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Cytogenetic and Morphophysiological Effects of Exogenous Triacontanol against Drought in Barley

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Abstract: Drought stress is one of the leading abiotic stresses that have a devastating impact on sustainable agricultural policy as a result of the decrease in crop yield. For this reason, hormones or growth regulators can be used to grow plants that can adapt to morphological and physiological changes caused by stress. Triacontanol (TRIA) is a hormone that takes part in abiotic stress tolerance mechanisms and performs this task by ensuring the continuity of growth, productivity, development and vital metabolic processes. In this study, the morphophysiological and cytogenetic effects of exogenous TRIA application in barley (*Hordeum vulgare* cv. Avcı) under drought stress were investigated. The results showed that drought stress negatively affected barley both morphophysiologically and cytogenetically. Application of exogenous TRIA had an increasing effect on germination percentage, radicle number, coleoptile length and percentage under drought stress. Moreover, in the same environment, with the effect of TRIA, there was an increase in the mitotic index (44%) and a decrease in chromosomal abnormalities (46%). The conclusion drawn from this study is that the application of 10 µM TRIA application causes physiological and cytogenetic improvements in barley under drought stress, resulting in the plant's stress tolerance.

Key words: Drought, Mitotic activity, Morphophysiological effects, TRIA.

1. Introduction

One of the biggest risks to plant growth is drought stress, which is caused by increasing temperatures due to climate change around the world, decreasing precipitation, and using the wrong tactics such as inconsistent irrigation. Crop development and productivity are severely harmed by drought stress because it lowers the quality of the morphological, physiological, and biochemical features of plants [1]. According to research conducted in a variety of countries, the drop in yearly precipitation produced by global warming has had a deleterious impact on plants, including a fall in relative water content and loss of turgor [2]. Drought also interferes with growth, photosynthesis, and other vital physiological and biochemical functions [3]. Previous studies has demonstrated that the stress caused by drought increases chromosomal aberrations, decreases the cell cycle and mitotic index, damages biological membranes and macromolecules, and generates oxidative stress [4-7].

Adverse environmental conditions such as drought threaten the survival of plants that depend on soil and inhibits plant growth, development, and product quality [8]. Numerous plant investigations have demonstrated the detrimental consequences of drought stress at the physiological [9–12] and cytogenetic levels [6, 13, 14]. However, plants produce a range of organic substances, including plant hormones, that aid in their adaptation in response to these changes through a variety of biochemical, molecular, and structural alterations [15].

Plant hormones are a class of signaling molecules that are present in minute quantities in cells. They are crucial for regulating metabolism in response to biotic and abiotic stresses and for improving a plant's capacity to morphologically and physiologically adapt to unfavorable environmental circumstances [16]. Although prior research has established the roles of certain hormones, including gibberellin, ethylene, auxins, ABA, and salicylic acid, in abiotic stress tolerance, it is recognized that the mechanisms of action of these hormones vary depending on the degree of stress, the type of plant, and the tissues that are under stress [17–19]. TRIA is a plant growth regulator that helps plants adapt to harsh environments like salt and drought by balancing a variety of physiological and biochemical processes. After exogenous application of TRIA, which is in the form of a 30-carbon primary alcohol, the increase in photosynthesis, water and nutrient assimilation, membrane stability, gene regulation and activities in vital processes has attracted the attention of researchers [20]. TRIA, which is present in plant epicuticular waxes, increases nitrogen fixation when applied exogenously to plants. In addition, it raises the levels of photochemical pigments, sugars, soluble proteins, free amino acids, carbonic anhydrase, transpiration rate, photosynthetic rate, and water usage efficiency [21]. The exogenous injection of TRIA boosts growth, dry mass, photosynthetic pigments, photosynthesis rate, suitable osmolytes, and antioxidant enzyme activities, hence mitigating the harmful effects on plants under abiotic stress conditions. This is because TRIA actively regulates the processes of plants in changing environmental conditions [22–24].

Aim of the work was designed to comprehensively test of the efficiency level of exogenous TRIA against effects physiological, cytotoxic and genotoxic in caused by drought stress in *Hordeum vulgare* cv. Avcı meristem cells and to contribute to the gap in the literature. That is, it is aimed at clarifying to what extent exogenous TRIA tolerates drought stress, whether it encourages cells to enter the mitosis division, and whether it causes any changes in the structure and behavior of chromosomes.

2. Material and Method

Hordeum vulgare cv. Avcı seedlings were obtained from Ankara Field Crops Central Research Institute and employed in the study's experimental phases. In addition, PEG 6000, among the chemicals used to reveal the effect of drought stress in plants, was supplied by Merck Firm (S7455691, 1 kg) and the TRIA Fluka Firm (90275, 100 mg) was supplied. To generate a 500 ml stock with a 100 µM concentration for the investigation, the TRIA was dissolved in distilled water and kept at +4 °C in the refrigerator. In a preliminary investigations conducted by us, the most proper concentration of TRIA in alleviation of the drought stress at the germination were determined as 10 µM and also 22% PEG 6000 was the most suitable concentration for drought stress. 22% PEG 6000 concentration was prepared fresh each use by dissolving it in distilled water.

2.1. Identification of physiological properties

Every germination experiment involving the barley seeds utilized in the study was conducted in a dark, sterile incubator with a temperature setting of 20 °C. During the first phase of the germination trials, seeds that appeared physically alive, plumpness, and similar characteristics were chosen. Prior to use, these chosen seeds were surface sterilized by washing them with distilled water for 24 hours and pre-treated in 1% sodium hypochlorite for 10 minutes. The sterilized seeds were placed in conical flasks with 50 ml of distilled water and 10 µM TRIA, and they were soaked for a full day at room temperature. Following that, these seeds were placed in 10 cm sterile, disposable petri plates that were double-layered with blotting paper on the bottom and contained 7 ml of distilled water and 22% PEG 6000. The germination percentage, fresh weight, radicle numbers and lengths, coleoptile percentage, and lengths of the seedlings sprouting from the germinated seeds were measured after the planted petri dishes were maintained in the incubator for seven days. The length of the radicle was measured by using a ruler to measure the portion of the germinated seeds from the point where the root and stem separated to the tip of the longest fringe root. By using a ruler to measure the length of each germinating seed, from the point where the root and coleoptile separate to the tip of these organs, the coleoptile length was ascertained. The number of radicles and the percentage of coleoptiles in the seedlings were determined by counting the germinated roots and coleoptiles based on their concentrations at the end of the seventh day in the incubator. A precision scale was used to weigh all of the germinated seeds after they had sprouted for seven days. The average fresh weight, given in grams per plant, was then calculated by dividing the weight by the total number of seeds that germinated [25].

2.2. Identification of cytogenetic characteristics

The root tips of seeds belonging all concentrations in the research after they reached 0.5–1 cm in length, the roots were chopped and placed in glass bottles containing pretreatment solution. Then, the root tips pretreated for 4 h. at paradichlorobenzene. Following a distilled water wash, root tips extracted from the paradichlorobenzene solution were kept in acetic alcohol (3 parts ethyl alcohol/1part glacial acetic acid) solution at +4 °C for a full day. After being taken out of the acetic alcohol, the root tips were placed in 70% alcohol solutions and refrigerated at +4 °C until the cytogenetic procedures were carried out [26].

2.3. Root tip staining procedures and preparation

After the root tips, which had been kept at +4 °C and 70% alcohol, had been washed several times with tap water were kept in 5 N HCl at room temperature for 20-25 minutes for hydrolysis. Hydrolyzed root tips were stained with Feulgen at room temperature for 1h. The root tips were then let to sit in little petri dishes filled with water for 15 min. to remove any leftover dye. Finally, after the dying process, the slayts were prepared by the crushing method for the preparation process and examinations were made [26].

2.4. Calculating chromosomal aberrations and mitotic index

The 100X magnification of the microscope was used to scan the slides prepared for the purpose of examining the stages of mitosis. Three iterations of counting the prepared slides were performed. After counting at least 3000 cells for each application (3x1000), mitotic index, phase indices and chromosomal abnormalities were calculated using the following equations. Images of chromosomal anomalies were photographed (100X) using a C-5060 WZ digital camera and an Olympus CX41 research microscope.

$$\text{Mitotic index (\%)} = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells}} \text{X100}$$

$$\text{Chromosome aberrations (\%)} = \frac{\text{Number of abnormal cells}}{\text{Number of cells in mitosis}} \text{X100}$$

$$\text{Phase indices} = \frac{\text{Number of cells belonging to phase}}{\text{Total number of dividing cells}}$$ [27,28].

3. Results and Discussions

In this study, the physiological characteristics and cytogenetic effects of exogenous TRIA application in barley under normal and drought conditions were investigated and the results were compared. According to the results obtained, the scores of physiological changes in control and PEG 6000 applied seeds is presented in Table 1. The germination percentage value of barley seeds planted in petri dishes in distilled water (control group) environment after seven days was 79.00±5.03 However, with TRIA application alone to stress-free environment reached value of 92% increasing in the germination percentage by approximately 16% compared to the control group. This increase, in addition to being numerical, also revealed a statistically significant different. Drought stress showed its negative effect by decreasing from 79.00±5.03 to 42.00±4.00 (approx. 47%) the germination percentage compared to the control group. Exogenously applied TRIA were ameliorated the negative effect caused by drought on germination percentage, achieving value almost close to the stress-free environment Drought+TRIA application compared to alone drought on the germination percentage was showed an increase of 70% and the germination percentage value was 72.00±7.30. As a result of the study, it was determined that the fresh weight of barley seeds germinated in distilled water environment was 257.00±5.72 mg. TRIA administered alone showed an inhibitory effect of approximately 5% on fresh weight. After the application of drought stress, fresh weight down to 111.00±2.16 mg by decreasing approximately 60% compared to the control group. After Drought+TRIA applied, there was a decrease in the amount of fresh weight compared to the control group, as in the non-stress environment. It was observed that the root length values in the control group were both statistically and numerically close to each other when TRIA was applied alone in a non-stressed environment. Accordingly, while the radicle length in the control group was 10.00±2.21 cm, it was 10.93±1.81 cm after TRIA application. However, drought stress and TRIA application values under stress also showed very close values to each other. After applying drought stress alone, radicle length decreased to 4.89±1.52 cm, a 52% decrease compared to the control group. Likewise, after TRIA applied in a stressful environment, radicle length decreased to 4.22±1.27 cm.

In the study conducted to determine the number of radicles in barley seeds, this value was determined to be 4.75±0.59 in the control group. After the addition of TRIA, the number of radicles increased by approximately 5% and reached 4.99±0.72. As expected, the number of radicles decreased after drought stress. As a results of the study, the number of radicles decreased by approximately 30% under stress, with a value of 3.34±0.76. After exogenous TRIA application in the presence of drought stress, the number of radicles increased slightly, up to 3.40±0.91. When the coleoptile percentage values were examined in the study, it was determined that the results obtained after the control group and TRIA application were very close to each other. According to the results obtained, the coleoptile percentage values in the control group and after TRIA application were determined to be 98.69% and 100%, respectively. Application of drought stress caused the coleoptile percentage in barley plants to decrease by approximately 32%, down to 68% compared to the control group. However, TRIA applied in the presence of stress showed a stimulating effect on this parameter. According to the results obtained, the coleoptile

percentage increased by approximately 25% compared to the control group and rose to 83.80%. When the results obtained were examined, it was determined that the results of coleoptile lengths were parallel to the results of coleoptile percentage. Accordingly, the coleoptile length values in the control group and after TRIA application were 10.13±1.99 and 10.21±1.84 cm. Drought stress had a negative effect on this parameter, as in all other parameters. Application of drought stress caused the coleoptile length to decrease by 82% to 1.92±1.32 cm. TRIA applied under drought stress showed an encouraging effect on coleoptile length by increasing the coleoptile length (approx. 12%) and reached 2.18±1.31cm (Table 1).

Table 1. Effects of exogenous TRIA on physiological changes in barley under drought stress and normal conditions (*Shows values with insignificant difference ($p<0.05$) for each column shown with same letters, ± standard deviation).

Applications	Germination percentage (%)	Fresh weight (mg)	Radicle length (cm)	Number of radicles	Coleoptile percentage (%)	Coleoptile length (cm)
Control	*79.00±5.03^{b}	257.00±5.72^{c}	10.00±2.21^{b}	4.75±0.59^{ab}	98.69±2.63^{c}	10.13±1.99^{b}
10 µM TRIA	92.00±5.66^{c}	248.00±9.09^{b}	10.93±1.81^{b}	4.99±0.72^{b}	100.00±0.00^{c}	10.21±1.84^{b}
%22 PEG 6000	42.00±4.00^{a}	111.00±2.16^{a}	4.89±1.52^{a}	3.34±0.76^{a}	68.00±2.37^{a}	1.92±1.32^{a}
%22 PEG 6000 +10 µM TRIA	72.00±7.30^{b}	108.00±2.94^{a}	4.22±1.27^{a}	3.40±0.91^{a}	83.80±11.10^{b}	2.18±1.31^{a}

Under normal conditions, where there are no biotic or abiotic stresses, there is no need to use additional external hormones or growth regulators for the seeds to germinate. In the absence of any stress conditions, growth regulators given externally to plants have positive effects on seed germination and seedling growth but may also have negative effects. According to the findings obtained in this study, it was determined that drought stress had an inhibitory effect on all physiological parameters. There are many studies showing that there is a decrease in germination percentage, fresh weight, root number, root length, coleoptile length and percentage as a result of applying drought stress [29–32]. In order to alleviate or eliminate such negative effects of drought stress, researchers have applied TRIA exogenously to various plants and at different drought stress intensities and shared the results. In one of these studies, 30% TRIA application to *Triticum aestivum* L. seedlings resulted in physiological developments due to increased seed germination, seedling growth, and free amino accumulation after PEG-based drought stress application [33]. In another study, it was reported that TRIA application in *Vigna unguiculata* (L.) seeds under drought stress improved the stress tolerance of the plant by improving germination parameters such as germination ability, relative seed germination, germination rate, radical and hypocotyl length, total seedling length and growth [34]. Sanadhya et al. [35] stated that TRIA pre-application to mung bean seeds significantly increased root and shoot length, germination percentage, fresh and dry weight of seedlings, thus increasing tolerance to PEG-induced drought stress. In another study conducted on rice plant, it was stated that TRIA application created a defense mechanism against drought stress by improving seed germination, seedling length, fresh mass, dry mass and biochemical enzyme activities [36]. After detailed literature research, no study was found investigating the effects of exogenous TRIA application on morphophysiological characteristics of especially barley plants under drought stress conditions. Therefore, the effects of TRIA application on the germination percentage,

fresh weight, number of radicles, radicle length, coleoptile length and coleoptile percentage in barley plants under drought and normal conditions are presented for the first time in this study. As can be seen from the results, drought stress caused negative effects on all parameters, causing a decrease in the values. Additionally, it is clearly seen that exogenous application of TRIA together with drought stress also creates a response mechanism to drought stress. It has been concluded that the increase in germination percentage, radicle length, radicle number, coleoptile percentage and length values caused by TRIA application has significant effects on coping with drought stress, especially in barley plant and other plants that can be used.

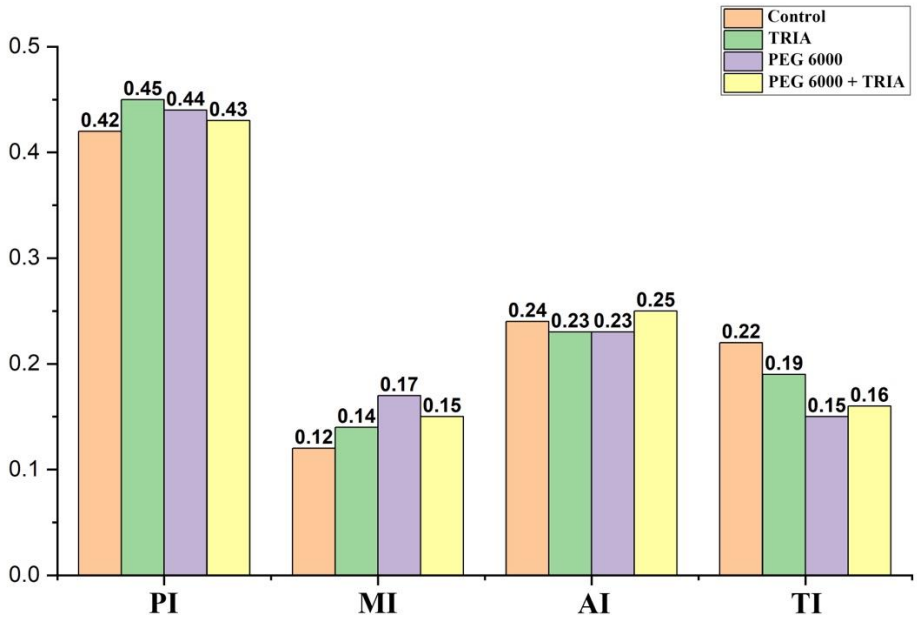

Figure 1. Effects of exogenous TRIA on mitotic division stages in barley under both drought and normal conditions (PI: Prophase index, MI: Metaphase index, AI: Anaphase index, TI: Telophase index)

Mitotic index and chromosome abnormality values according to the data obtained in this study are shown in Table 2. The mitotic index value was determined to be 10.20±0.76% in the control group germinated in distilled water environment. With the application of drought stress to the control group, TRIA applied in both normal and drought environments revealed statistically and numerically significant differences. TRIA application alone increased the mitotic index value by approximately 8% (11.04±0.21 %) compared to the control group. The lowest mitotic index value was observed after the application of drought stress. That is, drought stress was an inhibitory effect by reducing the mitotic index value by 20% (8.18±0.61%). With Drought stress+TRIA application, the mitotic index was the highest value reached. The findings revealed that TRIA applied under stress had a stimulating effect by increasing the mitotic index value by approximately 44% (11.85±0.12%) compared to alone drought stress conditions.

Following the determination of the mitotic index, the indices at each stage of mitosis were determined by taking into account the dividing cells of all concentrations (Figure 2). While the concentration with the highest index in prophase, which is the first phase of mitosis, was the TRIA application alone, the concentration with the lowest index was seen in the control group. When the metaphase indices were examined, the concentration with the highest index was the drought stress application, while the concentration with the lowest phase index was the control group. At anaphase indexes, Drought stress+TRIA application had the highest phase index compared to all other concentrations, while TRIA and drought stress applications alone showed the lowest anaphase index values. At

telophase the highest index value occurred in the control group. In addition, Drought stress+TRIA and alone drought stress had the lowest telophase index values (Figure 1).

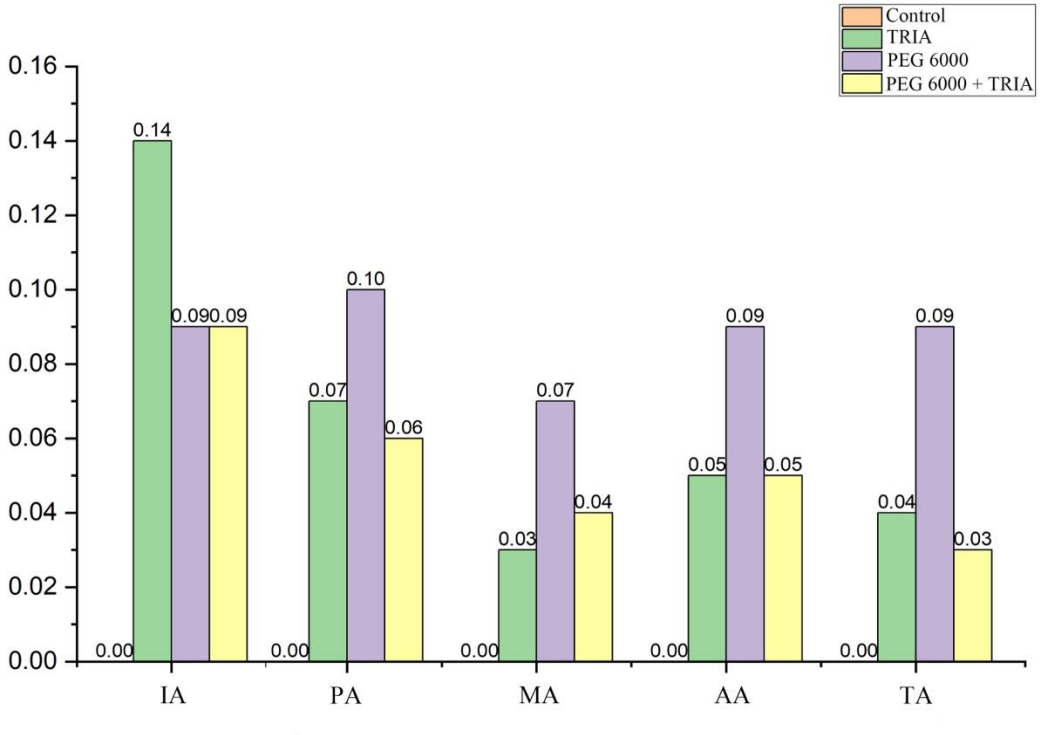

Figure 2. Distribution of chromosome abnormality indices in mitosis stages of exogenous TRIA applied to barley under drought and normal conditions (IA: Interphase abnormalities, PA: Prophase abnormalities, MA: Metaphase abnormalities, AA: Anaphase abnormalities, TA: Telophase abnormalities).

The chromosomal aberration frequencies data obtained from barley root tips germinated both distilled water and drought stress in the absence or presence of 10 μM TRIA are summarized in Table 2. After alone TRIA application, chromosomal abnormalities frequency was 19.97±0.46%. After the application of drought stress, chromosomal abnormalities increased significantly and an abnormality percentage of approximately 35% was reached. Drought stress+TRIA application significantly reduced chromosomal abnormalities while there was a decrease of approximately 50% compared to alone drought stress, and the chromosomal abnormality frequency decreased to approximately 19%.

After chromosome abnormalities were determined, chromosomal abnormality indices at each stage of mitosis were determined by taking into account dividing cells in all the concentrations. Since there are no chromosomal abnormalities in a stress-free environment, control group has the lowest chromosomal abnormality indices value in all phase. According to the results obtained, the application in which prophase, metaphase, anaphase and telophase abnormalities were most observed was the application of alone drought stress. In the interphase, the application with the highest chromosomal abnormality indices value was alone TRIA application (Figure 2).

In this study conducted on the root tips of barley plants to determine chromosome abnormalities, although no chromosome abnormalities were found in any of the seeds germinated in the control group, various chromosome abnormalities were found at samples belonging other concentrations. Microscopic images of a wide range of

chromosome aberrances observed in the preparations prepared with root tips belonging to all other application groups are shown in Figure 3-5.

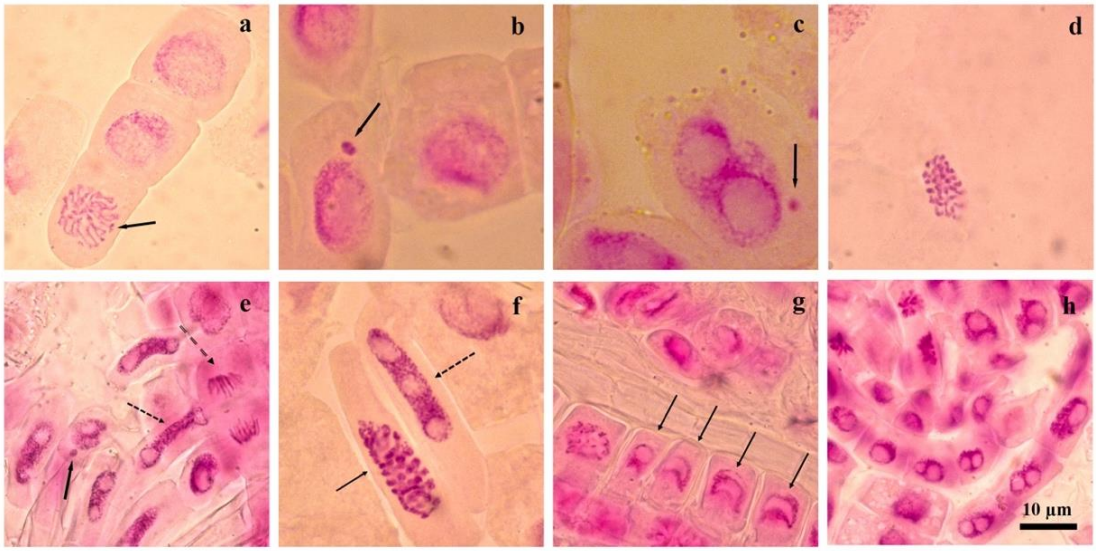

Figure 3. Interphase and prophase stages of exogenous TRIA applied under normal conditions and with drought stress and images of the chromosome abnormalities. a: normal prophase (arrow), b: micronucleus (arrow), c: micronucleus (arrow) in two-vacuolated cell, d: granulation, e: micronucleus (arrow), elongated nucleus with two vacuoles (short spaced arrow), anaphase (long spaced arrow), f: granulation (arrow), elongated nucleus with three vacuoles (striped arrow), g: sickle-shaped nucleus (arrows), h: two-vacuolated cell in interphase.

Chromosomal abnormalities, which occur spontaneously or as a result of the adverse effects of environmental stress, reflect the deleterious effects of a toxic agent on plant cells [37]. The aneugenic and clastogenic effects, which account for a significant proportion of the chromosome abnormalities observed in this study, may be largely due to spindle thread disruption and chromosome breakage, respectively. Micronucleus (MN) assay is agreed to be the most effective endpoint for the analysis of the mutagenic effects of toxic agents. The presence of a large MN in a cell is indicative of an aneugenic effect resulting from chromosome loss. Conversely, the presence of a small MN in a cell is indicative of a clastogenic effect due to chromosome breaks [38]. Briand and Kapoor [39] have indicated that the MNs (Figure 1a, c, e, 2 b, 3b) are a possible consequence of vagrant chromosomes and fragments. It is widely acknowledged by the research that a number of chromatin regulation-related factors, including histone modification enzymes, linker histone H1, HMG proteins and ATP-dependent chromatin remodelling factors, play a role in plant abiotic stress responses [40, 41]. Chromatin granulation observed at interphase (Figure 1d, f) is likely the result of the deformation of the nuclear material caused by toxic agents. This is thought to be a consequence of the aforementioned factors and abnormal chromatin condensation, and indicative of potential abnormalities that may occur in future mitosis phases. Sickle-shaped nucleus and elongated nucleus with vacuoles may have occurred due to absence of NuMA (Nuclear Mitotic Apparatus) in interphase, which is a protein required for the formation of spindle poles in mitosis. Sun et al. [42] and Merdes and Cleveland [43] asserted that the absence of NuMA in interphase nuclei was associated with non-spherical, elongated or beaded nuclear morphology, suggesting that NuMA may act as a non-fundamental nucleoskeletal element during interphase. The formation of sticky chromosomes (Figure 4 e,f) may be attributed to a number of factors, including aberrant DNA condensation, anomalous chromosomal wrapping and the inactivation of the axes [44]. It has also been proposed that such abnormalities may be caused by an incorrect folding of the chromatin fibres

[45]. Some researchers posit that the presence of sticky chromosomes indicates a high degree of toxicity affecting the chromatin, as well as an irreversibility of the change [46, 47]. Chromosome aberrations, such as bridges and breaks, are indicative of a clastogenic action, whereas chromosome losses, laggards, sticky, multipolarity and C-metaphase originate from aneugenic effects [48]. Given that kinetochore attachment is a stochastic process, it is susceptible to errors and can result in chromosome malorientation [49]. Mitodepressive actions, including spiralized chromosomes extending from pole to pole in metaphase, disorderly pro-anaphase, alignment anaphase, ring chromosome in anaphase, multipolar anaphase, bridges and polar slip in ana-telophase, star chromosome in ana-telophase, may be primarily attributable to the aforementioned factors. Furthermore, Tabur and Demir [50] proposed that the nucleoplasmic bridges observed in ana-telophase may have resulted from inversions. Similarly, Bonciu et al. [51] suggested that these bridges may originate from dicentric chromosomes or result from a faulty longitudinal break of sister chromatids during anaphase. Additionally, Fiskesjö [52] posited that bridges are clastogenic effects, resulting from both chromosome and chromatid breaks. Vagrant chromosomes (Figure 5i) and lagging chromosomes (Figure 5m) occur during the anaphase, whereby one or more chromatids become detached from the rest of chromatids and are unable to move towards the poles. Patil and Bhat [53] have put forth the hypothesis that laggard chromosomes may originate from the failure of the spindle apparatus to organize in the typical manner. Also, the laggard of chromosomes may have occurred due to a weak mitotic impress. It is hypothesised that irregular chromosome contractions may result in uncoiling of chromosomes in prophase and metaphase cells. Consequently, it is postulated that TRIA and/or drought stress may have been the cause of the aforementioned abnormalities, due to their ability to stimulate or inhibit enzymes and proteins that are essential for normal cell division, and thereby disrupt the spindle mechanism.

In addition to the substances found in the plant, substances such as hormones and growth regulators given externally can be perceived as stress in plant growth development and toxicity tests. This may result in mitodepressive effects such as inhibition of mitosis or suppression of DNA synthesis, depending on the dose of the substance administered or the duration of application [28]. Thanks to the active properties of the substances exogenously administered to plants, the fact that they cause different levels of clastogenic responses on chromosome structures and behavior varies according to plant species [54]. Researchers have done very little to examine the effects of TRIA on mitotic activity, and this is a major gap in the literature. In one of the rare studies conducted, they stated that TRIA application against the negative effect of salt stress on mitotic activity did not improve mitotic activity, but caused a decrease in chromosome abnormalities [55]. In another study, it was reported that TRIA application under ABA hormone decreased mitotic activity and chromosome abnormalities [56]. This study is the first in the literature to determine the clastogenic effects of exogenous TRIA on especially barley root tip meristems under the effects of drought stress. According to the results of the study, it has been determined that exogenous TRIA application promotes mitotic activity both in a stress-free environment and despite the negative effects of drought stress, and also helps maintain chromosome stability by reducing mutational damage that may occur in DNA or chromatids under drought stress. Although they generally occur as a result of environmental stresses, chromosomal abnormalities may also occur, albeit very rarely, as a result of changes occurring within the plant. This situation occurs especially under the influence of substances that have a toxic effect on plant cells [37]. Along with abiotic factors, biotic toxic agents cause chromosomal abnormalities as a result of aneugenic (changes in the total chromosome number) and clastogenic (changes in chromosome structure) effects. As a result of this study, it has been proven again that drought stress causes various chromosome abnormalities. Considering that chromosome abnormalities

occur as a result of stimulation/inhibition of enzymes and proteins necessary for normal cell division and disruption of the spindle mechanism [57], it is clear that stress has negative effects on these mechanisms. TRIA applied under drought stress showed ameliorative effects on chromosome abnormalities. This means that exogenous TRIA has a stimulating feature on the mechanisms mentioned above.

Table 2. Effects of exogenous TRIA applied on cytogenetic changes in barley under drought stress and normal conditions (*Shows values with insignificant difference ($p<0.05$) for each column shown with same letters, ± standard deviation).

Applications	Mitotic index	Chromosome aberrations
Control	*10.20±0.76^{b}	0.00±0.00^{a}
10 µM TRIA	11.04±0.21^{bc}	19.97±0.46^{b}
%22 PEG 6000	8.18±0.61^{a}	35.03±2.32^{c}
%22 PEG 6000 + 10 µM TRIA	11.85±0.12^{c}	18.98±1.25^{b}

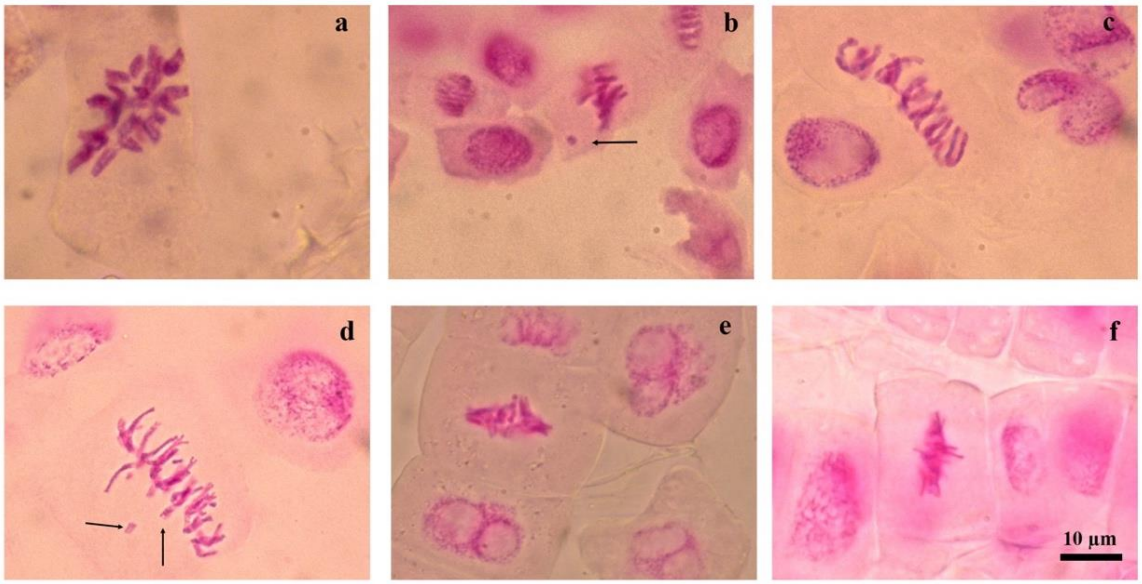

Figure 4. Images chromosomal aberrations of the metaphase stage of exogenous TRIA applied under normal conditions and drought stress. a: normal metaphase, b: micronucleus in metaphase (arrow), c: spiralized chromosomes extending from pole to pole in metaphase, d: fragmented chromosomes (arrows), e and f: sticky chromosomes.

4. Conclusion

As a result of this study, the morphophysiological and cytogenetic effects of exogenous TRIA application on *H. vulgare* cv. Avcı, a barley cultivar under both distilled water and drought stress were examined. Drought stress fatally affects plant growth and development, and it is obvious that agricultural areas in almost all parts of the world will be damaged by these negative conditions in the near future. The results clearly show that TRIA application both under drought stress and alone showed encouraging effects on the mitotic index. While TRIA hormone application alone and drought stress caused chromosome abnormalities, TRIA application under stress reduced chromosome abnormalities. This study supports that the pre-application of TRIA given externally in drought conditions can eliminate the negative conditions in the studied parameters. In order to fully reveal the cytogenetic role of TRIA, it is very important and necessary to determine the effects of biosynthetic inhibitors involved in cell division on chromosome

behavior in different plant species. Consequently, investigating the effects of TRIA on basic metabolic events that may directly or indirectly affect cell division and chromosome configuration will be important to form the basis of strategies to combat drought stress.

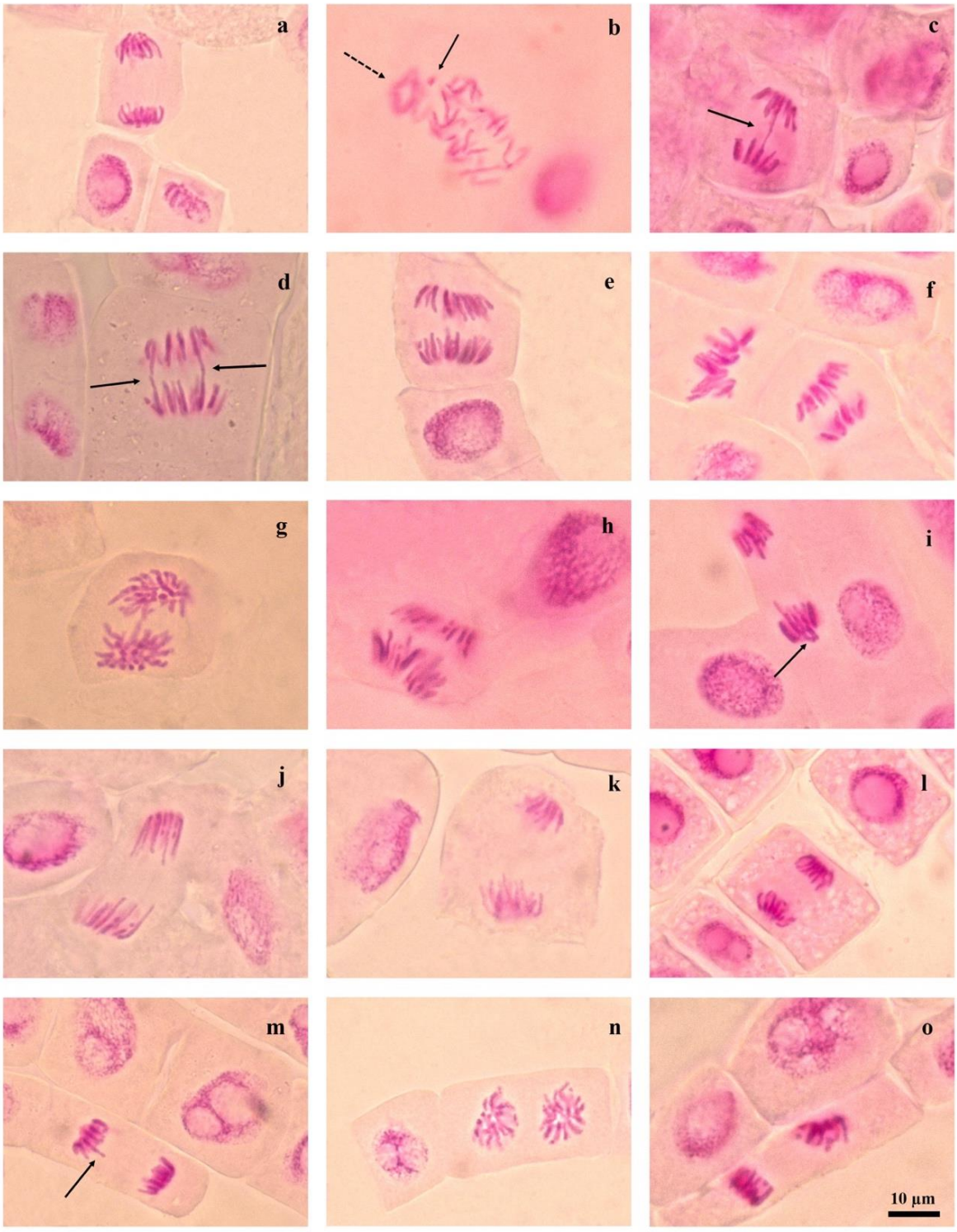

Figure 5. Images of the anaphase and telophase stage of exogenous TRIA applied under normal conditions and drought stress and the chromosome abnormalities. a: normal anaphase, b: micronucleus (arrow), disorderly anaphase and ring chromosome (striped arrow), c: bridge in anaphase (arrow), d: alignment anaphase and double bridge in anaphase (arrows), e and f: alignment anaphase, g: star anaphase, h: multipolar anaphase, i: vagrant chromosome (arrow), j and k: pole slip in anaphase, l: early telophase, m: lagging chromosome. (arrow), n: star telophase, o: pole slip in telophase.

Authorship contribution statement

S. Tabur: Investigation, Original Draft Writing, Review and Editing

S. Özmen: Data Curation, Original Draft Writing; Visualization

A. Yiğit: Assistance in the experimental stages.

Declaration of competing interest

There is no conflict of interest.

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Ethics Committee Approval and/or Informed Consent Information

As the authors of this study, we declare that we do not have any ethics committee approval and/or informed consent statement.

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