphanus sativus* L., while JA levels below 1500 µM decreased the cortex zone thickness and increased the epidermis cell width and length. Therefore, the anatomical results of the present study are very important.

CONCLUSIONS

The multiple action mechanisms of JA at different doses applied externally were investigated in detail in this study. These results showed that exogenous JA is a potent inhibitory substance that affects various physiological, cytogenetic, biochemical parameters and root anatomical structure of *Allium cepa* bulbs. In addition, these results strengthened the idea that the *Allium* test is a helpful model, especially in understanding the activities of inhibitory substances.

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