Role of β-carotene on alleviation of salt-induced stress in *Allium cepa* L.

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Abstract

Effects of β-carotene on the seedling growth (fresh weight, radicle length and radicle number), seed germination, chromosomal aberrations (CAs) and mitotic index (MI) in *Allium cepa* L. germinated in both normal and salt stress conditions were investigated in this study. In only β-carotene medium, the fresh weight and radicle number of the seedling were reduced partially compared to the control seedling grown in the distilled water medium. While their germination percentage indicated statistically the same values as the control, their radicle number was decreased according the control. Besides, the MI in the root tip meristematic cells of *Allium cepa* seeds germinated in alone β-carotene medium statistically showed the same value compared to the control seeds germinated in the distilled water medium, whereas the CAs exhibited increase significantly compared to the control. On the other hand, salt stress significantly inhibited the seedling growth and seed germination of *A. cepa*. What's more, it markedly reduced the MI in the root tip meristems of the seeds and increased the number of CAs. Nonetheless, inhibitive effects of salt on the MI, seedling growth, seed germination and CAs significantly decreased with the application of β-carotene.

Key words: *Allium cepa* L.; β-carotene, chromosomal aberrations; mitotic index; salt stress; seed germination; seedling growth

INTRODUCTION

Soil salinity was long before people and agriculture, but the current problem was worsened by agricultural practices such as irrigation. Almost half of all irrigated areas with around 20 % of the cultivated land in the world today are affected by salt (Rhoades and Loveday, 1990). In plants, high salt concentrations cause hyperosmotic stress, ion imbalance and secondary stresses (oxidative damage) often occur as a result of these primary effects (Zhu, 2001).

Carotenoids, are natural isoprenoid pigments including several aromas in plants, responsible for orange, red and yellow colors of plants, fungi, bacteria and animals are as well as a large group of fat-soluble natural pigments of other organisms and high plants (Just et al., 2007). The color depth symbolizes the increasing amount of carotene with maturity and an index of very good carotene content (Slinger & Bird, 1978). These compounds play an important role in plant reproduction with their role in attracting seed dispersants and pollinators. These pigments are second the most common pigments in nature and the first and basic function in plants is to contribute to photosynthesis (Polivka & Frank, 2010). The other is that they can protect plants overexposed to sunlight (Cazzonelli, 2011). Some plant physiologists believe that carotenoids as regulators of certain developmental responses may have an additional function in plants. Carotenoids are not only natural food colorants because of their pro-vitamin and antioxidant activities that provide additional value to the target products, but also use as phar-

maceutical (Benítez-García et al., 2014). Moreover, intake of carotenoid-rich products plays important role in health and human nutrition, particularly in cancer and cardiovascular diseases (Diretto et al., 2007). Carotenoids commercially are important in health, food, the cosmetic industries and agriculture (Benítez-García et al., 2014). Carotene's anti-aging effect is due to its effect on free radicals (Leung, 1980).

Nutraceutical industry produces synthetically 5 carotenoids on an industrial scale (e.g. β-carotene, lycopene) as feed additives for fish, livestock, crustaceans and poultry use in various cosmetic and food products such as health products, vitamin supplements (Jackson et al., 2008). Carotenes does not have intrinsic vitamin A activity but convert into vitamin A by enzymatic movements that take place in the intestinal mucosa and liver (Morales-González, 2009). The body converts β-carotene into fat soluble vitamin A, which is available from natural (broccoli, sliced carrots, fresh apricots, cantaloupe, asparagus, raw endive, mustard greens, kale, watermelon, leaf lettuce, liver, spinach, sweet potatoes, winter squash, pumpkin) and synthetic sources Winter-Griffith (1988) states that β-carotene is a pre-vitamin A compound found in plants. The anti-infective effect of β-carotene may be due to the activity of provitamin A, which is due to the fact that vitamin A has an important role in the immune function preservation. In addition, β-carotene might has an independent effect(s) on immune responses apart from pro-vitamin A activity (Burton & Ingold, 1984). There are some studies suggesting that β-carotene added in the diet has shown some evidence of antitumor action (Allende-Martínez, 1997).

The *Allium* test, which was applied in this study, has been used for a long time to investigate the genotoxicity of many compounds (Ateeq et al., 2002) and it's usableness in studying the mutagenicity of different compounds is unquestionable. The test an excellent tool for easy, rapid and reliable evaluation of disturbances that occur during cell divisions and by inter alia (Majewska et al., 2002). Furthermore, the importance of the *Allium* test is supported by the very similar of the mutagenic activity of numerous compounds on mammalian cells and *Allium* test cells (Fiskesjö, 1979). For this reason, *A. cepa* was used as a test material in this study. There is no study on the effects of β-carotene on the seedlings growth, seed germination, chromosomal aberrations, mitotic activity under saline and normal conditions. For this reason, this study was carried out to reveal the first time whether β-carotene affected these parameters at normal and salinity conditions. Therefore, the results of this study are important especially for the first time.

MATERIALS AND METHODS

The seed, β-carotene, and salt concentrations 3-4 cm in diameter small onion (2n=16, *Allium cepa* L.) were used as test material in the assay. By a preliminary investigation carried out in the present study, β-carotene concentration was determined as 300 mg/L and salt (NaCl) concentration was determined as 0.175 M.

Germination of the seeds

The assay was carried out with *A. cepa* seeds, that are physiologically homogeneous. Onion seeds were germinated at incubated at 20˚C in the darkness and were surface-disinfected in 2.5 % sodium hypochloride solution for ten minutes then, washed with in ultra-distilled water for 24 hours. For germination, 20 seeds from each treatment group were placed in 1700-mL plastic boxes. For 7 consecutive days, boxes with seedlings were divided into 4 groups: The seeds in the control (Group I) were germinated in distilled water, Group II, Group III and Group IV as application groups were treated with alone 0.175 M NaCl, 300 mg/L dose of β-carotene and 0.175 M NaCl + 300 mg/L dose of β-carotene, respectively.

Plastic boxes were germinated at incubated. When the roots reached about ten cm in length (approximately 7 days after the beginning of the assay), their radicle numbers, germination percentages were determined. The radicle lengths measurements were made in mm and the fresh weights in g/seedling were determined.

Cytological analysis

For cytological and physiological studies, root tips were excised after a few days. Cytological preparations pretreated with saturated para-dichlorobenzene for 4 hrs, carried out by fixation of roots in a mixture of ethanol-glacial acetic acid (3-1) overnight at room temperature. Then, they stored at 4°C

in 70 % ethanol until used. Microscopic slides made permanent by mounting in balsame. After cold-hydrolysis in 5 N HCl for 40 min, root tips (1 mm) were washed, were stained with Feulgen, squashed and smashed in a drop acetic acid of 45 %. Mitotic index was expressed in percentage by counting cells of different mitotic phases in total number of cells. Mitotic phases were also expressed as percentage of total number of cells. The mitotic index was calculated by means of this formula, 2,000 cells (three slides = 6,000 total cells).

Mitotic index (%) = Number of cells in mitosis × 100 / total number of cells

Chromosomal aberrations also were recorded. Counted cells in each application groups were determined under an Olympus CX41 microscope and photographed at X500 magnification.

Statistical analysis

The statistical calculations were done using SPSS program according to DMRT in triplicate at level of significance $P < 0.05$.

RESULTS

Effects of β-carotene on the seedling growth and seed germination

Table 1 results clearly demonstrated that while the germination percentage of group III seeds statistically showed the same value as group I (control) seeds, their fresh weight and radicle number decreased partly according to group I seedlings. In addition, their radicle length increased compared to group I seedlings.

NaCl showed an inhibitory effects in all growth parameters examined. For instance, the control (group I) seeds germinated in distilled water after 7 days showed germination 100 %, whereas this value was 23 % in group II seeds germinated at 0.175 M salinity. That is to say, NaCl prevented 77 % *Allium* seed germination. The inhibitive effect of NaCl stress on the seed germination markedly mitigated by β-carotene application. Group IV seeds treated with β-carotene at the mentioned salt level showed 77 % germination (Fig. 1). In addition, β-carotene continued its success on the seedling growth parameters such as the fresh weight, radicle number and radicle length. The radicle number, radicle length and fresh weight of group II seedlings grown in 0.175 M salted were 12.7, 10.3 mm and 7.0 g, respectively while these values became 24.0, 12.8 mm and 11.0 g in group IV seedlings (Tab. 1).

Effects of β-carotene on the chromosomal aberrations and mitotic activity

Exposure to 0.175 M salt revealed significant inhibition of the MI and induction of the CAs. That is to say, the MI in *A. cepa* root tip meristems germinated in containing 0.175 M salt media showed a 90

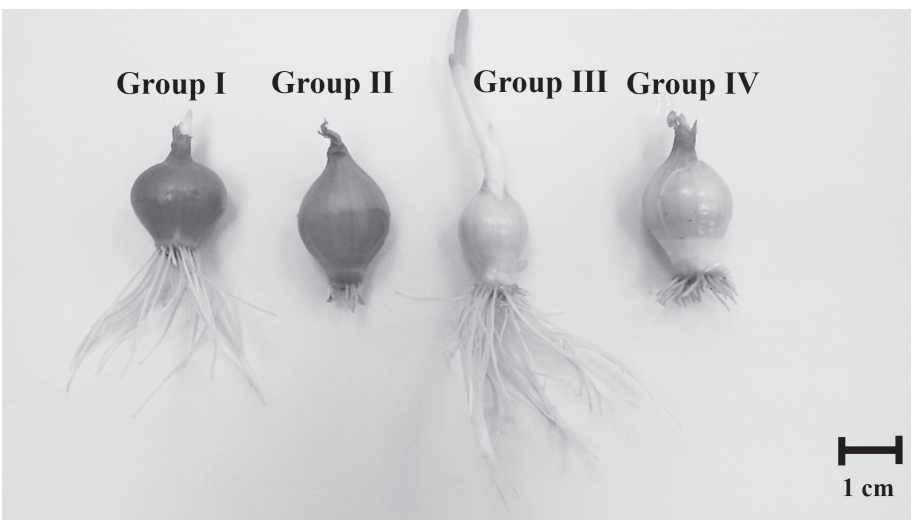

Figure 1. Root tip cells of *Allium cepa* showing germination situations at the end of 7 day.
Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 300 mg L^{-1} β-carotene and Group IV: 300 mg L^{-1} β-carotene + 0.175 M NaCl. Scale bar = 1 cm

% reduction compared to the control (group I) seeds and the mitotic aberration frequency increased significantly. The MI of group III seeds germinated in only β-carotene medium was remained the same compared to group I (control) samples. This application increased the CAs (Tab. 2). Simultaneous β-carotene+NaCl treatment (group IV) may be successful in improving reverse effects of salt on

Table 1. Effect of β-carotene on some growth parameters of *Allium cepa* L.

Groups	Growth parameters			
	Germination percentage (%)	Radicle length (mm)	Radicle number	Fresh weight (g/seedling)
Group I	*100 ± 0.0^{c}	63.5 ± 0.5^{c}	63.2 ± 0.6^{d}	14.2 ± 0.8^{c}
Group II	23 ± 2.8^{a}	10.3 ± 0.3^{a}	12.7 ± 0.5^{a}	7.0 ± 0.5^{a}
Group III	100 ± 0.0^{c}	69.2 ± 0.8^{d}	52.5 ± 0.2^{c}	12.2 ± 1.0^{b}
Group IV	77 ± 2.8^{b}	12.8 ± 0.1^{b}	24.0 ± 0.7^{b}	11.0 ± 0.3^{b}

* The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 300 mg L^{-1} β-carotene and Group IV: 300 mg L^{-1} β-carotene+0.175 M NaCl.

Table 2. Effect of β-carotene on some cytogenetic parameters of *Allium cepa* L.

Groups	Mitotic index (%)	Chromosome aberration (%)
Group I	*11.6 ± 1.0^{c}	0.0 ± 0.0^{a}
Group II	1.2 ± 0.2^{a}	17.0 ± 0.4^{d}
Group III	13.0 ± 0.8^{c}	3.1 ± 0.3^{b}
Group IV	8.2 ± 0.8^{b}	10.8 ± 0.3^{c}

* The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 300 mg L^{-1} β-carotene and Group IV: 300 mg L^{-1} β-carotene + 0.175 M NaCl.

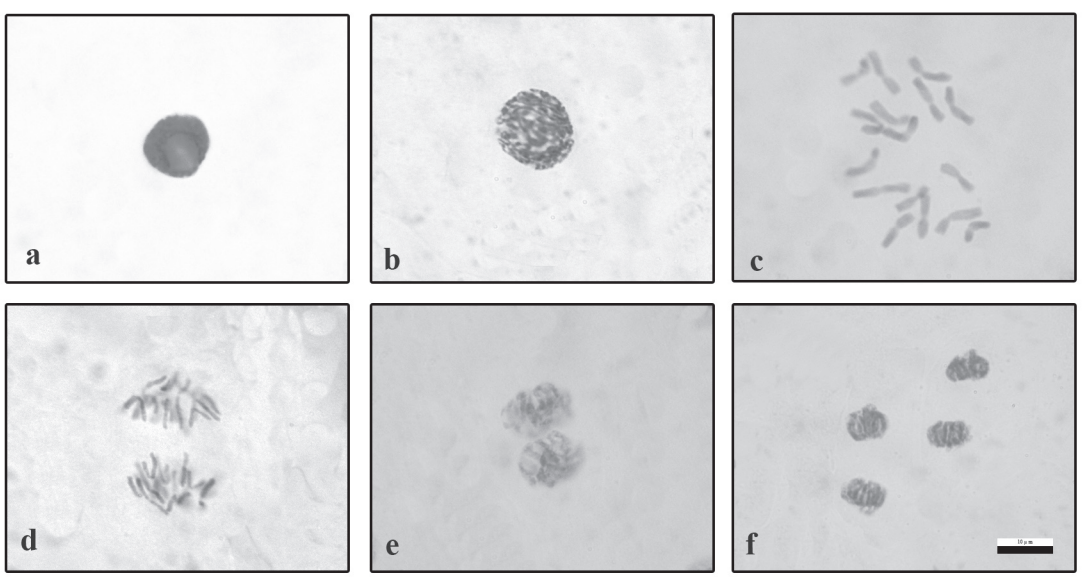

Figure 2. Normal mitosis phases in *A. cepa* root tip meristematic cells Scale bar = 10 μm a- interphase b- prophase c- 2n = 16 metaphase d- anaphase e- early telophase f- telophases.

the CAs and MI. In the same time, simultaneous β-carotene+NaCl application (group IV) couldn't be succeeded in ameliorating the harmful effects of salt on the CAs, if also it can't seriously be until alone β-carotene. Statistically, all values mentioned here are highly significant. Table 2 summarizes all cytogenetic parameters obtained from the control and other treated seeds.

The normal mitotic phases observed during the microscopic examination of root tip meristematic cells of *A. cepa* are shown in Fig. 2 and abnormal mitosis phases are shown in Fig. 3. The chromosomal aberrations were majorly noticed anaphases with chromosome loss and ring chromosomes in this study. Other abnormalities observed in the cells are as follows: binucleolars, disturbed anaphases, bridges in anaphase, metaphase with lagging chromosomes, pole to pole arrangement with chromosomes, go into division of metaphase plate, chromosome loops, breakage in metaphase, nuclear peak.

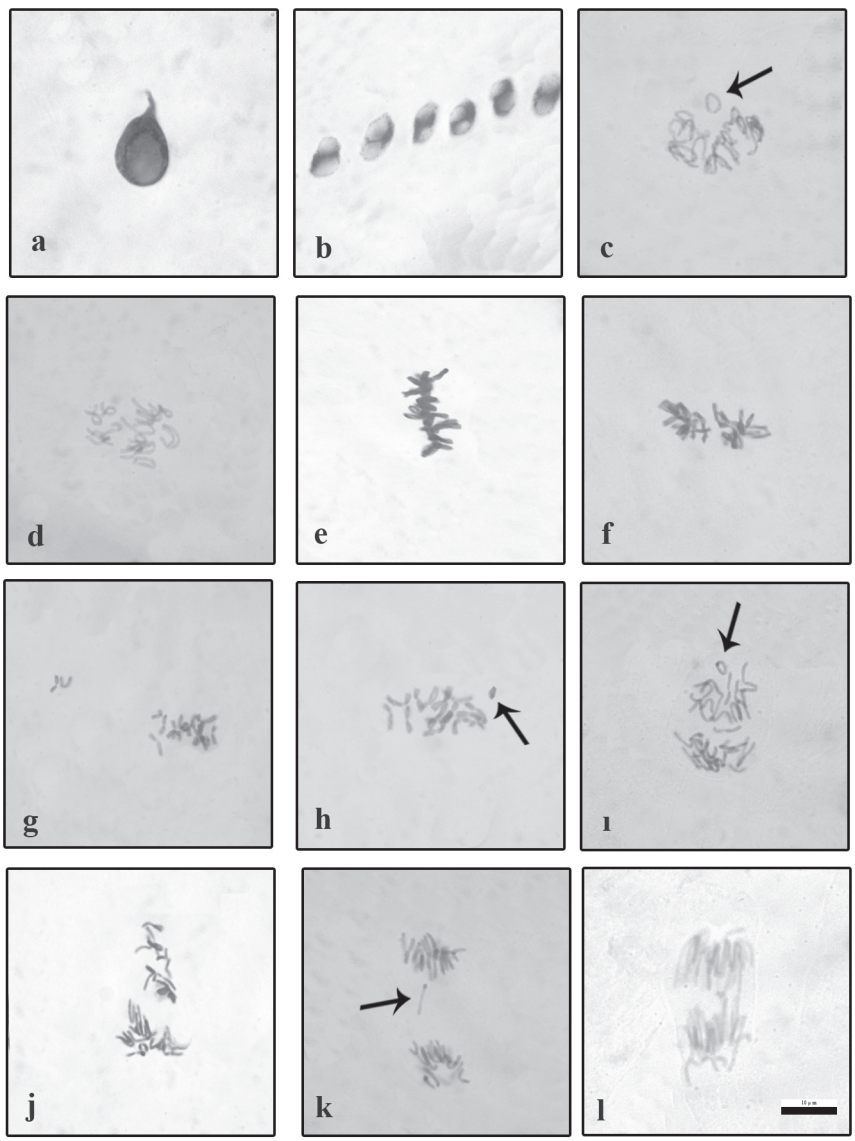

Figure 3. Chromosomal aberrations; a: nuclear peak b: binucleolars c: ring chromosome d: chromosome loops e: pole to pole arrangement with chromosomes f: go into division of metaphase plate g: metaphase with lagging chromosomes h: breakage in metaphase ı: anaphase with a ring chromosome j: disturbed anaphase k: anaphase with chromosome loss l: bridges in anaphase (Scale bar = 10 μm)

DISCUSSION

Physiological and cytogenetical effects of β-carotene under normal conditions

Unless there generally are stress conditions, there is no need for exogenously add any plant growth regulator during germination. Exogenously addition of a plant growth regulator in non-stress conditions can cause negative or positive effect on the seed germination and seedling growth (Çavuşoğlu et al., 2017; 2019). But, there is a few study about the effects of β-carotene on the seedling growth and seed germination under normal conditions. Therefore, the effects of β-carotene application on the mitotic activity, seedling growth, chromosomal aberrations and seed germination under normal conditions requested to be tested in the laboratory study. The results of this study demonstrated that the fresh weight and radicle number of the seedlings grown in only β-carotene medium were partially reduced but the radicle length of the seedlings increased compared to those of the control seedlings grown in distilled water. In addition, the germination percentages of the mentioned seeds statistically showed the same value as the control seeds (Table 1). Udengwu (2012) determinated that 200μM β-carotene application increased the germination percentage, fresh weight and radicle length of *Amaranthus hybridus* under normal conditions. However, these results aren't consistent with this present research findings, so β-carotene can have different effects on the seedling growth and seed germination depending on application method used, concentration and plant species.

Furthermore, some growth regulators may cause particularly cell disortions, chromosomal aberrations and mitotic irregularities especially if stress conditions aren't present (Çavuşoğlu, 2020a; 2020b). There is no extent study relating to the effects of β-carotene on the chromosomal aberrations and mitotic activity subject to normal conditions. For this reason, this study was examined the first time whether β-carotene affected these parameters at normal conditions. The findings of this study stated that MI in the root tip meristematic cells of *Allium cepa* seeds germinated in alone β-carotene medium showed the same value as the control seeds germinated in distilled water medium, whereas the CAs frequency exhibited an important increase according to the control (Table 2). In this case, some abnormalities can be said to be caused by this stimulator.

Physiological and cytogenetical effects of β-carotene under saline conditions

Zhu (2002) reported that saltinity stress is known to have severe effects on plant development and growth. Salt stress occurs due to evapotranspiration, which is usually causing an increase in the ground water formed by salinity. Growth of plant severely is affected if plant is exposed to more salt than other stresses in the natural environment. Salt effects development and growth of the plant by impairing certain biochemical and physiological processes such as phytohormone levels water-nutrient imbalance, protein synthesis, photosynthesis (Kuiper et al., 1990).

The results from table 1 clearly demonstrated that as expected, the seedling growth and germination of *A.* seeds inhibited under saline medium. Salinity stress can be preventive in many ways. Seed germination can be prevented by causing to change in water situation of the seed, thus prevent water intake (Ali, 2000). Results of the present study displaying the diminish in the fresh weight and water content of the seedlings in salted conditions can be explained by the inability of roots to receive enough water due to high of osmotic pressure in medium. Salt inhibitive effect on the radicle number, fresh weight and radicle length may result from reducing protein synthesis, nucleic acid and cell division (Mccue & Hanson, 1990).

On the contrary, β-carotene application significantly eliminated the inhibitory effect of salt stress on the seedling growth, seed germination, fresh weight and radicle number (Tab. 1). Unfortunately, to date, there isn't extant literatüre data relating to effects of β-carotene on the seedling growth and seed germination exposed to saline conditions. β-carotene alleviates salt stress on the seedling growth and seed germination can be understood from the decrease in the salt's osmotic effects. For example, at 0.175 M saline medium, β-carotene application partly increased the fresh weight of seedlings compared to the control indicates this probability. Moreover, it reduced the preventive effect of salt on the seed germination and seedling growth by stimulating mitotic activity of the embryo (Tab. 2).

Effects of the determined concentrations of a test chemical on the chromosome aberration and mitotic index are used respectively as parameters of cytotoxicity and genotoxicity (Nefic et al., 2013). The cytotoxic and inhibitory effects of salt stress on the mitotic activity have long been known. A high concentration of salt causes total inhibition

of the chromosomal abnormalities and mitotic activity in root-tip cells according to some researchers (Radic et al., 2005). With this study, it should be noted that salinity affects the mitotic activity and chromosome behaviors negatively in *A. cepa* root-meristem cells. Data of this study stated that salt showed a greater number of the chromosomal abnormalities compared to controls and reduced the mitotic index by 90 % and this reduced was achieved by reducing the number of cells entering mitotic division. For example, the chromosomal aberration frequency in the root-tip mitotic cells of the seeds germinated exposed to distilled water was 0.0 % while it was 17.0 % at 0.175 M saline (Tab. 2). Besides, simultaneous β-carotene+NaCl application can be successful in alleviating of the detrimental effect of saline on the chromosome aberration and mitotic activity. Limit of mitotic inhibition by this treatment reached to 8.2 %. So, the frequency of CA with the application of simultaneous β-carotene+NaCl decreased by 36 %. This result exhibited β-carotene repair role against salt injuries during *A. cepa*'s mitosis. Shortly, β-carotene may be function as a stimulator protein synthesis triggering required for the normal cell division and accelerate the mitotic cycle.

Chromosome aberration is a change in chromosomal material or exchange in the structure of the chromosome resulting from breakage. CA induction could affect the fertility, vigour, competitive or yield ability of the exposed plants (Kara et al., 1994). Anaphases with chromosome loss and ring chromosomes were the most common abnormalities in the present study. Several abnormalities have been observed as a result of the structural deviations in the chromosomes at all stages of the mitotic division. Mitotic spindle formation failure may cause chromosome loss (Fig. 3c, ı) when they can not bind to the spindle and therefore are not separated (Gisselsson et al., 2004). Abnormal pole to pole arrangement with chromosomes (Fig. 3e) in metaphase as a result of the equatorial seperation of the chromosomes in anaphase is an acute abnormal condition that occurs as a result of abnormal spindle assembly and irregular pathways of the spindle mechanism (Waters & Salmon, 1997). Break (Fig. 3h) is indicator of a clastogenic action (Leme & Marin-Morales, 2009). Disturbed anaphases (Fig. 3j) might be due to spindle apparatus disturbance which allows that the chromosomes to spread irregularly over the cell (Amer & Alı, 1974). Lagging chromosomes is direct results of breaks and fragmentation, which lead to the centromere losses and stopping of their movement (Gari et al., 1998). The loss of chromosomes (Fig. 3k) is typically associated with mitotic spindle malfunction (Leme & Marin-Morales, 2009). The bridges (Fig. 3l) are probably caused by joining and interruption to chromatids or chromosomes (Türkoğlu, 2007). Ring chromosomes (Fig. 1r) can spontaneously occur after breakage of the chromosomal ends and after the joining of the raw ends of the chromosomes (Singh, 2003).

CONCLUSIONS

There are no literature data on the effects of β-carotene application under salted conditions on the cytogenetical and physiological parameters examined in this study. Therefore, this study results have been reported for the first time particularly in saline conditions. As a result, this study showed that β-carotene can significantly increase the activations such as the seedling growth and seed germination, either alone or in saline conditions. But the mechanisms by which salt inhibits growth are controversial and complex, they might also vary according to cultivar and species. An universal mechanism hasn't been established yet. While the causes of salty have been determined, it is still very poor to understand the mechanisms by which salty prevents plant growth. Therefore further work should be done to learn more about the effect of β-carotene on the cell division, cell cycle and germination molecular metabolism. This literature study can serve to present new conceptual tools for designing salt tolerance hypotheses in plants.

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