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Comparative studies between growth regulators and nanoparticles on growth and mitotic index of pea plants under salinity

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To our knowledge, no research study has been carried out on the effects of ascorbic acid (ASA), 5-Aminolevulinic acid (ALA) and Nano selenium (N-Se) on the cytological parameters of pea seedlings under salinity stress. Salinity treatment (60 and 120 mM NaCl) was applied. Two concentrations of ASA (50 and 100 ppm), ALA (25 and 50 ppm), and N-Se (10 and 20 ppm), respectively were used individually and in combination with NaCl (60 and 120 mM). Modifications in shoot length, number of leaves, leaf area, chromosomal aberrations and mitotic index were determined. Salinity treatment (120 mM) caused the highest reduction in shoot length, leaf area and mitotic index. A significant increase of chromosomal abnormalities percentage (%) was detected in salinity treatments compared with control. ASA (100 ppm), ALA (50 ppm) and N-Se (10 ppm) treatments significantly reduced the damaging effect of salinity stress on growth attributes, mitotic index and chromosomal abnormalities percentage (%) and improved seedlings' performance. These treatments can be recommended for the improvement of pea plants' productivity under salt stress.

Key words: Ascorbic acid, 5-aminolevulinic acid, nano selenium, salt stress, mitosis, chromosomal aberrations, *Pissum sativum* L.

INTRODUCTION

Salt stress adversely affects the morphological, physiological and biochemical responses of plant species (Nazar et al., 2011). Several researchers found that the chlorophyllian pigments were reduced with an increase in salinity level. This may be due to the disruption of the fine structure of chloroplasts and pigment-protein complex or chlorophyll stability, which can result in chlorophyll oxidation (Saha et al., 2010; Helaly et al., 2016; Elsheery et al., 2020c) and disturb plant growth and development (Sairam and Tyagi, 2004). Tang et al. (2017b) established that salinity inhibits plant growth, reduces yield in many crop plants and affects their commercial value (Helaly et al., 2016; Elsheery et al., 2020c). So, salinity stress inhibits growth of basil plants by

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decreasing a significant number of leaves/plant and plant height (Khan et al., 2009; Nassar et al., 2019). Also, the retardant effects of salinity stress on growth, physiological aspects and productivity were also recorded on other different plants species; for instance, Reda (2007) on *Senna occidentalis*, Dawood et al. (2014) on Faba bean, Bargaz et al. (2016) on *Phaseolus vulgaris*, Nassar et al. (2016) on Leucaena and Elsheery et al. (2020b) on mango. There are many ways to improve salinity tolerance in plants such as using of biofertilizer and amino acids (Helaly et al., 2016) and grafting in vegetable crops (Elsheery et al., 2020a; Helaly et al., 2016; Al-Mayahi, 2016). This study was carried out to investigate the effects of ascorbic acid (ASA) under salinity stress on growth of pea plant. Some biochemical constituents that can promote growth and increase productivity of many species of plants grown under normal or abiotic stress conditions are highly recommended (Sharma et al., 2019). Ascorbic acid (ASA) is a small water soluble antioxidant molecule which acts as an essential substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide. Ascorbic acid (ASA) is a naturalist product that acts as an antioxidant and enzyme and also improves cofactor. It engages in a variety of procedures. It correlates with chloroplasts in the oxidative stress of photosynthesis (Latif et al., 2016). Furthermore, ASA has a number of roles in protein modification and cell division in plant cells (Hussein et al., 2019). Nowadays, it plays an essential role in a series of physiological processes such as cofactor of key enzyme, plant defense against oxidization, growth, development, cell division, cell extension, senescence and counteracts the deleterious effects of biotic and abiotic stresses (Zhang and Sonnewald, 2017). Therefore, it is chosen to be one of the substances of the subject of our present study.

5-Aminolevulinic acid (ALA) is a type of non-protein amino acid that supports plant stress tolerance. However, the underlying physiological and biochemical mechanisms are not entirely understood (Anwar et al., 2020). ALA is found in all plants and animals. 5-aminolevulinic acid (ALA) is and a key precursor for the biosynthesis of porphyrins such as chlorophyll, heme and plant hormones. In addition, it has newly been reported that ALA regulates the expression level of fructose-1, 6-bisphosphatase (FBP), triose-3-phosphate isomerase (TPI), and ribulose-1, 5-bisphosphate carboxylase/ oxygenase small subunit (RBCS), which activate the Calvin cycle of photosynthesis under drought stress (Liu et al., 2016). It was found that, ALA is one of plant growth regulators (PGRs) and mitigates salinity stress effect in germinating seeds and ameliorates seedling growth. Foliar application of 5-aminolevulinic acid at low concentrations has been shown to promote salt tolerance in a lot of plants (Tang et al., 2017a). On the other hand, ALA is involved in the chlorophyll biosynthesis pathway under salt stress conditions (Wu et al., 2011) and motivates antioxidant enzyme efficiency and accumulation of endogenous hormone under many stress factors such as low-temperature in cucumber seedlings (Anwar et al., 2018). Under drought stress, spray application of ALA up-regulated the chlorophyll fluorescence indexes in oilseed rape (*Brassica napus* L.) (Liu et al., 2014) and gas exchange indexes, such as net photosynthetic average (Pn), stomatal behavior (gs), intercellular CO_2 concentration (Ci) and the rate of transpiration (Tr), which were adversely influenced by abiotic stress (Wu et al., 2018). It is also reported that foliar application of ALA may confer plant tolerance to diverse abiotic stresses, such as chilling, high temperature, salinity, drought, weak light, and heavy metals (Wu et al., 2019a). Previous studies demonstrated that ALA encourages abiotic stress tolerance by activation of numerous types of transcription factors, signal transduction, and chlorophyll and carbohydrate biosynthesis (Nishihara et al., 2003; Anwar et al., 2020). These results submit that ALA can broadly minimize the harmful effects of environmental stress. Increasing attention has been paid to the beneficial impacts of many nanoparticles (NPs) used in low doses on diverse crops (Jampílek and Kráľová, 2017; Rastogi et al., 2019; Kumar et al., 2020; Elsheery et al., 2020c). A lot of researchers like Sonkaria et al. (2012) and Prasad et al. (2014) established that, using of NPs can promote plant growth, warrant food goodness and decrease waste. Nano-Selenium (N-Se) as Nano fertilizer has been recently used in the field (Shang et al., 2019; Elsheery et al., 2020a; Elsheery et al., 2020b). There is less documented information on the biological effects of N-Se and its application (Chau et al., 2007; Cushen et al., 2012). Bhattacharjee et al. (2014) and Kamle et al. (2020) suggest that N-Se plays a role as a reactive oxygen species (ROS) scavenger in plants under stress conditions. So, the purpose of our study was: To evaluate the (ASA, ALA and N-Se) morphological and cytological effect of application of our treatments (Soaked and foliar) on pea plants under salinity stress using hydroponic methods.

MATERIALS AND METHODS

Plant material

The pea variety used in this study was obtained from El Korma Company, Egypt for seeds import. The experiment was carried out at the greenhouse of the Agriculture Botany Department, Faculty of Agriculture, Tanta University, Egypt, during winter of the two growing seasons of 2018 and 2019. In both growing seasons, the average of the daily temperature ranged between 11 and 26°C and relative humidity between 60 and 65%.

Hydroponic experiment for evaluating responses of tested cultivar of pea to salt stress

The seeds were washed and soaked for 6 h before they were

planted in the treatment solutions [ASA (50, 100 ppm), ALA (25, 50 ppm) and N-se (10, 20) ppm]. Then they germinated in polyethylene bags (8 seeds per bag) filled with washed sand on a half-strength Hoagland,s nutrient solution used as macronutrient sources (Hoagland and Arnon, 1950). Then, the bags were placed in dishes containing 1 L of Hoagland,s solution (pH 5.8) in greenhouse at a temperature range of 20 to 26° during the day and 11 to 16° during the night. Nutrient solutions were added every day and renewed every 3 days. Four replicates (each with 10 polyethylene bags containing 8 plants) were planted. After the emergence of the first real leaves (15 days after germination), the number of plants per bag was adjusted to five, by thinning out weak and less vigorous ones. Seedlings were exposed to two levels of salinized water (salt mixture of 60 and 120 mM) of a mixture salts. When the 4th true leaf emerged, foliar spraying was done twice (after 30 and 33 days from sowing) at the same concentrations [ASA (50,100 ppm), ALA (25, 50 ppm), N-Se (10, 20 ppm)]. Foliar treatments were not applied on nine pots: The first 3 pots were treated with saline; the second pot was treated with a salt mixture of 60 mM and the third was treated with a salt mixture of 120 mM.

The plants were collected after seven days of foliar application and their morphological parameters were recorded.

Growth parameters

The following parameters were recorded: Shoot length (cm), number of leaves and leaf area per plant (cm^2) using the formula:

Leaf area /plant with weight method (cm^2/plant) = B=L*S/ Z

B = Green leafy area on one plant; S = Circle space for tablets; L = Total weight of the leaves on the plant; Z = Weight of tablets.

For shoot length from each treatment, shoots of 10 pea plants were separated from roots; they were washed using distilled water and dried carefully with wish tissue paper. The number of leaves per plant was counted.

Cytological parameters

For mitotic screening physiologically, uniform and healthy seeds of *Pisum sativum* L. were used to study the effect of ASA, ALA and N-Se on the growth of pea plants under salinity stress conditions (Darlington, 1976). The dividing cells were observed and recorded. Cells were examined under a light microscope for mitotic index, numbers and types of abnormalities. At least 3000 cells were examined per treatment (1000 cell/replicate). Mitotic index (MI) and percentage of abnormal cells were calculated using the following formulas:

$$\text{Percentage of abnormal cells} = \frac{\text{Total abnormal cells}}{\text{Total dividing cells}} \times 100$$

$$\text{Mitotic index (MI)} = \frac{\text{Total dividing cells}}{\text{Total dividing and non dividing cells}} \times 100$$

RESULTS AND DISCUSSION

Growth parameters

Plant height (shoot length), fresh and dry biomass of shoot and root per pea plant, number of leaves and leaf area per plant were affected by salt stress levels. ASA, ALA, and N-Se soaking and foliar application improved the plant's tolerance to salt stress (Table 1).

The data presented show that increased salt levels induced significant (mean value for two seasons) stress which resulted in a significant reduction of all growth parameters compared to control. ALA, ASA and N-Se treatments reduced the harmful effect of salinity on plants treated compared with non-treated plants.

Salinity is an essential environmental factor which curbs crop plants from attaining their full genetic potential; therefore, salt stress in pea plant induces a lot of growth limitation (Gama et al., 2007). Pea plant subjected to higher salt will experience delay in growth which could be attributed to the inhibition of cell elongation (Taïbi et al., 2013). These results are consistent with those obtained on rice by Yu et al. (2019), on flax by Wu et al. (2019b) or other species.

The inhibitory effects of salt stress on pea plant and the reduction in dry mass might be due to the toxic effect of salt stress as a result of high osmotic pressure. Salinity stress causes a significant increase of growth inhibitors and decrease of growth promoters. Disturbance of water in plants grown under salinity restricts absorption or plants are not able to uptake the water and nutrients required by them (Memon et al., 2010) (Colla et al., 2006a, b).

A similar tendency was observed by Shi et al. (2013). The suppression effects of salinity lead to disturbance in ionic homeostasis, stomatal closure, reduction in photosynthesis, accumulation of toxic ions (Na^+, Cl^-) which restricted the absorption of water (Shahzad et al., 2020; Kamran et al., 2020) and inhibited growth and productivity. The same trend was observed by Nassar et al. (2019) and Bargaz et al. (2016).

The results presented in Table 1 show that all growth characteristics of "leaf area, shoot height and number of leaves" decreased significantly with increased salt stress level.

Leaf area

Data indicate that the leaf area has been significantly affected by different salinity concentrations (Table 1). Soaking and foliar applications significantly increased the leaf area under salinized and non-salinized conditions ($P < 0.05$).

Under salinity level of 120 mM, the highest increase in leaf area was with ASA (100 ppm) (99.96 cm^2) followed by ALA (50 ppm) (96.71 cm). In contrast, the lowest amounts of leaf area recorded using N-Se (20 ppm) (62.50 cm^2) were 99.96, 96.71 and 62.50 cm^2, respectively, compared with control plants.

Foliar application of ASA and ALA increased all previous parameters, improved plants' tolerance to NaCl toxicity and minimized reduction in growth caused by

Table 1. Effect of ASA, ALA and N-Se soaking and foliar application on growth parameters of field pea plant grown under different salt stress levels.

Salinity level (mM NaCl)	Treatments	Concentration (ppm)	Leaf area (cm^2)	Shoot length (cm^2)	No. of leaves
Control 0 mM NaCl	Control		131.07	28.96	6.00
	ASA	50	135.33	29.64	7.00
		100	168.26	38.81	9.00
	ALA	25	132.77	30.61	7.00
		50	140.04	35.66	8.00
	N-Se	10	137.50	32.70	7.00
		20	137.18	29.24	6.33
60 mM NaCl	Control		97.08	22.73	6.00
	ASA	50	133.52	29.03	7.00
		100	167.14	35.82	8.00
	ALA	25	99.98	27.95	7.00
		50	138.57	34.68	7.00
	N-Se	10	137.01	30.84	7.00
		20	122.33	26.45	6.00
120 mM NaCl	Control		49.84	17.75	5.00
	ASA	50	68.11	25.34	6.00
		100	99.96	32.69	7.00
	ALA	25	58.64	24.79	6.00
		50	96.71	28.00	6.00
	N-Se	10	85.39	27.24	6.00
		20	62.50	22.39	5.00
L.S.D. (0.05)					
Salinity			1.491**	0.403**	0.540**
Treatment			2.277**	0.616**	0.825**
Salinity x Treatment			3.944**	1.067**	0.040**

NaCl. Meanwhile, it is evident that ASA and ALA play a vital role in the regulation of a number of metabolic processes in plants exposed to salinity. It has been concluded that, the typical effect of salt stress on plants is growth retardation due to the inhibition of cell elongation (Hanafy et al., 2013).

ALA is one of the existing PGRs used for the improvement of plants' stress tolerance (Hotta et al., 1997; Naeem et al., 2012) and an essential precursor for the biosynthesis of tetrapyrroles such as heme and chlorophyll. It was found that ALA is formed in all animals and plants. Recently, it was found that low concentrations of ALA had a promotive effect on growth and yield of several crops and vegetables (Watanabe et al., 2000).

Ascorbic acid is an essential main metabolite in plants that is utilized as an enzyme cofactor, an antioxidant and a cell signaling modulator in crucial physiological processes, in biosynthesis of the cell wall, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth (Elkelish et al., 2020).

Selenium spraying treatment had a considerable positive effect on all studied characteristics such as plant height, number of leaves, fresh weight of shoots and roots, dry weight of leaves and shoots and chlorophyll content. It could be proved that foliar application of nano selenium at 10 and 20 ppm increased vegetative growth, yield and quality as well as mineral contents in leaves of pea plants. Furthermore, the best selenium used as a foliar spray is the nano type because it is safer and more environmental friendly compared to the chemical form. These results agree with Shedeed et al. (2018).

Shoot length

Shoot length was significantly affected by different salinity concentrations. ASA (100 ppm) showed higher shoot length followed by ALA (50 ppm) (32.69 and 28.00 cm, respectively). The lowest shoot length was obtained with

N-Se (20 ppm) treatment (22.39 cm) compared to untreated plants exclusively under high level of salt mixture (120 mM). Previous studies show that the application of some natural bio-stimulants used as a foliar spray and/or seed soaking improved growth and yield constituents of pea grown under salinity stress (Desoky et al., 2017).

Number of leaves

The data in Table 1 show that in both growing seasons, salinity caused a significant reduction in the number of leaves by different salinity concentrations. In contrast, in the treatments of ASA (100 ppm) and ALA (50 ppm), the number of leaves was higher (7 leaves and 6 leaves respectively). The lowest number of leaves was obtained with N-Se (20 ppm) treatment (5 leaves) in comparison to untreated plants, especially under high level of salinity (120 mM).

The decrease in growth and productivity could be attributed to the osmotic effect of salinity stress which caused increase of growth inhibitors and decrease of growth promoters, disturbance of water in plants grown under salt stress. It indicates that these inhibitory effects of salinity lead to stomatal closure, reduced photosynthesis, unrest in ionic homeostasis, accumulation of toxic Na^+, Cl^- and finally inhibit growth and productivity. Moreover, the decrease in the shoot length of stressed plants is actually due to senescence which is accompanied by loss and withering of plant organs as well as the transport of elaborated materials to the reproductive organs.

Pea seed and plant treated with ASA, ALA and N-Se used as foliar spray and seed soaking significantly promoted plant growth and productivity under the adverse effect of soil salinity. One of the compounds which has antioxidative characteristic is ASA (Zhang, 2013). This compound can reduce the harmful effects in plants under environmental stress. The ASA treatments influenced the passive effect of salinity on growth and productivity. This could be referred to as the biochemical functions of ASA which can be divided into categories, that is, antioxidant, that changes the lipophilic antioxidants such as tocopherol, vitamin E, and enzyme cofactor for hydroxylase enzymes involved in the synthesis of rich-hydroxyproline glycolproteins, and cell wall structural proteins (Desoky et al., 2017). It was observed that, foliar treatment with ALA stimulated growth and also partially enhanced the effects of toxic caused by high levels of salinity in root and shoot. Also, salinity damage can also be attributed to the physiological drought generated by salt stress (Hopkins, 1995; Sajid et al., 2020), due to the reduction in osmotic potential and relative water content. ALA application encouraged an increase in osmotic potential and relative water content of the stressed seedlings (Naeem et al., 2011). Many studies have shown that ALA can stimulate crop resistance, yield and quality and can be used as a new type of plant growth regulator (Rafaqat et al., 2015; Tang et al., (2017b). A concentration of 50 mg/L of ALA could significantly mitigate seeds' deterioration and seedlings of *Perilla frutescens* under NaCl stress and encourage salt resistance (Zhang et al., 2011). It was indicated that a high level of salinity damages cellular electron transport, leading to the generation of reactive oxygen species (ROS). This activates lipid peroxidation and cell membrane damage (Shalata et al., 2001).

Cytological parameters

The cytological effects on pea plants treated with ASA, ALA and N-Se under saline conditions are shown in Table 2.

Our results showed that, the mitotic index in root tip meristems of *P. sativum* treated with salt mixture (60 and120 mM) significantly decreased compared to seeds (in control treatment). Table 2 proves the effect of ASA, ALA and N-Se on mitotic index (%) in *P. sativum* root tips. The total number of proliferating cells and the numbers of cells at various mitotic stages of *P. sativum* meristemic cells were scored in root tips. Cytological analysis showed that, under harmful stress conditions (120mM NaCl), the highest value of mitotic index (%) was observed in pea treated with ascorbic acid at a concentration of 100 ppm (13.53%) followed by ALA 50 ppm (13.19%); nano selenium at a concentration of 20 ppm gave the lowest value (10.12%), compared to control (10.02%).

The mitotic index of root tips of seeds treated with ASA, ALA and N-Se remarkably increased in salinity samples. At the same time, ASA, ALA and N-Se + NaCl application indicated serious performance in improving the passive effects of salt stress on the mitotic activity. Çavuşoğlu et al. (2007, 2013) established that, growth regulator (exogenous application) may have a positive or negative effect on seed germination and seedling growth under non-stress conditions.

Thus, the study aims to test the effects of ASA, ALA and N-Se application on seedling growth in non-stress and stressed conditions. Our results indicated that the shoot length, number of leaves and leaf area of treated plants were generally amplified in comparison to the control. It was stated previously that saline conditions harmfully affect growth and development actions in general, even in halophytes (Çavuşoğlu et al., 2017; Ghoulam and Fares, 2001). The seedling growth and germination of *P.sativum* seeds, as anticipated, were prevented under saline conditions (Table 1).

Ali (2000) demonstrated that salinity could fulfill/replace its harmful effect in numerous ways. It may intervene with seed germination by converting the water status of the seed so that water reuptake is inhibited. Our outcomes

Table 2. Mitotic index and mitotic phase (%) of tip root cells of *pissum sativum* under salinity stress conditions and different applied treatments (ascorbic acid - ASA, 5-aminolevulinic acid - ALA and Nano selenium - N-Se).

Salinity level (mM)	Treatments		No. of screened cells	Mitotic phase (%)				Total no. of normal dividing cells	Mitotic index (%)
				Prophase	Metaphase	Anaphase	Telophase		
Control (0)	Control		1072.67	38.333	27.67	22.67	18.00	106.67	12.11
	ASA	50 ppm	1135.00	61.667	45.33	31.00	20.00	158.00	14.03
		100 ppm	1061.00	83.000	71.33	57.00	38.33	241.67	22.79
	ALA	25 ppm	1056.67	51.333	44.67	30.67	20.00	146.67	13.97
		50 ppm	1052.67	72.667	59.33	49.00	32.33	221.33	21.06
	N-Se	10 ppm	1007.00	67.667	50.00	38.33	24.67	180.67	17.95
		20 ppm	1021.67	47.667	33.33	23.33	15.33	119.67	12.11
60 mM	Control		1054.00	21.000	24.33	20.00	20.00	85.33	10.31
	ASA	50 ppm	1092.67	45.000	26.00	17.00	9.33	97.33	11.72
		100 ppm	1028.00	71.667	62.33	38.33	26.33	198.67	20.24
	ALA	25 ppm	1034.00	40.000	21.67	17.67	10.33	89.67	10.92
		50 ppm	1164.33	61.333	54.33	31.33	20.33	167.33	15.63
	N-Se	10 ppm	1058.33	54.333	41.00	24.67	15.33	135.33	14.17
		20 ppm	1131.33	37.333	27.33	15.67	9.00	89.33	10.74
120 mM	Control		1103.67	17.000	16.67	17.33	12.67	63.67	10.02
	ASA	50 ppm	1177.00	21.000	19.33	18.00	12.67	71.00	10.62
		100 ppm	1048.00	37.000	38.00	15.33	8.67	99.00	13.53
	ALA	25 ppm	1261.00	19.333	19.67	16.00	13.33	72.00	10.23
		50 ppm	1029.00	35.667	34.67	12.33	6.00	88.67	13.19
	N-Se	10 ppm	1077.33	30.667	27.67	14.67	7.33	80.33	12.12
		20 ppm	1066.67	21.667	18.67	16.33	15.33	68.33	10.12
L.S.D. (0.05)									
Salinity			62.618	1.624**	0.997**	1.065	1.058**	2.435**	0.429**
Treatment			95.650	2.481**	1.523**	1.626	1.616**	3.719**	0.636**
Salinity x Treatment			165.670	4.298**	2.638**	2.817	2.798**	6.442**	0.964**

showed the decrease in stem length, number of leaves and leaf area under saline conditions. Other studies showed that the inhibitive effect of salt on root might result from decreasing cell division (McCue and Hanson, 1990), protein synthesis and nucleic acid (Prakash et al., 1988). This may be demonstrated by the failure of the roots to receive enough water due to high osmotic pressure of the medium (Al-Karaki, 2001).

On the other hand, ASA, ALA and N-S treatments noticeably removed the inhibitor effect of salinity stress on growth parameters, so our

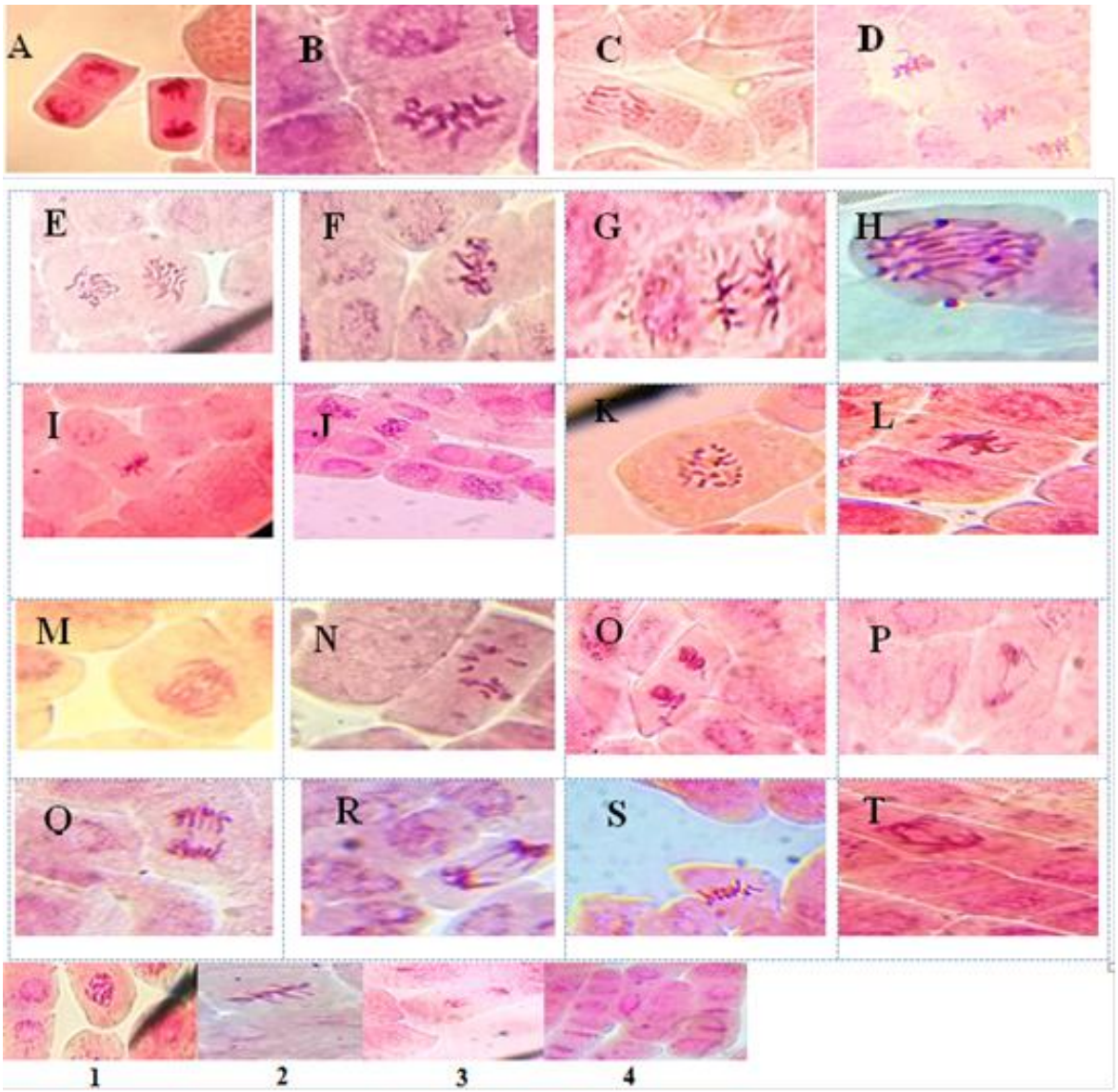

Figure 1. A: Sticky Anaphase, B: C- Metaphase stage, C: Vagrant chromosome in Anaphase, Chromosomal Laggard in late Anaphase, D: Overlapping chromosomes in Metaphase stage, E: Irregular prophase, F: Sticky Metaphase, G: Irregular Metaphase, H: Alignment anaphase, I: Vagrant chromosome in Metaphase, J- Irregular prophase. K: Granulation, L: Clumped metaphase, M: Multiple bridges in anaphase, N: Disrupted equatorial plate, O: Telophase with a fragment, P: Anaphase with laggard chromosome, Q: Anaphase with chromosome breakage, R: Anaphase with a single bridge and sticky chromosome, S: Metaphase with a fragment, T: Anaphase with a single bridge. 1: normal prophase, 2: normal metaphase, 3: normal anaphase, 4: normal telophase.

treatments alleviate salt stress on roots due to the reduction in the salts osmotic effects. ASA might have been effective in decreasing the inhibitive impact of salinity stress on the seed germination and seedling growth by rising nucleic acid and protein synthesis, providing steadiness of cell membranes or by raising the activity of antioxidant enzyme (Al-Kaisy et al., 2018). ASA has also received special attention because it is a highly efficient antioxidant and has free radical scavenging capacity (Acosta-Motos et al., 2020).

There are no present studies on the effects of ALA application under salinity conditions on cytogenetical parameters as studied here.

In Figure 1, It was concluded that aberrations in chromosomes induced by salinity at different stages of mitosis such as prophase, metaphase, anaphase, and telophase in root meristem cells are variable. In addition, a gradual decline in mitotic index and an increase in the abnormality index were observed as the concentration of salt mixture application duration was raised. Aberrations in chromosomal behavior such as sticky and disturbed chromosomes in metaphase and anaphase, c-metaphase,

bridges, laggard, and disturbed telophase were also observed. Bhattacharjee et al. (2014) confirmed that a significant reduction by N-Se in chromosomal aberration in bone marrow, and DNA damage in lymphocytes and bone marrow in mice treated with cyclophosphamide - induced hepatotoxicity and genotoxicity.

Types of chromosome aberrations in *P. sativum* root tips treated with ASA, ALA and N-Se under saline condition

In Table 3, various chromosomal aberrations like chromosomal bridges, stickiness, vagrant, broken, and lag chromosomes were recorded. In our study, the lowest value of abnormalities (%) under severe stress conditions (120 mM) was observed when the pea was treated with ascorbic acid at a concentration of 100 ppm (13.63%) followed by ALA 50ppm (14.01%); nano selenium at a concentration of 20 ppm gave the highest value. It was 17.45% compared with control which recorded 23.42%. Cytological effects and the data obtained from the analysis of root tips treated with ALA, ASA and N-Se are summarized in Tables 1 and 2 and Figure 1.

Previously, it was reported that salt stress conditions adversely affect growth and development events. It is recognized that salinity inhibits seedling growth (Abdul Qados, 2011; Ghezal et al., 2016).

Salinity that inhibits shoot length and leaf number may result from decreasing cell division (McCue and Hanson, 1990). The kinds of mitotic abnormalities detected in the present study were disturbed chromosomes, sticky, vagrant, laggard, bridges and C- shaped Metaphase.

On the other hand, ASA, ALA and N-Se application that markedly removed the inhibitor effect of salinity on growth parameters (Tables 1 and 2) is due to the decrease in the salts osmotic effects compared to the control. In addition to all these, ASA, ALA and N-Se might have been efficacious in decreasing the inhibitive effect of salt stress on the plant growth by increasing nucleic acid and protein synthesis, stabilizing cell membranes or raising the activities of antioxidant enzyme (Liu et al., 2014; Ekanayake et al., 2015; Germ et al., 2007).

According to some researchers, the harmful effects of salinity stress on mitotic activity have been known for a long time. Furthermore, the negative effects of salinity stress on chromosomal abnormalities have been planned in the last decade (Radic et al., 2009; Tabur and Demir, (2009, 2010a, b). It was demonstrated that a high concentration of salt entirely suppressed the activity of mitotic division and facilitated chromosomal abnormalities in root-tip meristem cells (Radić et al., 2009).

It is worth mentioning that salinity adversely affected the mitotic activity and chromosome behaviors in root meristem cells of *P. sativum*. Briefly, the results proved that under salinity, ASA, ALA and N-Se might act as a stimulator, triggering the protein synthesis necessary for the normal division of cells and acceleration of the mitotic cycle. Previous researches also proved that nanomaterials with low concentrations are better than high concentration alone or in a group with stress (Zedan and Omar, 2019). It was indicated that, the protective effect of selenium as nanoparticles versus numerous material induced cytotoxicity and genotoxicity effects (Bhattacharjee et al., 2014). A significant reduction by N-Se in chromosomal aberration was found in bone marrow, and DNA damage in lymphocytes and bone marrow in mice treated with cyclophosphamide -induced hepatotoxicity and genotoxicity.

Barakat (2003) established that, treating the roots with vitamin B6 or ascorbic acid presented a considerable increase in the mitotic index and reduced the inhibition effect of NaCl in wheat cultivars.

The previous results showed that ASA (100 ppm) in combination with NaCl does not only antagonize the inhibitory action of salinity but also activates the cells to inter mitosis and encourages a high mitotic activity. These results are in harmony with those obtained by Autifi et al. (2018) who proved that vitamin C reduces the influence of lead acetate on the mitotic activities. No perversion from the normal was seen in roots treated with vitamin C or vitamin B6; parallel results were observed by Bronzetti et al. (2001).

Harmful effects of salinity or any other stress conditions were attributed to lower endogenous levels of cytokinins and endogenous hormonal imbalance by some researchers (El-Mashed and Kamel, 2001). Plant growth inhibition refers to disturbances in natural growth regulators and mitotic chromosomal irregularities as additional factor (Hoda et al., 1991). So, the application with ascorbic acid can reform the genotoxic effect of the salinity which delays the cell in entering mitosis.

Conclusions

Considering the data of the present study it appears that when applied at high concentrations of salinity shows cytotoxic activity. In this study some growth regulators such as ASA, ALA and N-Se were used. It was concluded that salinity treatments stimulated the genotoxic effect to throw ROS generation inside the tissues which ultimately cause oxidative disturbance. This leads to redox homeostasis imbalance and genotoxic in addition to mito-depressive effects. Using these treatments (ASA, 100 ppm; ALA, 50 ppm and N-Se, 10 ppm) causes induction of mitotic index and reduces the chromosomal aberrations.

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Table 3. Effect of salt mixture (60 and 120 mM) ascorbic acid (ASA 50 and 100 ppm), 5-aminolevulinic acid (ALA 25 and 50 ppm) and Nano selenium (N-Se 10 and 20 ppm) and the combination between salinity and ASA, ALA and N-Se on frequency of chromosomal aberrations in *Pissum sativum* root tip meristems.

Salinity level mM	Treatments		No. of examined cells	No. of dividing cells	No. of abnormal cells	Mitotic aberration (%)									Abnormalities %
						Granulation %	C-metaphase%	Laggard %	Break%	Stickiness %	Vagrant %	Irregular %	Alignment %	Bridge %	
Control (0)	Control		1072.67	106.67	3.00	0.00	0.00	0.67	0.67	0.67	0.33	0.33	0.00	0.33	3.31
	ASA	50 ppm	1135.00	158.00	1.00	0.00	0.00	0.33	0.00	0.00	0.33	0.33	0.00	0.00	0.64
		100 ppm	1061.00	241.67	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.13
	ALA	25 ppm	1056.67	146.67	1.00	0.00	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.33	0.68
		50 ppm	1052.67	221.33	0.33	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.15
	N-Se	10 ppm	1007.00	180.67	0.67	0.00	0.00	0.33	0.00	0.00	0.33	0.00	0.33	0.00	0.55
		20 ppm	1021.67	119.67	1.33	0.00	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.33	1.11
60 mM	Control		1054.00	85.33	20.00	2.33	2.67	4.00	3.33	3.33	3.00	1.50	0.00	1.33	18.99
	ASA	50 ppm	1092.67	97.33	16.67	1.50	2.00	1.67	1.67	3.00	3.33	3.50	0.00	1.67	14.36
		100 ppm	1028.00	198.67	10.00	0.00	0.67	1.33	1.50	1.67	2.00	3.33	0.00	1.67	4.84
	ALA	25 ppm	1034.00	89.67	17.67	1.33	1.33	2.67	1.67	1.33	2.00	3.33	0.00	4.00	15.68
		50 ppm	1164.33	167.33	12.33	1.33	1.67	1.67	1.67	0.67	1.50	2.33	0.00	2.00	6.87
	N-Se	10 ppm	1058.33	135.33	11.00	1.67	1.00	1.00	1.67	2.00	0.67	1.33	1.33	1.67	7.36
		20 ppm	1131.33	89.33	18.00	1.00	2.00	1.67	2.00	2.00	2.67	3.00	0.33	3.67	16.56
120 mM	Control		1103.67	17.000	31.33	5.33	7.33	3.00	5.67	4.00	1.33	2.67	2.67	2.00	23.42
	ASA	50 ppm	1177.00	71.00	20.67	2.67	4.67	1.00	2.00	5.00	2.33	1.67	4.33	1.33	16.52
		100 ppm	1048.00	99.00	19.33	3.00	3.00	1.33	3.00	1.00	2.33	2.67	1.33	3.00	13.63
	ALA	25 ppm	1261.00	72.00	21.33	3.00	4.33	1.00	1.33	4.33	2.33	2.67	4.33	2.33	17.02
		50 ppm	1029.00	88.67	19.00	2.67	2.67	1.33	3.00	1.67	2.33	2.33	3.00	3.00	14.01
	N-Se	10 ppm	1077.33	80.33	20.33	3.67	4.00	0.33	1.33	2.67	2.67	2.67	3.67	3.00	15.61
		20 ppm	1066.67	68.33	22.33	2.67	3.67	2.00	1.67	4.67	2.67	2.00	4.33	3.00	17.45
L.S.D. (0.05)															
Salinity			62.618	2.435**	1.083**	0.551**	0.542**	0.582**	0.498**	0.710**	0.651**	0.628**	0.411**	0.487**	0.791**
Treat			95.650	3.719**	1.655**	0.842*	0.827**	0.889**	0.761**	1.084**	0.994	0.960	0.628**	0.743**	1.208**
Salinity x Treat			165.670	6.442**	2.866**	1.459	1.433**	1.540	1.319**	1.877	1.722	1.662	1.088**	1.288*	2.092**

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