

COMPARISON OF CYTOGENETIC ANTAGONISM BETWEEN ABSICISIC ACID AND PLANT GROWTH REGULATORS

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Abstract

In the present work, antagonistic effect of abscisic acid (ABA) and various plant growth regulators on cytogenetic activity in root meristem cells of barley (*Hordeum vulgare* L. cv. Bülbül 89) was compared. The seeds germinated in medium with ABA alone (25µM, micromolar), mitotic phases and mitotic aberrations were mounted on an Olympus CX41 microscope and photographed (100X) with a digital camera (Olympus C-5060). The results showed that mitotic index (MI) significantly decreased while chromosomal aberrations increased by approximately 70% as compared to control. However, all of the plant growth regulators (PGRs) studied [gibberellic acid- GA₃, Kinetin- KIN, benzyladenine- BA, ethylene- E, 24-epibrassinolide- EBR, triacontanol-TRIA and polyamines- PAs (cadaverine-Cad, putrescine- Put, spermidine- Spd, spermine- Spm)] revealed to a successful performance in ameliorating of the negative effect of ABA on these parameters. The data obtained in the present work showed that all stimulators used overcame the mitotic activity-preventive effect of ABA and the most effective stimulators were GA₃, KIN, BA and EBR, respectively. In addition, this study indicated that almost all stimulators were able to largely remove detrimental effect of ABA on chromosomes and the most prominent effect on this parameter was obtained with GA₃, KIN and TRIA.

Introduction

Abscisic acid (ABA) is one of the major plant growth regulators that functions by inhibiting growth activities in times of environmental stress rather than by promoting growth. It often serves as an antagonist to the other growth promoting hormones in plant. The phytohormone ABA has been also involved in various physiological processes of plants, e.g., adaptation to stressful environments, seed germination and seedling growth (Finkelstein *et al.*, 2002; Shafī *et al.*, 2011; Khan *et al.*, 2012). Several studies of various plant tissues have indicated that ABA has an important role in blocking or slowing the cell cycle progression (Müller *et al.*, 1994; Liu *et al.*, 1997; Shabbir & Khan, 2000). Such studies have not conclusively established whether ABA blocks the cell cycle in G₁, G₂ or both. In some studies of root meristems in maturing seeds (Liu *et al.*, 1997), ABA arrested cells in G₁, whereas in other studies, ABA arrested root meristems cells in both G₁ and G₂ (Müller *et al.*, 1994). In addition, Mambelli and Setter (1998) reported that cell division was decreased 50% with the exogenously applied ABA in maize endosperm. Jacqmard *et al.*, (1995) explained that ABA slowed DNA synthesis by inactivating some DNA replication origins. Świątek *et al.*, (2002) suggested that exogenous ABA application inhibited G₁/S transition in synchronized BY-2 cells, but had no effect on further cell cycle progression when applied during S-phase.

Cytokinins (CKs) have important roles in plant growth and development by promoting cytokinesis, regulating mitosis cell division (Carle *et al.*, 1998) and increasing mitotic activity (Tomaszewska-Sowa *et al.*, 2002). Cytokinins have been used in alleviation of ABA inhibition caused by stress (Pospíšilová *et al.*, 2000; Khan *et al.*, 2003). Gibberellin (GAs) stimulates cell division, cell elongation (Besnard-Wibant *et al.*, 1983; Gupta *et al.*, 1993) and mitotic activity (MacDonald & Little, 2006). Effect of ethylene (E) on cell division and mitotic activity are not known well. Some authors hold that cell division and elongation were not triggered by

ethylene (Stange & Osborne, 1988; Ponce *et al.*, 2005), while others contend that ethylene showed promotive effects on elongation (Kazama *et al.*, 2004). In recent years, a new group called polyamines (PAs) has been added to the list of plant growth regulators. Four types of polyamines, namely putrescine (Put), spermidine (Spd), spermine (spm) and cadaverine (Cad), are found in all living organisms. The effects of exogenous PAs on cell division are controversial since they affected positive or negative on cell proliferation (Rost *et al.*, 1996; Hu *et al.*, 2000). It has been also suggested that some PAs lead to many chromosomal aberrations (Ünal *et al.*, 2002; İsmailoğlu *et al.*, 2004). Recently, Mahajan and Sharma (2009) have studied antagonistic effect of ABA and polyamines on mitosis in root tips of *A. cepa*. They reported that ABA caused a considerable decrease in the mitotic index and the higher concentrations of PAs lead to cell distortion. Their data indicated the possibility of ABA- PA interaction in the regulation of mitosis. It is known that brassinosteroids (BRs) promote root elongation and mitosis in low concentrations but not high concentrations (Hu *et al.*, 2000; Howell *et al.*, 2007). Tabur and Demir (2009) reported that exogenous 24-epibrassinolide (EBR) decreased approximately 50% the mitotic index and showed higher number of chromosomal abnormalities. Kartal *et al.*, (2009) also suggested that homobrassinolide (HBR) application increased mitotic activity and mitotic abnormalities when compared with control. Triacontanol (TRIA) is a new plant growth regulator discovered for the last 2 or 3 decades such as polyamines and brassinosteroids. Many researchers did not consensus about whether TRIA has preventive or stimulative effect on plant growth and development (Somen & Seethalakshmi, 1991; Kumaravelu *et al.*, 2000). So far, there are very few studies on the effect of TRIA on mitotic activity and chromosomal aberrations. Tabur and Demir (2008) reported that mitotic index remarkably decreased with TRIA pretreatment in root meristem of barley and also chromosomal aberrations increased.

Eventually, the hormonal explanation of mitotic activity and understanding of antagonism between ABA and plant growth regulators seem to be far more difficult at present with limited and stochastic information. In the present work, the performances of the plant growth regulators aforementioned in determining effect of ABA on mitotic index and mitotic aberrations were elaborately studied in root meristem of barley. Also, comparison of ABA- stimulators antagonism on these parameters was tested. It was thought that these results provide the conceptual basis for extending the study of ABA-stimulator antagonisms to mitotic activity and chromosomes affected by ABA

Materials and Methods

Hordeum vulgare L. cv. Bülbül 89 which was the cultivar of barley was used in this study. As test solutions 900 μ M (micromolar) GA₃, 100 μ M KIN, 100 μ M BA, 400 μ M E, 3 μ M EBR, 10 μ M TRIA and 10 μ M polyamines (Cad, Put, Spd and Spm) were used. The concentrations of these stimulators were at levels which were the most successful in alleviation of the ABA-induced inhibition on the germination. Concentration of ABA was 25 μ M. The concentrations of the stimulators and level of ABA preventing germination of seeds in a great extent were determined in a preliminary study (Çavuşoğlu & Kabar, 2007).

Ten dry seeds uniform sized of barley were placed in Petri dishes covered with two sheets filter papers moistened with 7ml of solution of ABA or distilled water (control), and with one of the plant growth regulators mentioned above. The Petri dishes were transferred in an incubator to germinate at 20 \pm 1 $^{\circ}$ C, in continuous dark for several days. When roots reached about 1cm length, they were excised, pretreated with a saturated solution of paradichlorobenzene for 4 h at 20 $^{\circ}$ C, fixed with Carnoy (ethanol:acetic acid, 3:1) for 24 h, and stored in 70% alcohol at 4 $^{\circ}$ C until required. Root tips were hydrolyzed

in 1 N HCl at 60 $^{\circ}$ C for 18 min., stained with Feulgen, and squashed in 45% acetic acid (Sharma & Gupta, 1982). The mitotic phases and mitotic aberrations were photographed (100X) with a digital camera (Olympus C-5060) mounted on an Olympus CX41 microscope.

Mitotic index was evaluated by analyzing at least 15,000 cells per treatment (5,000 per slide). Chromosomal aberrations were calculated for each concentration as the percentage of 350 dividing cells counted. Statistical analysis for both parameters were performed using SPSS programme according to Duncan's multiple range test at level of significance $p \leq 0.05$ (Duncan, 1955).

Results

The mitotic index of barley seeds germinated on the medium with ABA alone remarkably decreased as compared to control (in distilled water) and the frequency of mitotic abnormalities increased approximately 70% (Figs. 1-2). Mitotic index value was 0.40 in root tip cells of control seeds, while it was 0.15 in seeds germinated on the medium with ABA. Statistically, this value is substantially significant. In the present work, all the plant growth regulators used overcame the preventive effect of ABA on mitotic index. In other words, these stimulators were effective to a great extent in the breaking of the negative effect of ABA on mitotic index. GA₃ and KIN were more effective in comparison with the other stimulators and control. In this context, the most effective antagonists of ABA on mitotic activity were GA₃ and KIN (0.49), BA (0.45) respectively. ABA-induced inhibition of mitotic index was alleviated partly by TRIA (0.20) and Spm (0.22) and largely by EBR (0.31). The values of success of the other stimulators- Put and Spd (0.25), E (0.26) and Cad (0.28) were close to each other and showed less success. The details of the ABA-stimulator antagonism on mitotic index are indicated in Fig. 1.

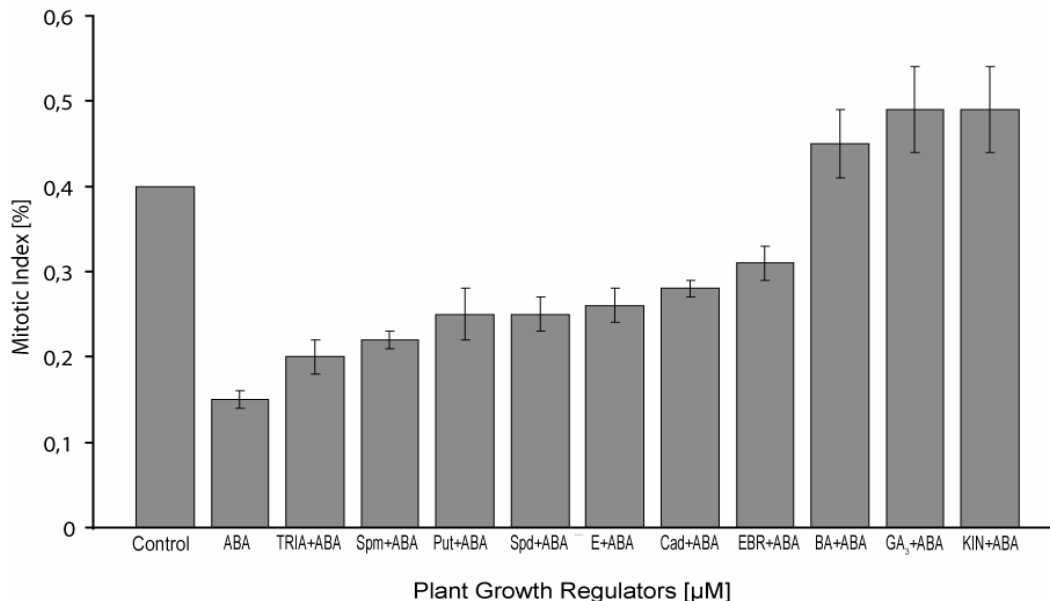


Fig. 1. Effects of ABA and stimulator antagonisms on the mitotic index in root meristems of barley. The concentrations of solutions were 900 μ M GA₃, 100 μ M Kin, 100 μ M BA, 400 μ M E, 3 μ M EBR, 10 μ M TRIA and 10 μ M polyamines (Cad, Put, Spd and Spm) and 25 μ M ABA. Both ABA and stimulators were exogenously applied to germination medium. Data are the means of three replications.

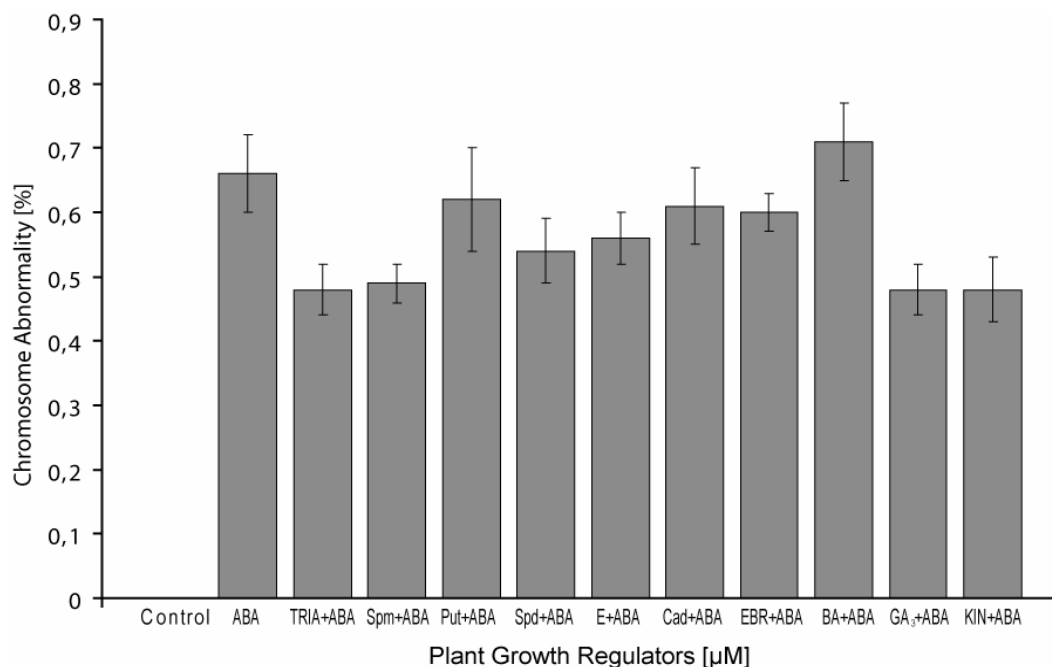


Fig. 2. Effects of ABA and stimulator antagonisms on the chromosomal aberrations in root meristems of barley. The concentrations of solutions were 900 µM GA₃, 100 µM Kin, 100 µM BA, 400 µM E, 3 µM EBR, 10 µM TRIA and 10 µM polyamines (Cad, Put, Spd and Spm) and 25 µM ABA. Both ABA and stimulators were exogenously applied to germination medium. Data are the means of three replications.

All mitotic phases were normal in root-tip cells of barley seeds germinated in distilled water (Fig. 3). However, the frequency of chromosomal aberrations in meristem cells of seeds germinated in the medium with ABA alone (0.66) was approximately seven times higher than in control (0.00). Although most stimulators studied except BA (0.71) were successful in varying degrees in alleviating of detrimental effect of ABA on chromosomal aberrations in comparison with one in the medium of ABA alone (Fig. 1). GA₃ (0.48), KIN (0.48), TRIA (0.48) and Spm (0.49) showed statistically a significant success on detrimental effect of ABA. ABA+ BA antagonism (0.71) lead to the highest frequency of mitotic abnormalities in total (Fig. 2). The success rate for the rest of the stimulators on the chromosomal aberrations were 0.54 for Spd, 0.56 for E, 0.60 for EBR, 0.61 for Cad and 0.62 for Put, respectively. The performances of the ABA-stimulator antagonisms on the mitotic abnormalities are presented in Fig. 2.

The chromosomal abnormalities observed were irregular metaphase, uncoiled chromosomes, adherent chromosome, fault polarization and alignment anaphase. Moreover, adherent chromosome and bridges in telophase were also observed (Fig. 4a-g). The greatest frequency of abnormalities in root tip-cells in total was irregular metaphase and uncoiled chromosomes (Fig. 4a, b, c).

Discussion

ABA inhibits plant growth and development by limiting the cell wall extensibility (Kutschera & Schopfer, 1986). Also, it is well known that ABA prevents cell

division by blocking or slowing down cell cycle (Liu *et al.*, 1994; Müller *et al.*, 1994; Świątek *et al.*, 2002) and by inactivating some DNA replication origins (Jacqumard *et al.*, 1995). On the other hand, several of stimulator substances plays an important role in plant growth and development by regulating and stimulating cell division (Gupta *et al.*, 1993; Carle *et al.*, 1998), increasing mitotic activity (MacDonald & Little, 2006; Kartal *et al.*, 2009) and triggering cell elongation (Kazama *et al.*, 2004; Howell *et al.*, 2007). However, effect of many stimulators such as E, PAs, TRIA, BRs on aforementioned parameters is still controversial (Hu *et al.*, 2000; Kumaravelu *et al.*, 2000; Tabur & Demir, 2008, 2009).

Recently, much interest has been focused on the cytogenetic activity studies of plant growth regulators under the toxic effect of abiotic stress conditions (Mahajan & Sharma, 2009; Tabur & Demir, 2008, 2009). It presented evidence indicating that mitotic index was decreased by all these growth regulators under without stress conditions, but chromosomal aberrations were increased (except GA₃, BA and Spd) (Demir, 2007). This case indicates that many of the stimulators inhibit the cell division and also can produce abnormalities in chromosome structure and behaviors. In the event that, there is no need to add exogenously any growth regulators unless the stress conditions are present.

The present work demonstrates that the mitotic activity prevented largely with effect of ABA asserted as a stress factor. This case can be explained with irregular mitosis. In addition, high number chromosomal aberrations in seeds germinated in the medium with ABA may be a sing for that cell division could be slow, retard or irregular.

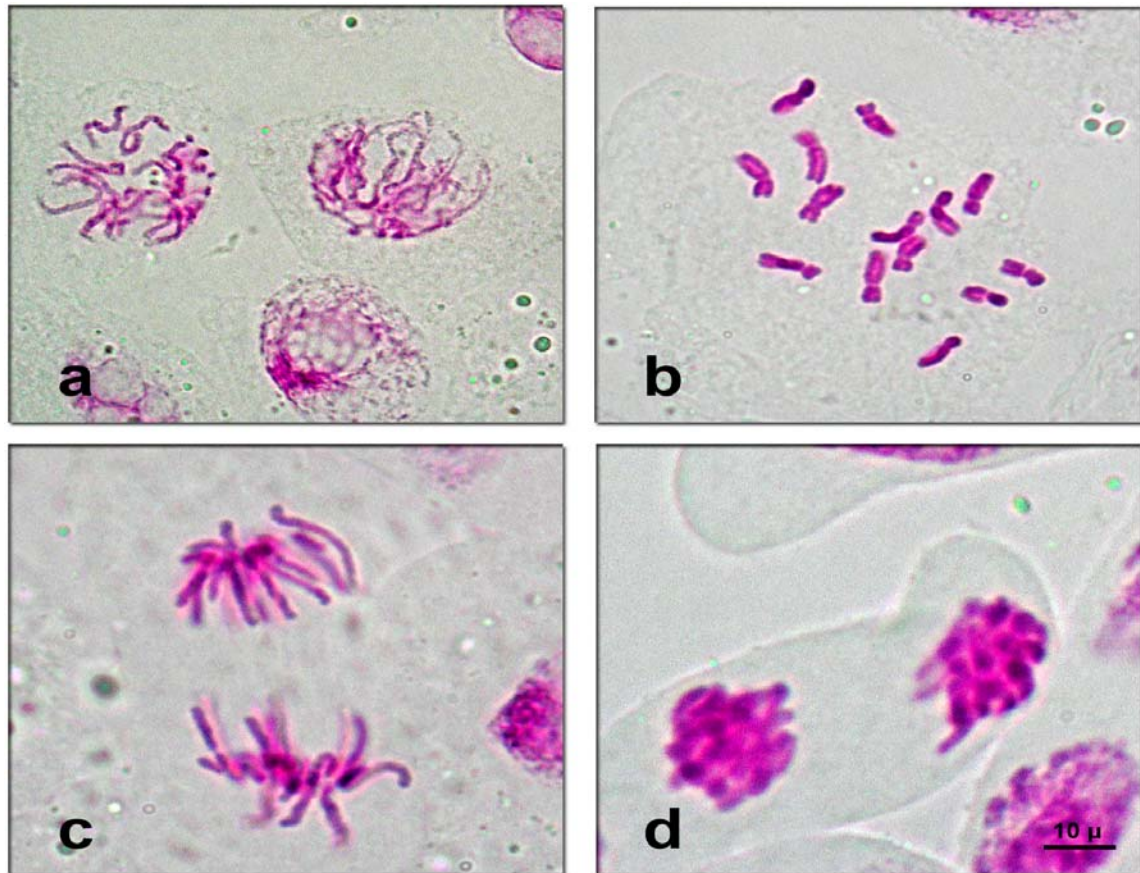


Fig. 3. Normal mitosis phases in root tips meristems of barley germinated in distilled water (control). (a) prophase; (b) metaphase $2n = 14$; (c) anaphase and (d) telophase. Scale bar = 10 μ m.

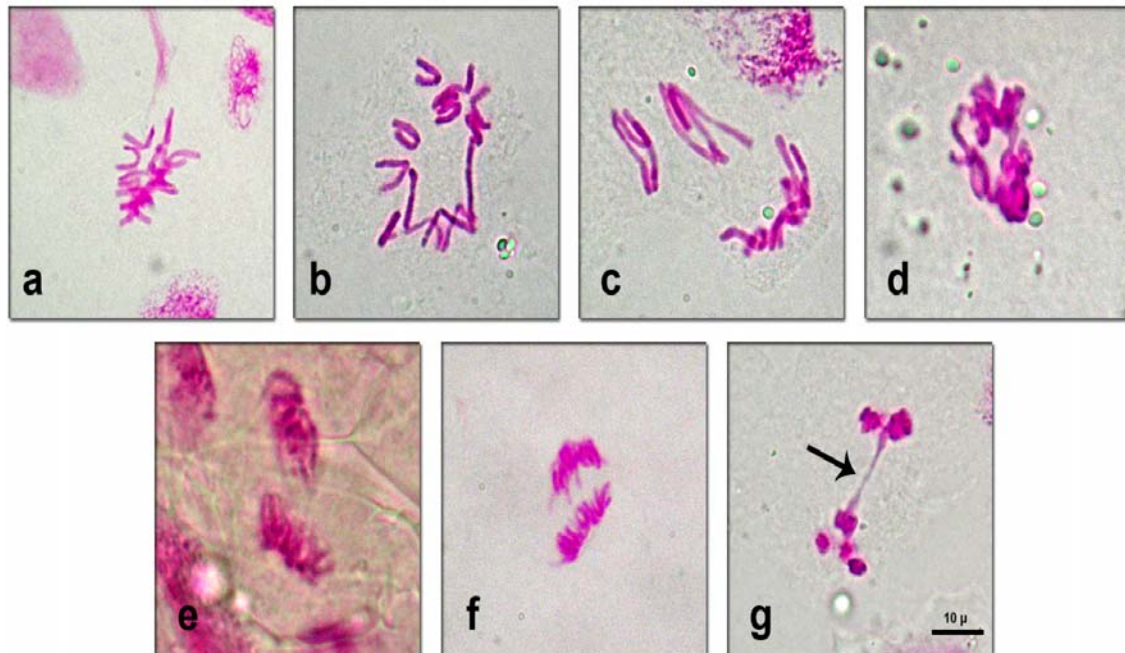


Fig. 4. Chromosomal aberrations in root tip meristems of barley after ABA alone and ABA-stimulators treatments. (a) irregular metaphase; (b, c) uncoiled chromosome; (d) adherent chromosome; (e) fault polarization in anaphase; (f) alignment anaphase; (g) adherent chromosome and bridge in telophase (arrow = bridge). Scale bar = 10 μ m.

In relation to ABA-stimulators antagonism on the mitotic activity and chromosomal aberrations, all of the stimulators studied exhibited noteworthy results. One of the common responses of cell to ABA is inhibition of cell division (Liu *et al.*, 1994). However, the amounts and concentrations of the stimulators used overcome excellently ABA-induced-inhibition of mitotic activity (Fig. 1). The most effective stimulators overcoming the mitotic activity-preventive effect of ABA were GA₃ and KIN (0.49), BA (0.45) EBR (0.31), respectively. PAs, TRIA and E, still under debate, showed also an important performance on this parameter. All available evidence suggested that all growth regulators used in present work is partly or completely found success in alleviating ABA inhibition on the mitotic activity of barley seeds. We can say that all stimulators studied in here could be regarded as the antagonists of ABA in cell division.

As for chromosomal aberrations, there is no extent literature data relating to detrimental effect of ABA-induced. In the present study, we determined that chromosomal aberrations of barley seeds germinated on the medium with ABA alone remarkably increased as compared to control (Fig. 2). These results are reported for the first time in this work.

As known, some growth regulators increase the cell distortion and chromosomal aberrations even at without stress conditions (Ünal *et al.*, 2002; İsmailoğlu *et al.*, 2004; Demir, 2007; Tabur & Demir, 2008, 2009, 2010; Kartal *et al.*, 2009; Mahajan & Sharma, 2009). In more recent studies, we have seen that exogenous PAs, BRs and TRIA lead to many chromosomal abnormalities. Therefore; it may be possible that these growth regulators completely did not respond to ABA. Some aberrations may also result from these stimulators.

Accurate chromosome segregation in mitosis requires that sister kinetochores attach to microtubules emanating from opposite spindle poles. Because kinetochore attachment is a stochastic process, it is error prone and can result in chromosome malorientation (Rieder & Salmon, 1998). Mitotic irregularities such as irregular metaphase, fault polarization, alignment anaphase and bridge may be mainly the result of these reasons or spindle dysfunction. Adherent chromosomes could be because of sub-chromatid linkage between chromosomes or chromosomes lose their movement abilities due to presence of ABA and some growth regulators. Therefore, they get stuck in anywhere and can not go to final destination. This is also explained as physical adhesion of the proteins of the chromosome (Patil & Bhat, 1992). Adherent chromosomes are accepted as an indicator of toxicity which results in cell death (El-Ghamery *et al.*, 2000). Anaphase bridges could happen during the translocation of the unequal chromatid exchange or due to dicentric chromosome presence (El-Ghamery *et al.*, 2000). The metaphase cells with uncoiled chromosomes may be the result of disorderly chromosome contractions. Shortly, ABA might be function as an inhibitor preventing the synthesis of protein necessary for the normal cell division and delaying mitotic cycle.

In this study, although most plant growth regulators used was able to remove to a great extent detrimental effect on chromosomes of ABA, but not completely, only

BA did not sufficiently success on this parameter. EBR, Cad, E and Put partly prospered in ameliorating of the negative effects of ABA on chromosomes. The most drastic and positive effect on chromosomal aberrations was obtained with GA₃, KIN, TRIA and Spm.

The plant growth regulators perfectly overcoming the inhibitive effect of ABA on both mitotic index and chromosomal aberrations was undoubtedly GA₃ and KIN.

Conclusion

Mitotic activity and chromosomes in root meristem of barley was adversely affected by exogenous ABA application. However, this negativity may be largely removed by plant growth regulators using convenient methods and techniques. The obtained results may serve to a new conceptual tool to dissolve many contradictions particularly in relation to effects of ABA and PGRs on mitotic activity and chromosomal aberrations. Further studies should be carried out in order to gain more knowledge about effect of ABA- stimulator antagonisms on molecular metabolism of cell division and cell cycle.

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