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Role of ten different exogenous plant growth promoters in regulating cytotoxic and genotoxic processes in barley exposed to high temperature stress

SELMA TABUR*, Ş. BETÜL YILMAZ-ERGÜN, SERKAN ÖZMEN

Faculty of Engineering and Natural Sciences, Department of Biology, Süleyman Demirel University, Isparta 32260, Turkey

*Corresponding author. Email: taburs@gmail.com

Abstract. Agricultural crop affected preliminary and the most prominent by the adverse effects of global climate change have to adapt to various abiotic factors that will occur as a result of climate fluctuations in the near-future and struggle to survive. Among abiotic factors, the one of the greatest impact on plant stress is high temperature. Therefore, the most important step to take action against the global threat is the development of new temperature tolerant varieties. Barley, which is the fourth most important cereal in the world after wheat, maize, and rice are affected by high ambient temperatures. In this work, the effects of their alone and in double, triple combinations of ten various plant growth regulators (PGRs) on mitotic activity and chromosome behaviors in root meristems of barley exposed to high temperature (30°C) were investigated. In the experiments, *Hordeum vulgare* L. cv. Bülbül 89 variety and GA₃, KIN, BA, E, EBR, TRIA and PAs (Spm, Spd, Put, Cad) as growth regulators were used. The results obtained were compared with each other and with those of the seeds germinated at optimum temperature (20°C). Consequently; it has been determined that most of the PGRs studied, especially the GA₃ and their combinations with GA₃, exhibit a very successful performance on mitotic activity and cytogenetic aberrations in barley seeds germinated under high temperature stress- HTS conditions. The effects of these PGRs (except for EBR) and their combinations on mitotic activity and chromosome behaviors under HTS have been presented in this study for the first time.

Keywords: chromosomal aberrations, heat stress, *Hordeum vulgare* L., mitotic index, plant growth regulators.

INTRODUCTION

Climate change is an inevitable phenomenon globally, which affects all aspects mankind, including agricultural production worldwide today. According to IPCC report (2021) projects that in the coming decades climate changes will increase in all regions and for 1.5°C of global warming there will be increasing heat waves- longer warm seasons and shorter cold seasons. And,

this temperature increases is particularly predicted to increase by about 1–3°C by the mid and by about 2–5°C by the late twenty-first century. The report shows that at 2°C of global warming, heat extremes would more often reach critical tolerance thresholds for agriculture and health. Therefore, it is staminal that a lot of countries especially in the south of 40° north latitude including Turkey located in the geographic region where the adverse effects of climate change are possible struggle these climatic fluctuations, take action against anticipated threats, and revive strategies in this direction (Budak 2022). It have predicted to climatological extremes are caused various abiotic stresses and have a general negative effect on plant growth and development as also likely all living. Thus, future agricultural crop production and thus global food security will encounter additional challenges with human population increase competing for environmental resources (Bita and Gerats 2013; Pereira 2016). In this respect, performing genome-wide analyzes of stress-resistant genotypes from agricultural crop, revealing their tolerance and selective mechanisms to against adversely conditions, and cultivating new varieties are of great importance to tackle all these challenges.

Barley (*Hordeum vulgare* L.) is one of the most significant cereal crops farmed in Europe, the Middle East, North and South Africa, and Asia. This cereal, great economic value due to its use in both animal feed and the food industry are a cereal required grown in abiotic stress conditions that limit plant growth due to global climate changes in our current period. Furthermore, it is preferred as a model plant in cytogenetic researches for reasons such as its effortless supply, in vitro germination of seeds and small genome (Tabur and Demir 2010b; Özmen et al. 2022, 2023). Cytogenetic researches play an important role in understanding the chromosomal and genetic architecture of plant species. In particular, the chromosomal aberrations (CAs) have been accepted as an indicator of genetic damages and for those alterations which ultimately lead to mutations (Saxena 2022). Therefore, performing the CAs test has vital significant to determine whether a test substance or abiotic stress factors can cause various types of mutations over time.

Among the abiotic stresses, temperature increase has major negative impact on agricultural crops susceptible to changes in temperature. Temperature stress occur result of the cumulative effect of the temperature severity, the time the plant is exposed to these unfavorable condition, and the degree at which the temperature is increasing and cause significant and irreversible damage to plant growth and development (Hill and Li 2022). High temperatures are absolute effective as a stress factor in plants during germination, and the measures tak-

en by plants and their molecular responses under these stress conditions are completely different. Each plant species has a temperature range represented by a minimum, maximum, and optimum in which it functions optimally, and outside this range all cellular metabolisms and thus plant growth are adversely affected (Hatfield and Prueger 2015). High temperature stress (HTS) disrupts the vital cellular phenomena by damaging generally physiological, biochemical and molecular mechanisms in plants (Narayanan 2018; Jacott and Boden 2020) and production of toxic metabolites and reactive oxygen species (ROS) takes place in the injured cells occurred as a result of aberrant metabolism (Wahid et al. 2007). Hence this situation causes total crop failure by decreasing in growth, product and quality (Shrestha et al. 2022; Khan et al. 2023). Increasing temperature inhibits different stages of plant development especially seriously reduces the germination and early seedling growth in a number of plant species including barley (Wahid et al. 2010). Additionally, it has been reported that HTS negatively affects cell division and microtubule organization in tobacco, wheat and vetch thus leading to decreased mitotic index (MI) and irregular mitotic configurations (Abou-Deif and Mohamed 2007; Öney and Tabur 2013; Öney et al. 2015). Fareghi et al. (2015) asserted that *Vicia dasycarpa* that are normally diploid exhibit a mixoploid state with diploid, aneuploid and tetraploid cells after temperature shock (boiling the seeds at 90°C for 3 min.). However, the ability of a genotype to survive at high temperature depends on the type or variety of the plant, age, stage of development, the susceptibility of the cell types, the degree and duration of the elevated temperature (Wahid et al. 2007; Hasnuzzaman et al. 2013).

Plant hormones are essential for regulating the interactions between plants and their complex biotic and abiotic environments. Most of the physiological activities occurring in the plant are under the control of these hormones. The effects of hormones always appear in a balance as complementary to each other (synergistic) or reducing the effect of each other (antagonistic) (Aerts et al. 2021). Under single or multifactorial stress combination phenomenon, fluctuations in hormonal balance in plants bring about serious morphological, physiological, biochemical and molecular changes (Goharrizi et al. 2021; Zandalinas et al. 2022 and their cited). For example, ABA (abscisic acid) plays a major role in different stages of plant development such as stomata opening and closing, seed germination, and dormancy and triggers many physiological mechanisms in plants. The plant growth is severely retarded and it increases the ABA concentration in cells under drought conditions. ABA

accumulation during this period controls transpiration and inhibit stomatal disclosure (Dong et al. 2018).

There are many studies that phytohormones or various PGR agents have positive effects on plant growth and development, as well as various physiological and biochemical mechanisms, and increase plant resistance against many stresses. Because the role of different individual phytohormones under abiotic stresses is too board to be covered here, we can direct readers to up-to-date research articles and reviews on the subject (Huyluoğlu et al., 2008; Moumita et al. 2019; Younis and Ismail 2019; Emamverdian et al. 2020; Islam and Mohammad 2020; Mangena 2020, 2022; Kosakivska et al. 2022; Kothari and Lachowiec 2021; Sharma 2021; El-Beltagi et al. 2022; Fatma et al. 2022; Sarwar et al. 2022; Shao et al. 2022; Verma et al. 2022; Sultan et al. 2023). The constantly rising ambient temperature caused by rapidly changing climate warming is considered one of the most detrimental abiotic stresses and heat tolerance in plants can be achieved by exogenous application of various protectant substances (Rasheed et al. 2011; Qureshi et al. 2022). Therefore, since especially recent ten years, the exogenous application of protectant substances such as osmoprotectants, phytohormones, signal molecules, polyamines, trace elements and nutrients have studied by numberless researchers to alleviate the harmful effects of HTS on plant (Hasanuz-zaman et al. 2012; Waraich et al. 2012; Öney and Tabur 2013; Zaki et al. 2014; Öney et al. 2015; Kaur et al. 2018; Taheri and Haghighi 2018; Chen et al. 2019; Wu and Yang 2019; Alcázar et al. 2020; Jing et al. 2020; Li N. et al. 2021, Li Y. et al. 2023; Sharma et al. 2022; Wang et al. 2022; Wu et al. 2022; Huang et al. 2023; Hudelson 2023; Mei et al. 2023).

As mentioned above, there are many studies that phytohormones have positive effects on plant growth, development, physiological processes, and yield and increase plant stress resistance against various stresses. On the other hand, the effects of various phytohormones or PGRs on mitotic activity and chromosomal behaviors under normal conditions (in a stress-free environment) have also investigated by many researchers since the 1970s (Powell et al. 1973; Oh and Clouse 1998; Hu et al. 2000; İsmailoğlu et al. 2004; Huyluoğlu et al. 2008; Kartal et al. 2009; Truta et al. 2011; El-Ghamrey et al. 2013; El-Ghamery and Mousa 2017; Tabur et al. 2019; Tütünoğlu et al. 2019). It is a well-known fact that the exogenous application of both natural and synthetic PGRs contributes to the increase in the relative number of embryonic cells. Therefore most of these researchers agree that the exogenous phytohormones promotes cell division and proliferation and activates DNA replication and protein synthesis, but causes chromosomal aberra-

tions (CAs) by disrupting the mitotic balance. Furthermore, some of these researchers argue that PGRs are more effective on cell division at high concentrations, while others assert that they are more effective at low concentrations.

However, studies on PGRs effects on cell division, mitotic activity and chromosome behavior under various stress conditions (heavy metals, salinity and drought) are quite limited (Mansour and Kamel 2005; Tabur and Demir 2009; 2010a,b; Maraklı et al. 2014; Özmen et al. 2022). Moreover, a single study was found on how effective only EBR are on these parameters (MI and CAs), especially under heat stress conditions as a result of our detailed literature research (Pradhan and Gupta 2013). For this reason, the effects of ten different PGRs either alone or double and triple combinations, mentioned on MI and chromosome behaviors under HTS have been comprehensively revealed in this study for the first time. As a result, aims of this work are (1) to determine the effect of HTS on MI and CAs, (2) to determine the effects of exogenous application of various PGRs alone or in double, triple combinations on the mitotic activity and chromosome behaviors in barley root meristems under nonstress conditions, (3) to comparatively evaluate the effects of these PGRs on the mentioned parameters in barley root meristems under HTS and to fill the gap in the literature on this subject, (4) to clarify in detail to what extent all studied PGRs and their combinations can overcome HTS, whether they encourage cells to enter mitosis, and whether they cause any changes in the structure and behaviors of chromosomes.

MATERIALS AND METHODS

Preparation of the seeds and PGRs

The barley seeds (*Hordeum vulgare* cv. Bülbül 89) were kindly provided from Field Crops Research Institute, Ankara/Turkey. PGRs used in the experiments were obtained from Fluka and Sigma-Aldrich Firm. To forbid contamination before germination experiments, the barley seeds were surface sterilized by immersion in 1% (w/v) NaClO solution for 10 min, rinsed thoroughly five times with sterile distilled water and dried on filter papers at room temperature. Ten different PGRs were used in the study: GA₃ (gibberellic acid), KIN (kinetin), BA (benzyladenine), E (ethylene), EBR (24-epibrassinolide), TRIA (triacontanol), Spm (spermine), Spd (spermidine), Put (putrescine) and Cad (cadaverine). The concentration of each PGR (as μ M, micromolar), which reduces the damaging effect of HTS (30°C) on germination, was determined as a result of a preliminary study (Figure 1).

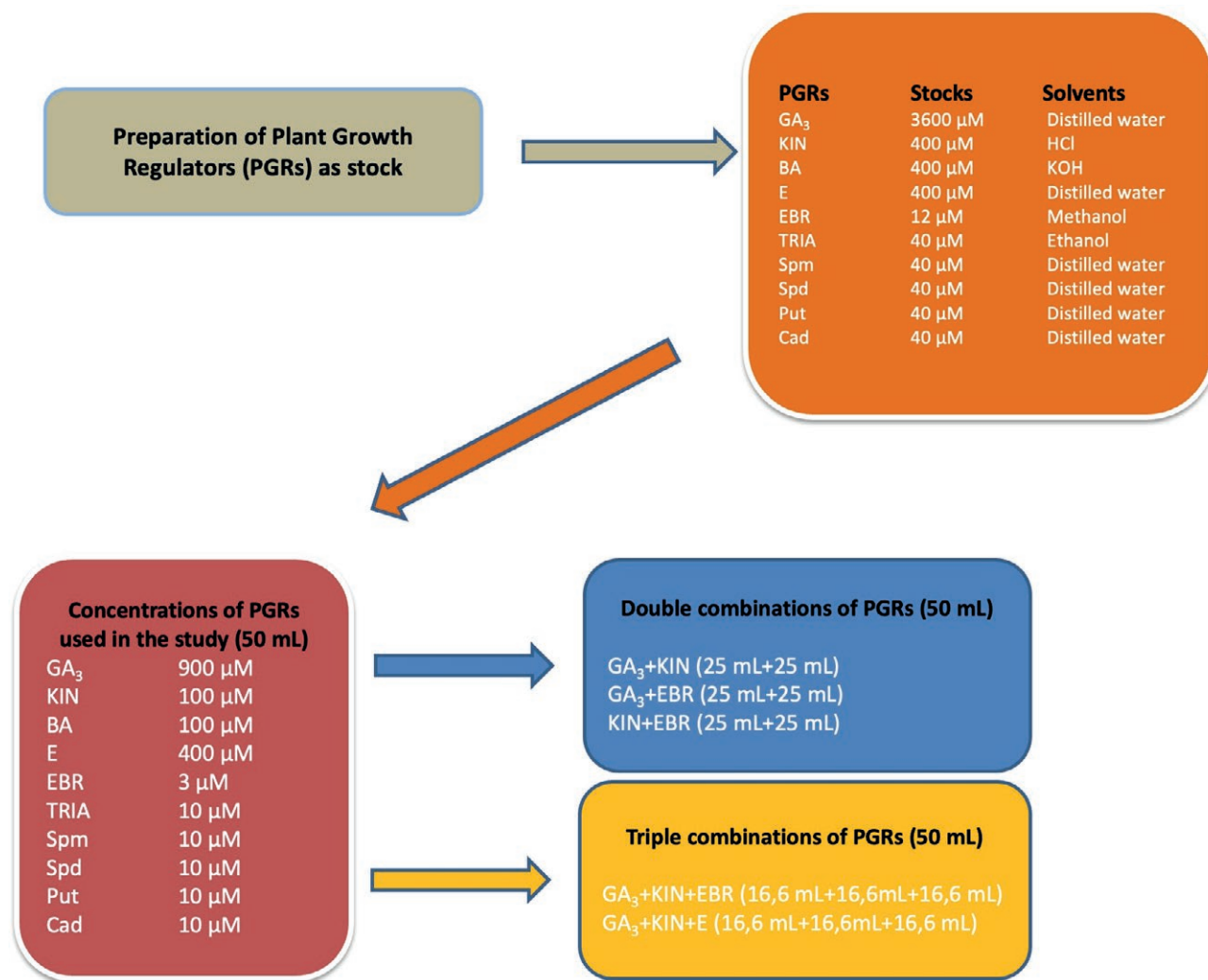


Figure 1. Diagram showing PGRs solvents, prepared stocks and concentration of solutions used in the study. Stock solutions were prepared by dissolving each of PGRs with appropriate solvents and made up to liter (µM, micromolar) with distilled water. The stock solutions were diluted and the concentrations of solutions used in the study were obtained. For this, PGR concentrations which reduce the damaging effect of 30°C, the tolerance limit of barley seeds against heat stress were used. Seeds were pretreated in 50 mL distilled water (control), PGRs alone and their double-triple combinations for 24 hours at room temperature. Germination process carried out at constant temperatures of 20°C (control) and 30°C in an incubator.

Germination experiments

First of all, germination experiments were carried out at different temperatures between 22 and 35°C and the tolerance limit of barley seeds against heat stress was determined as 30°C. Germination processes were carried out at constant temperatures of 20°C (control) and 30°C in the dark and in an incubator. For this process, full-looking, robust and uniform sized 20 seeds were selected first. These previously sterilized seeds were pretreated in 50 mL distilled water (control), GA₃, KIN, BA, E, EBR, TRIA, Spm, Spd, Put and Cad alone and in their double-triple combinations for 24 hours at

room temperature. At the end of this pretreatment session, the solutions were filtered and the seeds were vacuum-dried. Then, the seeds for each application were arranged in Petri dishes covered with two sheets of filter paper moistened with 7 ml of distilled water. Immediately after sowing, the Petri dishes were placed in the above-mentioned 20°C and 30°C constant temperature incubators for germination. At the specified temperatures, they could not be studied because suitable and sufficient germination did not occur in combinations other than double combinations GA₃+KIN, GA₃+EBR, KIN+EBR and triple combinations GA₃+KIN +EBR, GA₃+KIN+E.

Cytogenetic examinations

For cytogenetic examinations, the root tips reached about 1 cm length after 5-7 day were excised, pretreated with a saturated solution of paradichlorobenzene for 4 h at 20 °C, fixed in solution absolute ethanol:glacial acetic acid (3:1, v/v) for overnight, and stored in 70% alcohol at refrigerator until used again. The root tips were hydrolyzed in 1 N HCl at 60 °C for 15–18 min, stained for 1-2 h in accordance with the standard procedure for Feulgen staining, smashed in a drop of 45% acetic acid and squashed (Elçi and Sancak 2013). After 24 h, microscopic slides were made permanent by mounting with alcohol vapor exchange method. The best mitotic phases and mitotic aberrances were photographed (100X) with digital camera (Olympus C-5060) mounted on an Olympus CX41 microscope.

Data analyses and statistical evaluations

For detect the effect of PGRs and HTS on the MI, the prepared slides were examined under the microscope at 100X magnification, and MI, i.e. percentage of dividing cells were accounted by counting at least 6000 cells for per application (three repeat, 2000 per slide). The MI was calculated using the following equation:

$$MI (\%) = \frac{\text{total number of dividing cells}}{\text{total number of analyzed cells}} \times 100$$

At the same time, CAs occurring at all stages of mitosis during microscopic observation of the slides were calculated according to the following the equation for each per-application as the percentage of 350 dividing cells counted.

$$CA (\%) = \frac{\text{total number of aberrant cells}}{\text{total number of dividing cells}} \times 100$$

All experiments were repeated three times. Statistical evaluation concerning all obtained parameters was realized by using SPSS 14.0 program according to Duncan's multiple range test, at $p \leq 0.05$ level of significance (Duncan 1955).

RESULTS

As explained in detail in the Introduction, it is known that under normal conditions, GA₃, cytokinins (CKs= BA and KIN) and E generally promote cell division and cell elongation, thereby increasing growth.

However, the effects of EBR, TRIA and PAs (Spm, Spd, Put and Cad) on cell division have not yet been fully elucidated. Therefore, the present study aimed to clarify the counterchecks of these chemicals alone or in combination on MI and CAs, under both optimum (20°C) and HTS (30°C).

Counterchecks of PGRs against the adverse effects of HTS on MI

The MI values calculated as a result of cell counting of barley meristems after pre-application of various PGRs alone or in combinations at 20°C and 30°C are presented in Figure 2.

At optimum temperature (20°C), the application of GA₃, BA and Put, respectively, from the PGRs studied here showed statistically quite a lot successful effect on the MI of barley root meristems compared to the control group. Especially, at GA₃ the MI were reached to the highest value by increasing from 6,2±0,3 (at 20°C, in distilled water) to 15,1±0,8 (approx. 2.5 fold). While E, Spm and Cad applications were partially successful on this parameter KIN, EBR, TRIA and Spd applications exhibited an inhibitory effect on the MI. Considering the alone PGRs pre-applications, GA₃ treatment on the MI was more successful than all other treatments, while TRIA had the most negative effect. Among the double combinations of PGRs, GA₃+KIN showed a more successful effect on the MI than the control group and other double combinations. In addition, both of the triple combinations studied (GA₃+KIN+EBR and GA₃+KIN+E) displayed an excellent performance by showing a very successful effect on the MI compared to all other treatments except GA₃ alone. Considering all the PGRs applications both alone and in double/triple combinations, the most positive effect on the MI was obtained with the application of GA₃ alone and GA₃+KIN+E from the triple combinations. But, at the KIN+EBR, one of the double-combinations, the MI were recorded as the lowest value by decreasing from 6,2±0,3 to 1,5±0,5 (Figure 2).

At HTS (30°C), the MI in barley root meristem cells germinated in distilled water medium decreased by 35% compared to the control (at 20°C). When applied alone, it was determined that the PGRs, which showed a very successful performance on the MI of meristem cells at HTS compared to own control group (in distilled water/at 30°C), were GA₃, BA, E, Put and Spm, respectively. However, KIN, EBR, TRIA, Spd and Cad alone were not successful in alleviating the negative effect of HTS on the MI. Considering the pre-applications of all PGRs alone, GA₃ treatment increased from 5,7±0,3 (at 30°C, in distilled water) to 15,3±0,7 was more successful than the others in

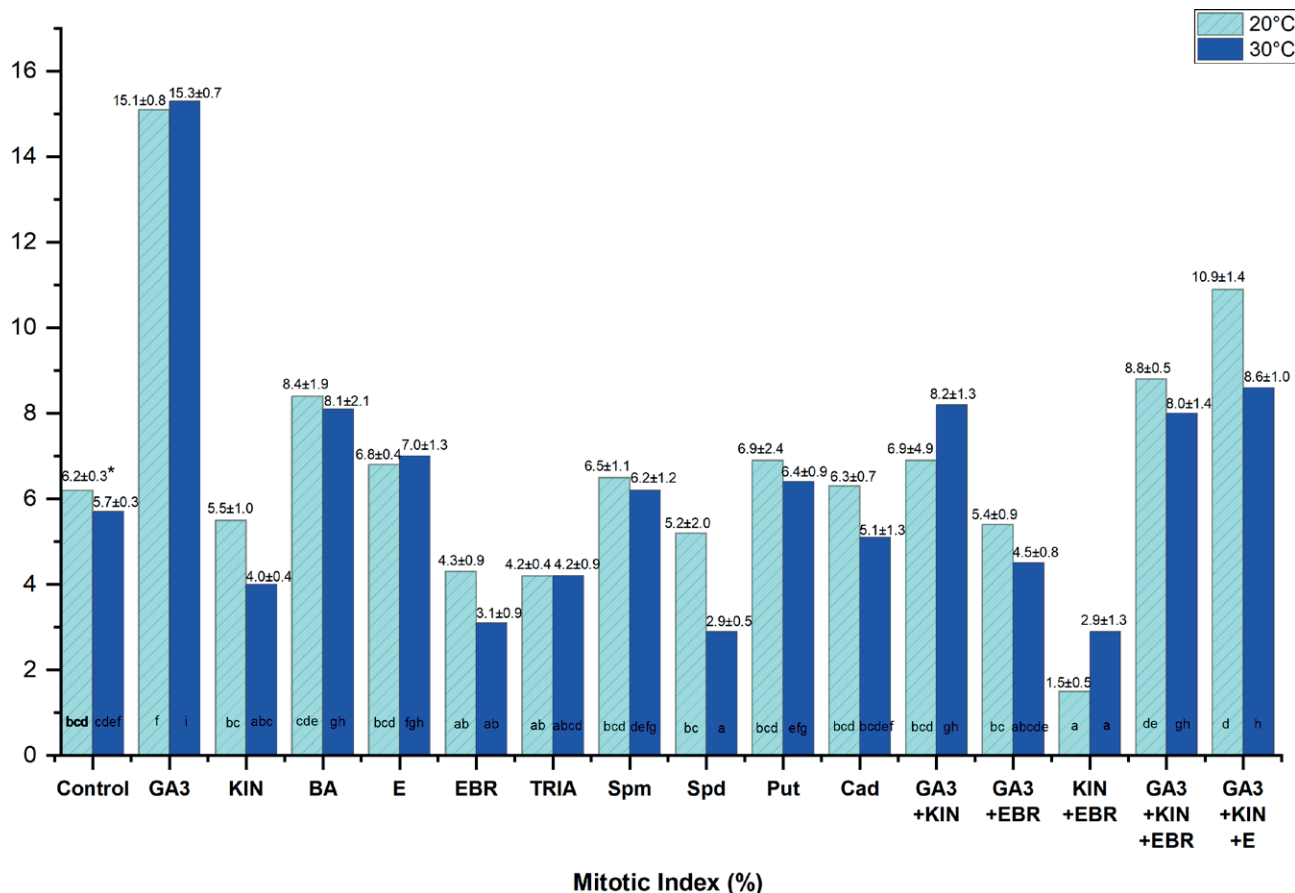


Figure 2. Mitotic index scores in meristem cells of barley exposed to high temperature stress after plant growth regulators supplementation. *Values with insignificant difference ($P \leq 0.05$) for each column are indicated with same letters (\pm Standard deviation). Seeds were germinated at constant temperatures of 20°C (control) and 30°C in the dark and in an incubator. As test solution, 900 μM GA₃, 100 μM KIN, 100 μM BA, 400 μM E, 3 μM EBR, 10 μM TRIA and 10 μM PAs (Spm, Spd, Put and Cad) were used. The pretreatment process of seeds was performed by soaking 24 h in constant volumes (50 mL) of distilled water (control) or each PGR. All data were evaluated as three replicates.

mitigating the negative effect of HTS on the MI, but Spd was extremely unsuccessful on this parameter. Among the double combinations of PGRs, again GA₃+KIN was quite a lot successful in mitigating the negative effect of HTS on the MI compared to its control group and other double combinations. However, the KIN+EBR double combination had the lowest MI value together with Spd from the single combinations. Similarly, both of the triple combinations studied resulted in a statistically significant increase in the MI under HTS (Figure 2).

Among all studied applications, GA₃, E, GA₃+KIN and KIN+EBR showed a more positive effect on the MI compared to those at optimum temperature in barley meristem cells exposed to HTS. None of the other applications under HTS could reach MI values under own self optimum conditions. Under these conditions, double combinations (except GA₃+EBR) were more successful

than single PGRs. Especially, while KIN+EBR had the lowest MI value (1,5±0,5) under optimum conditions, this value increased approximately twice and reached 2,9±1,3 at HTS (Figure 2).

Counterchecks of PGRs against the adverse effects of HTS on CAs

The percentages of CAs into barley meristem cells germinated in distilled water and at 20°C (control) and 30°C after pretreatment of various PGRs alone or in double/triple combinations were summarized in Figure 3. Representative images of CAs for all applications were given in Figure 4. As a result of cytological examinations, no aberration was found in the chromosome structures of barley meristem cells germinated in

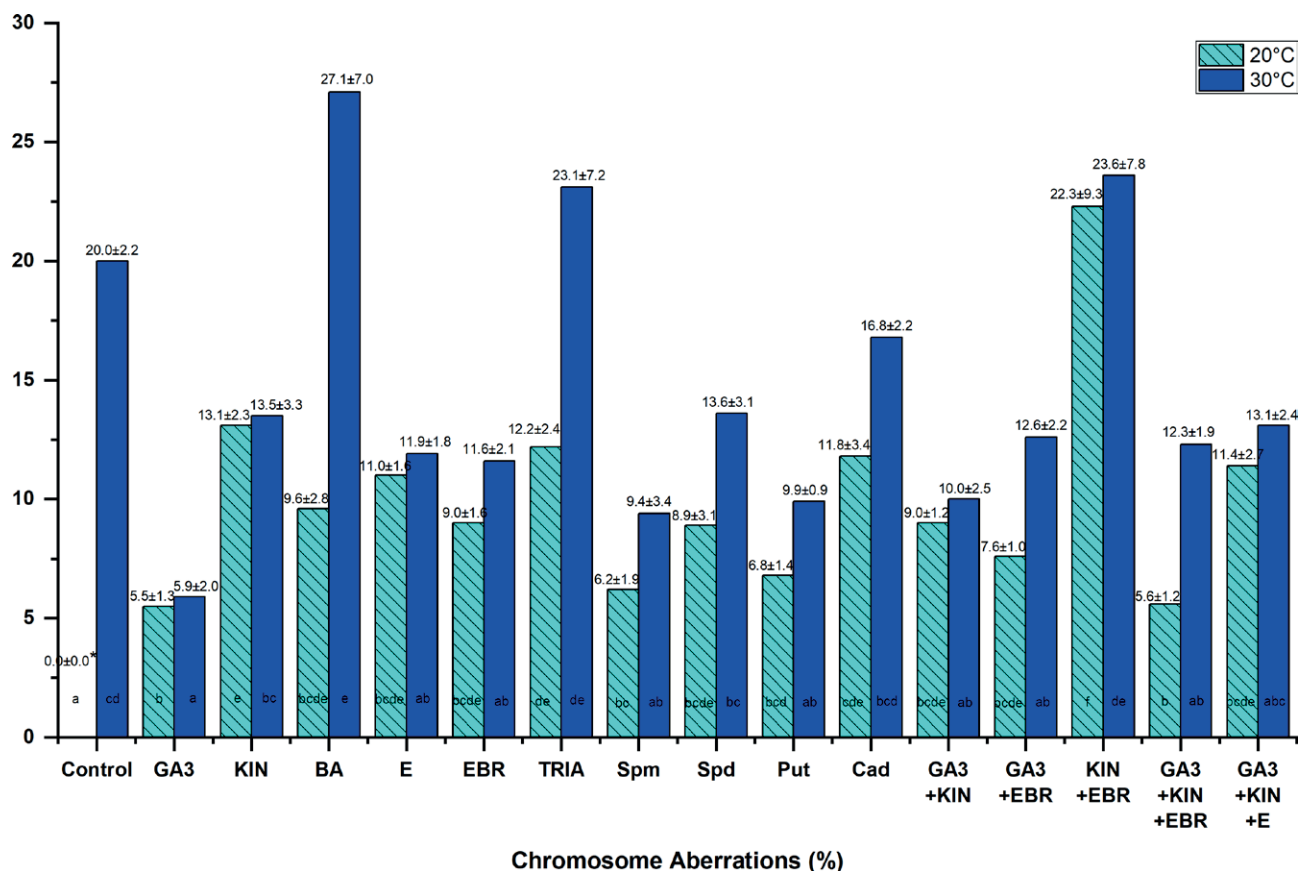


Figure 3. Frequency of chromosome aberrations in meristem cells of barley exposed to high temperature stress after plant growth regulators supplementation. *Values with insignificant difference ($P \leq 0.05$) for each column are indicated with same letters (\pm Standard deviation). Seeds were germinated at constant temperatures of 20°C (control) and 30°C in the dark and in an incubator. As test solution, 900 μM GA₃, 100 μM KIN, 100 μM BA, 400 μM E, 3 μM EBR, 10 μM TRIA and 10 μM PAs (Spm, Spd, Put and Cad) were used. The pretreatment process of seeds was performed by soaking 24 h in constant volumes (50 mL) of distilled water (control) or each PGR. All data were evaluated as three replicates.

distilled water and at 20°C and all of the mitotic stages were observed normally (Fig. 4 A-D). Whereas, the rate of CA in samples subjected to HTS (30°C) was determined to be 20.0±2.2%. Also, in terms of genotoxicity, these aberrations were observed to increase significantly ($p \leq 0.05$) with all PGR treatments compared to the control, as evidenced by the CA percentages. Under optimum conditions, the highest CA frequency was detected in KIN+EBR, one of the double combinations by increasing from 0.00 ±0.0% to 22.3±9.3%, followed by KIN (13.2±2.3%), TRIA (12.2±2.4%), Cad (11.8±3.4%) and GA₃+KIN+E (11.4±2.7%). The applications with the lowest CA frequency are also GA₃ alone (5.5±1.3%) and triple combination GA₃+KIN+EBR (5.6±1.2%) (Figure 3).

Under HTS, most of the PGR pretreatments studied greatly attenuated the negative effect on CA percentages in barley meristem cells. At the temperature level in mentioned, however, the percentage of CAs respec-

tively, at BA (27.1±7.0%), KIN+EBR (23.6±7.8%) and TRIA (23.1±7.2%), increased even more compared to own control group (20.0±2.2%). It was determined that the GA₃ application alone was the most successful application (almost the same as optimum conditions) compared to all other combinations studied by reducing the detrimental effect of HTS on the percentage of CA from 20.0±2.2% to 5.9±2.0% (Figure 3).

In all PGR applications studied, HTS significantly increased CAs compared to own optimum conditions. Especially with BA, GA₃+KIN+EBR and TRIA applications respectively, CA rates increased by 2 times or more compared to own optimum conditions in HTS.

Microscopic images of a wide range of CAs observed in the preparations prepared with root tips belonging to all application groups are shown in Figure 4. Generally, the most extensive aberrations observed in all application were micronucleus (Figure 4 a, b), disorderly pro-

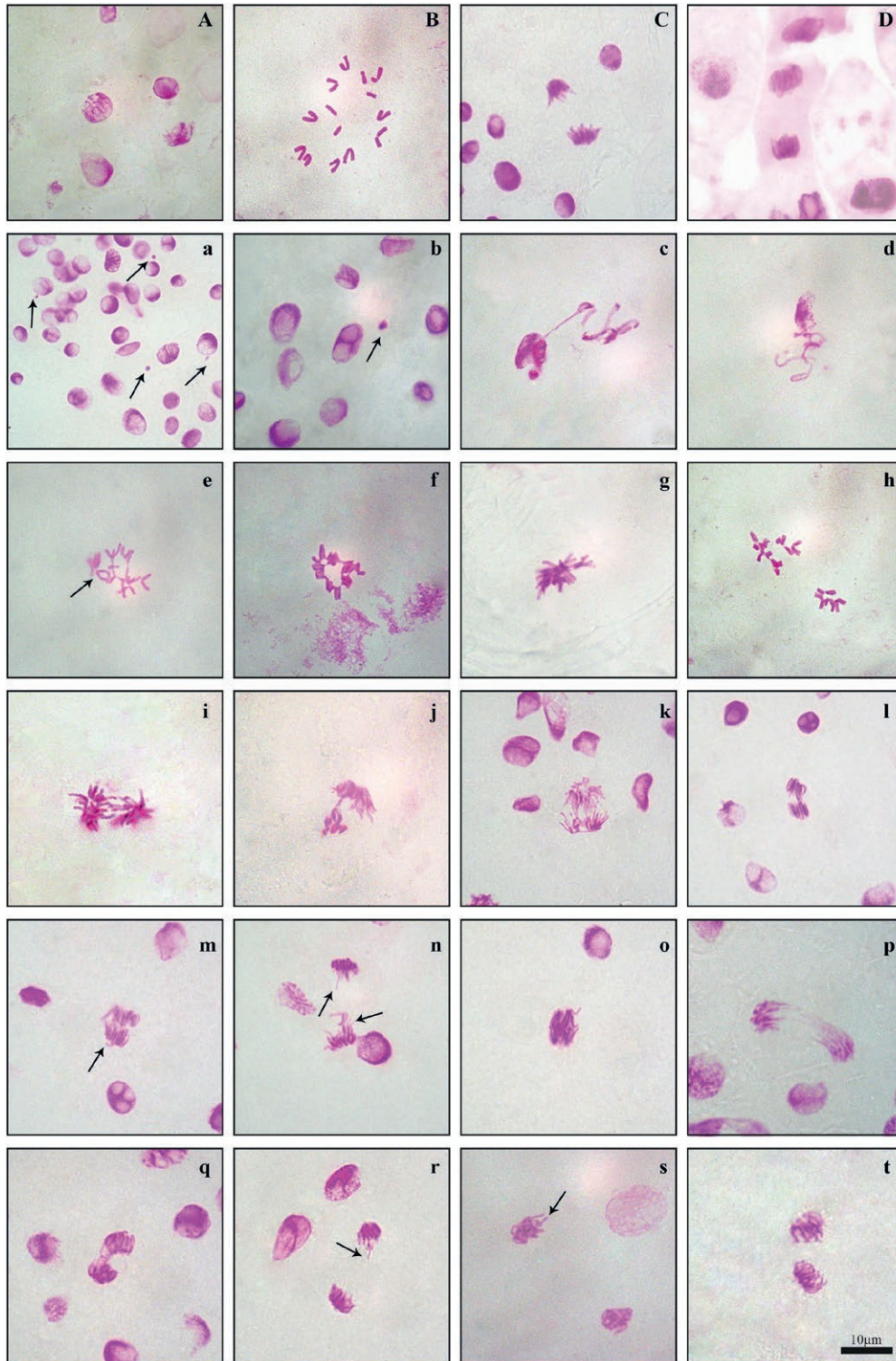


Figure 4. Representative images of microphotographs of normal mitotic stages (A-D) and aberrations (a-t) observed in all application groups studied in barley plant. A prophase, B metaphase ($2n=14$), C anaphase, D telophase. a, b micronucleus (arrows) c disorderly prophase with micronucleus d uncoiled chromosomes e chromosomal ringing (arrow) f vacuolated sequencing at metaphase g sticky chromosomes h chromosomal irregularity in the equatorial plane i stellar anaphase j disorderly anaphase k, l anaphase with multiple bridges m chromosomal alignment with vagrant chromosome (arrow) n laggards in anaphase (arrows) o multipolar anaphase p polar slip in anaphase q bridges in telophase r laggard in telophase (arrow) s vagrant chromosome in telophase (arrow) t polar slip in telophase. Scale bar = 10 μ m.

anaphase (Figure 4 c, j), uncoiled chromosomes (Figure 4 d), sticky chromosomes (Figure 4 g), chromosomal irregularity in the equatorial plane (Figure 4 h), alignment anaphase (Figure 4 m), multipolar anaphase (Figure 4 o), laggard and vagrant chromosomes (Figure 4 m, n, r, s), bridges (Figure 4 k, l, q) and polar slip (Figure 4 p, t) in ana-telophase. The minimal level aberrations recorded were chromosome ringing (Figure 4 e), vacuolated sequencing at metaphase (Figure 4 f) and stellar anaphase (Figure 4 i).

DISCUSSION

As a result of the extensive literature review, adequate study were not found on the effects of some of the PGRs studied here (especially EBR, TRIA and PAs) on cell division and chromosomal behaviors. Moreover, it was seen that there is still no consensus among researchers about the role of also GA₃, KIN, BA and E on these parameters. For this reason, it was found appropriate to compare the effects of these parameters under optimum conditions before moving on to the effects of the above mentioned PGRs under HTS conditions.

Effects of exogenous PGRs on cytotoxicity and genotoxicity at optimum conditions

In this part of the study, the effects cytotoxic and genotoxic the effects of hormone/with hormone-like activity shown chemicals such as exogenously GA₃, KIN, BA, E, EBR, TRIA and PAs in the barley meristem cells of at optimum conditions were investigated. The results were compared with the relevant literature and among themselves.

According to our findings, while the MI value of barley seeds in the control group (in distilled water, at 20°C) was 6,2±0,3%; KIN, EBR, TRIA, Spd, GA₃+EBR and KIN+EBR treatments could not reach this (see Figure 2). Similarly, some researchers suggested that externally applied GA₃, KIN, BA (Tabur and Demir 2010a; Tütünoğlu et al. 2019) and TRIA application (Tabur and Demir 2008a) under stress-free conditions reduced mitotic activity in barley root meristems. In that case, it can be said that exogenously application of some stimulatory growth regulators under normal conditions without stress may be useless. On the other hand, it has been reported that exogenous GA₃ (Mansour and Kamel 2005; MacDonald and Little 2006), low concentration BA (Huyluoğlu et al. 2008; Truta et al. 2011; El-Ghamrey et al. 2013) and TRIA (Hangarter and Ries 1978) applications promote cell division and thus MI

during germination under normal conditions. However, the effects of E, BRs and PAs on cell division have not been fully elucidated. It has been stated that these PGRs may have positive or negative effects on cell division. Some researchers asserted that these PGRs promote cell division and MI at low concentrations (Kartal et al. 2009; Maraklı et al., 2014), while at high concentrations reported that they had an inhibitory effect (Hu et al., 2000; İsmailoğlu et al., 2004; Tabur and Demir, 2009, 2010 a,b; Özmen et al. 2022). Our findings reveal that the application of GA₃, BA and Put alone had statistically a very successful effect on the MI of barley seeds compared to the control group and also E, Spm and Cad applications were had partially successful. But, TRIA, EBR, Spd and KIN applications had an inhibitory effect on this parameter (see Figure 2). Considering the PGRs studied, either alone or in double-triple combinations, it is seen that the most positive effect on the MI is obtained with GA₃ pre-application alone (15,1±0,8%) and generally the combinations with GA₃ are statistically more significant than the control group (6,2±0,3%). For example, the GA₃+KIN+EBR triple combination created with the addition of GA₃ to the KIN+EBR double application, where the most negative effect on the MI was observed, increased statistically significantly the MI compared to the control group. Moreover, considering all the PGR applications studied, the most positive effect on the MI was obtained with the application of GA₃ alone and GA₃+KIN+E from the triple combinations (see Figure 2). This indicates that GA₃ has an indispensable place in cell division. Data on double and triple combinations of PGRs studied here on MI under optimum conditions are presented for the first time in this study.

No any chromosomal abnormalities (CA) were came across in barley root meristems germinated under optimum conditions. However, as a result of the PGRs pretreatment studied here, either alone or in double/triple combinations, various types and percentages of CAs were generally observed (see Figure 3-4). This is due to the fact that even any externally applied stimulator under optimum conditions is perceived as a stress factor by the plant. The least percentage of CA was obtained with GA₃ pretreatment alone. In particular, it was determined that the percentage of CA in seeds with KIN pretreatment was higher than other PGRs applied alone. Among all the combinations studied, the most CA again was observed in the KIN+EBR application, which is one of the double combinations by increasing from 0,00±0,0% abnormal cells (at distilled water, control) to 22,3±9,3%. Moreover, the CA ratio was reduced in the GA₃+KIN+EBR triple combination (5,6±1,2%) formed by adding GA₃ to this double combination until to the

level in the GA₃ application alone (5,5±1,3%) (see Figure 3). In this case, as mentioned above, we can say that the negative effect of KIN+EBR double application on the MI also is due to these CAs caused by the mitotic irregularities during cell division. Again, data on double and triple combinations of PGRs studied under optimum conditions on CAs also are presented for the first time in this study.

In our study, it was observed that CKs and E caused the formation of CAs such as micronucleus, disorderly pro-anaphase, chromosome ringing, chromosomal irregularity in the equatorial plane, multipolar anaphase, sticky and uncoiled chromosomes, especially bridges in ana-telophase. In addition, CAs such as sticky chromosomes, chromosome ringing, laggard and vagrant chromosomes in ana-telophase, and alignment anaphase were frequently encountered in GA₃, EBR and TRIA applications. On the other hand, it has been determined that PAs cause CAs in the form of sticky chromosomes, disorderly anaphase, chromosome bridges in ana-telophase, and polar slip in ana-telophase (see Figure 4 a-t).

Information on the effects of various PGRs on chromosome behavior under optimum conditions is limited to only a few studies conducted in the last 20 years. It has been reported that high concentrations of CKs negatively affect chromosomal behaviors with a clastogenic effect and cause different types of genetic and chromosomal variations (Huyluoğlu et al. 2008; Truta et al. 2011; El-Ghamrey et al. 2013; El-Ghamery and Mousa 2017). However, Tabur and Demir (2010a) reported in their study that BA and GA₃ application did not cause any chromosomal abnormality, but KIN and E application increased CAs significantly compared to the control. According to Tütünoğlu et al. (2019) argue that increasing GA₃ concentrations depending on time and dose show cytotoxic and genotoxic effects and the difference between control and treatment groups is statistically significant, while Mansour and Kamel (2005) argue that there is statistically an insignificant increase in CAs. Again, some researchers reported that exogenous applied TRIA, EBR and HBRs under optimum conditions negatively affect chromosomal behavior in barley meristems (Tabur and Demir, 2008a, 2009; Kartal et al., 2009). Similarly, Ünal et al. (2002) on barley seeds, İsmailoğlu et al. (2004) on diploid, tetraploid and hexaploid wheat seeds in their studies stated that also PAs cause mitotic irregularities. Tabur and Demir (2010b) reported that PAs inhibited the MI in barley meristems, significantly increased the CAs of other PAs except Spd and Put had the highest abnormality rate in total. Özmen et al. (2022) also stated that PAs significantly increased the CA rate by causing various mitotic abnormalities, and

the PA with the highest abnormality percentage was Spm. If a comparison is made in the light of all these studies; it can be said that the effects of PGRs on MI and CAs under optimum conditions may differ depending on the plant species studied, plant development stages, genotype, used concentration, exposure time and pre-application method.

Effects of exogenous PGRs on cytotoxicity and genotoxicity at HTS conditions

HTS may inhibit seed germination and mitosis, thereby reducing germination rate (Çavuşoğlu and Kabar, 2007; Sharma et al. 2022) and mitotic activity (Öney and Tabur, 2013; Öney et al. 2015). It may be cause a decrease in the amount of protein and stop the synthesis of proteins that act as osmoprotectants that play a role in temperature tolerance (Xu et al. 2021). According to the results obtained from our study, it has been confirmed once again that HTS reduces the MI also in barley plant, and limited literature information on this subject has been contributed. The reason for the decrease in mitotic activity at high temperature may be directly or may be related to the loss of enzyme activation, which is responsible for mitosis, and also proteins denaturation and lipid peroxidation (Sheikhi et al. 2023).

At the same time, HTS showed quite unfavorable effects on the chromosome behavior of barley seeds. In our study, it was determined very high rate and various types of chromosome aberrations in barley root meristems germinated at 30°C (see Figure 3-4). These aberrations may be due to the damaging effects of HTS on microtubule organization (Wahid et al. 2007), which may have led to irregular mitotic configurations and CAs, mainly involving spindle fibers and metaphase (Abou-Deif and Mohamed, 2007). In addition, it has been reported that reactive oxygen species (ROS) such as hydroxyl (OH), superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and single oxygen (¹O₂), which occur due to HTS may be cause deaggregation of DNA, RNA and nucleic acids (Liu and Huang, 2000). Faraghi et al. (2015) suggest that *Vicia dasycarpa*, which is normally diploid, exhibits a mixoploid state with aneuploid and tetraploid cells after temperature shock. However, Öney and Tabur (2013) reported in their study that high temperature (30°C) did not cause any CA in *Vicia faba* root meristem cells. In the light of all these studies, we can emphasize again that heat stress may have different effects depending on the type of plant used, the severity and duration of the application of stress. Because the upper and lower threshold limits of abiotic stresses can show different effects in different species, sometimes even in different

varieties of the same species (Wahid et al. 2007; Heman-taranjan et al. 2014).

On the other hand, it was determined that PGRs were effective at different degrees in mitigating the negative effects of HTS on the MI, and this difference was statistically significant (see Figure 2). Especially KIN, EBR, TRIA, Spd, Cad, GA₃+EBR and KIN+EBR applications have not been successful in alleviating the negative effects of HTS on the MI. It was observed that the most negative effect on the MI occurred with Spd application alone and KIN+EBR application from double combinations. In this case, it would not be right to expect every stimulator to be successful in overcoming the heat stress on this parameter. Indeed, it has been emphasized by many researchers that the type(s) and concentrations of stimulators may vary from species to species in overcoming various abiotic stresses (Mirza and Bagni 1991; Tabur and Demir 2010a,b; Korek and Marzec 2023). However, considering all PGR pre-applications, either alone or in double/triple combinations, in our study, more than half of the tested applications showed a successful performance in alleviating the unfavorable effect of HTS on the MI. In particular, the most positive effect was obtained with the application of GA₃ alone, GA₃+KIN from double combinations, and GA₃+KIN+E from triple combinations (see Figure 2). In addition, in the case of HTS alone GA₃ and GA₃+KIN double combinations reached a higher value than they have shown success under optimum conditions. Moreover, considering the success of other combinations with GA₃ on the MI, it is seen that again GA₃ has an indispensable place in alleviating the negative effects of stress compared to their own control groups (at 30°C in distilled water). Similarly, GA₃+KIN (8,2±1,3%) combination was more successful than KIN alone (4,0±0,4%) and GA₃+EBR (4,5±0,8%) combination was more successful than EBR alone (3,1±0,9%) in overcoming HTS on the MI. Also, the excellent success of the GA₃+KIN+EBR (8,0±1,4%) triple combination on the MI compared to the KIN+EBR (2,9±1,3%) double combination indicates that GA₃ creates a noticeable synergism with these PGRs. It has also been emphasized in previous reports that combinations with GA₃ against abiotic stresses are more effective role on seed germination and MI (Çavuşoğlu and Kabar 2007; Tabur and Demir 2008b). With this together, considering that the internal amount of stimulators such as CKs (El-Mashad and Kamel, 2001) and GAs (Prakash and Prathapasenan, 1990) decreases in seeds under stress conditions, these externally applied promoters is not surprising that they increase mitotic activity; it can be expect.

Although the information about the response of PGRs to stress factors during cell division has not been sufficiently clarified, it is known that various priming

applications increase resistance to stress factors by promoting cell division, DNA replication (Giri and Schilinger 2003) and antioxidative defense (Afzal et al. 2006). The most common response under stress conditions is the acceleration of synthesis of protective components, especially osmoprotectants. Based on general literature information, it would be correct to say that the PGRs may have been successful in alleviating the damaging effect of HTS on the MI by increasing the activity of enzymes involved in cell division or by accelerating the synthesis of proteins that act as osmoprotectants that play a role in temperature tolerance. However, as mentioned in the Introduction, there is only one study (Pradhan and Gupta 2013) on the effects of these PGRs on mitotic activity, especially under high temperature conditions. In this previous study, it was reported that only EBR application was studied and increased the MI in *Brassica oleracea* var. *botrytis* root meristems germinated under low (4°C) and high (44°C) temperature stress. Contrary to our findings, these researchers suggested that increasing concentrations of EBR under high and low temperature stress increased MI. This paradox may be due to the type of plant, the concentration of EBR used and the applied temperature degree.

As for CAs, so far no studies have been conducted on the effects of all the above-mentioned PGRs on this parameter under HTS. Therefore, our study includes the first findings describing the data obtained on this parameter in detail. Accordingly, it was determined that the studied PGRs also showed statistically significant effects on the percentages of CAs in barley seeds germinated under HTS (see Figure 3). Although most of the PGRs applied alone or in double/triple combinations were successful in improving the CAs caused by HTS, only BA, TRIA and KIN+EBR applications could not show sufficient success on this parameter. Especially among all applications, the most positive effect on CAs was obtained again with the GA₃ application alone, while the most damaging effect was in BA application. Thus, the GA₃ application demonstrated once again on CAs its successful performance on the MI under HTS. For example; GA₃+KIN double application (10,0±2,5%) was more successful than KIN application alone (13,5±3,3%) in ameliorating the damaging effects of HTS by reducing the percentage of CAs.

Various mitotic aberrations were observed during microscopic scans of root meristem cells of barley seeds belonging to all application groups (see Figure 4 a-t). Aneugenic and clastogenic impacts that form an important portion of CAs might have been largely resulted from spindle dysfunction and chromosomal breaks respectively. The CAs, such as bridges and break, are

indicators of a clastogenic action, whereas chromosome losses, laggards, sticky, multipolarity and C-metaphase originate from aneugenic effects (Silveira et al. 2017). As known, accurate chromosome segregation in mitosis requires that sister kinetochores attach to microtubules emanating from opposite spindle poles (biorientation). Because kinetochore attachment is a stochastic process, it is error prone and can result in chromosome malorientation (Banerjee et al. 2020). Mitodepressive actions such as disorderly pro-anaphase, alignment anaphase, multipolar anaphase, stellar anaphase, bridges and polar slip in ana-telophase may be mainly the result of the above reasons. Moreover, Tabur and Demir (2010b) asserted that the nucleoplasmic bridges in ana-telophase might have been occurs as a consequence of inversions while Bonciu et al. (2018) have asserted originate from dicentric chromosomes or occur as a result of as faulty longitudinal break of sister chromatids during anaphase. Fiskesjö (1997) have claimed also that bridges are clastogenic effects, both resulting from chromosome and chromatid breaks. The large micronucleus (MN) in the cell indicates aneugenic effect resulting from chromosome loss while small MN indicates clastogenic effect due to chromosome breaks (Kontek et al. 2007). Briand and Kapoor (1989) have reported that the MNs are likely the consequence of vagrant chromosomes and fragments. Uncoiled chromosomes and chromosome ringings may be the result of a weak mitotic effect and irregular chromosome contractions (Tabur and Demir 2010b). Asita and Mokhobo (2013) asserted that sticky chromosomes could be originated from abnormal DNA condensation, irregular chromosomal wrapping and inactivation of the axes. At the same time, such aberrations may be a result of improper folding of the chromatin fibers (Klásterská et al. 1976). According to some researchers, sticky chromosomes are a marker of high toxic effect on chromatin and irreversibility of the change (Fiskesjö and Levan 1993; Türkoğlu 2007). Chromosomal irregularity in the equatorial plane and vacuolated sequencing at metaphase may originate from unequal distribution of chromosome and spindle dysfunction. Laggard and vagrant chromosomes occurs during the anaphase where one or more chromatids gets detached from the rest of the chromatids and is incapable of moving towards the poles. Aberrations of these kinds may have occurred due to a weak mitotic impresses a consequence of failures in chromosomal attachment to the mitotic spindle (Patil and Bhat 1992).

Generally, it was concluded that BA, E, Put and Spm, respectively, among the PGRs alone studied, including at first GA₃, showed a very successful performance statistically in mitigating the negative effect of HTS on the MI.

In addition, when the effects of the double/triple combinations of these PGRs on this parameter were evaluated, it was determined that all the studied combinations, except the KIN+EBR application from the double combinations, showed a superior success in overcoming the negative effect of HTS on the MI. In fact, this success was higher than most of the PGRs applied alone (see Figure 2). On the other hand, as a result of the statistical evaluations, it was proved that all PGRs studied, except for KIN+EBR, BA and TRIA applications, both alone and in double/triple combinations, showed an important successfully in the improvement of CAs (see Figure 3).

CONCLUSION

Various growth agents can be effective in different events in different species, even in individuals of the same species, and can be found in different amounts. Accordingly, which hormone is in effective concentration in any event in a plant this hormone would be responsible for growth and development events by performing its function. Indeed, as Khan (1971) points out, any event is unlikely to be governed by the absolute presence or absence of a hormone. In response to environmental conditions, some hormones in the plant may be more effective, some may be less effective or not effective at all. Therefore, it seems more plausible that whichever hormone is most effective, it functions in the relevant case.

In our study, the interactions between mitotic activity and mitotic irregularities and various stimulating growth agents, which can be counted as possible mechanisms of tolerance to increased heat stress as a result of global climate changes, were examined in barley, an important cereal crop. Thus, it has been tried to serve to fill a gap in the literature regarding these parameters. It is thought that the use of suitable PGRs for plants that will be grown in regions exposed to high temperatures will provide very beneficial results economically. However, a detailed investigation of the effects of these chemicals on basic metabolic events such as hydrolase synthesis and activity, nucleic acid metabolism, protein and enzyme synthesis, which can be directly or indirectly effective on mitotic activity, will help to elucidate the mechanism in question.

Consequently, thanks to these and similar studies, it can be contribute to the development of genetically temperature-tolerant products by changing the plant's sensing, signaling and regulatory pathways without disturbing other vital processes. In addition, a comprehensive explanation of the response of plants to high temperature tolerance and temperature tolerance mechanisms

and the development of possible strategies in this regard are mandatory. Therefore, it is necessary to map gene loci related to thermotolerance and to elucidate different genetic approaches that provide tolerance to heat stress (Asthir, 2015).

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AUTHOR CONTRIBUTIONS

ST and ŞBYE designed and performed the experiments. SÖ helped to conduct the experiments. ST wrote the manuscript. All authors read and approved the manuscript and have equal contribution.

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