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Effects of L-arginine on Some Cytogenetical and Physiological Parameters of *Allium cepa* L. Seeds exposed to Salinity

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Abstract— *In this study, L-arginine (Arg) effects on the seedling growth (fresh weight, radicle length and radicle number), seed germination, mitotic activity, chromosomal aberrations and micronucleus frequency in Allium cepa L. germinated in both salt stress and normal conditions were investigated. In only Arg medium, the radicle number of the seeds was partially reduced compared to the control seeds germinated in the distilled water medium, the fresh weight, radicle length and germination percentage indicated statistically the same values as the control. Besides, the mitotic index in the root tip meristems of A. cepa seeds germinated in the Arg medium alone showed a decrease compared to the control seeds germinated in the distilled water medium, whereas the chromosomal aberrations exhibited a significant increase compared to the control. Moreover, the micronucleus formation increased compared to the control. On the other hand, salt stress significantly inhibited the seedling growth and seed germination of A. cepa. In addition, it significantly reduced the mitotic index in the root tip meristems of the seeds and increased the number of chromosomal abnormalities and micronucleus frequency, which is the simplest indicator and the most effective of cytological damage. Nonetheless, the inhibitive effect of salt on the micronucleus formation, mitotic activity, seedling growth, seed germination and chromosomal aberrations significantly decreased with the application of L-arginine.*

Keywords— ***Allium cepa L., arginine, salt stress, seed germination, mitotic index.***

I. INTRODUCTION

Salinity or salt stress is an important abiotic factor that limits crop production. Soils with electric conductivity more than 40mM NaCl (about 4ds/m) are considered to be salty. Approximately 7 % of the earth's land [1] and 20 % of the irrigated land [2] are affected by salinity. More land is irrigated every year to increase crop production. Expansion of irrigated land, coupled with high salt content in irrigation water and poor drainage has increased salinity stress. Blumwald and Grover [3] predict that approximately 50 % of arable land will be affected by salt stress in 2050. Improvement of drainage to improve crop production in saline soils, irrigation management, use and development of salt tolerant varieties are suitable solutions. Salt tolerance is that plants complete their life cycles and grow with good yield potentials in salt conditions. There are two salt tolerance mechanisms; osmotic effect that minimizing salt ingress from root to leaf and ionic effect which minimizes intracellular toxicity due to higher salt concentration [4].

Arginine (Arg), one of the twenty standard amino acids necessary for the formation of proteins and peptides, is also used as a nitrogen storage compound in seeds. In addition, Arg is the precursor of polyamines and nitric oxide, which play an important role in response to various environmental stresses and in many developmental processes. Arg is ideal for storing nitrogen due to its very high N:4 / C:6 ratio, which is a basic amino acid with $H_2NC(=NH)NH(CH_2)_3CH(NH_2)COOH$ linear formula. Indeed, Arg can be represented as a free amino acid in seeds such as soybean, pumkin, broad bean, many other dicotyledonous plants, peach tree or as an important part of the nitrogen store in, other parts of plants or bulbs. And this is an important amino acid in the free amino acid ponds of seedlings of early growth of both seedling growth and megagametophine. Therefore, its biosynthesis may be of additional importance during seed development. Ornithine, is a precursor of Arg and Arg is required for polyamine production. These small positively charged organic molecules play an important role in senescence, root growth, division, response to plant stress, cell growth, ripening, fruit development and other processes. Arg which is the source of nitric oxide plays an important role in root growth, defense, germination, responses, flowering and hormonal marking in plants. Arg is a dietary precursor for nitric oxide formation and nitric oxide is a potent mediator of vascular tone, hence affects the cardiovascular system. The nitric oxide formed from dietary Arg has been linked to muscle repair and

optimal immune function [5]. Feirer [6] stated that the level of Arg increased twenty-fold during the embryo development in the seed. In addition, Arg which is a precursor of polyamines having a regulatory role in embryogenesis. Moreover, it has an important position in a transport compound and the urea cycle too [7].

Since the early 1920s, *Allium cepa* was used to assess the chromosome abnormalities. The method is a sensitive and easy tool to measure total toxicity caused by chemical treatments expressed by growth inhibition of onion bulb roots. *Allium* test was used as a standard test for cyto-genotoxicity monitoring [8]. The test has some advantages, such as being very inexpensive, easy to apply, simple and also as reliable as the method in which abnormalities are recorded in all types of mitotic cells. It combines two test targets: using for toxicity&genotoxicity monitoring, it is also an important fact that it shows a good correlation with mammalian test systems [9].

Although there are few published studies on the role of Arg on the seedling growth and seed germination under both saline and normal conditions, unfortunately, there are no studies on the effects of this amino acid on the micronucleus frequency, mitotic activity and chromosomal aberrations in saline and normal conditions. For these reasons, this work was designed to investigate the effects of Arg in reducing of the harmful effects of salt stress on the mitotic activity, seed germination, chromosomal aberrations, micronucleus frequency and seedling growth of *Allium cepa* L.

II. MATERIALS AND METHODS

2.1. Seed, arginine and salt concentrations

In the present work, *Allium cepa* L. seeds and 0.175 M NaCl (salt) concentration were used. The concentration of L-arginine used in the experiments was 10 mg/L. Arg was obtained from Merck. In a preliminary investigation of this study, Arg and salt concentrations were determined conducted.

2.2. Seed germination

Seed germination experiments were performed in a (fixed temperature) incubator set to 20°C in the dark. Approximately equal-sized and healthy onion seeds have selected. *Allium cepa* L. (*Amaryllidaceae*) seeds have sterilized with the aid of sodium hypochloride solution (2.5%) for ten minute and washed with ultra-pure water for 24 hour. Twenty seeds selected from each application group were placed in plastic containers. The bulbs have split in four groups:

- Group I (control) during 7 sequential days have treated by distilled water.
- Group II during 7 sequential days have treated by 0.175 M NaCl alone.
- Group III during 7 sequential days have treated by a 10 mg/L dose of Arg.
- Group IV during 7 sequential days have treated by a 10 mg/L dose of Arg + 0.175 M NaCl.

It is assumed that the seeds in plastic containers placed in the incubator for germination should have a length of 10 mm. After 7 days, the final germination percentage was taken, the number of radicle were recorded, the radicle lengths of onions were measured in mm, the fresh weights were also determined in g/seed. All experiments were repeated 3 times.

2.3. Cytological and statistical analysis

After a few days for cytogenetic analysis, 1-1.5 cm segment of germinated *A. cepa*'s root tips were excised. Initially, these have pretreated using saturated para-dichlorobenzene for four hours, afterwards were fixed in a solution (3:ethanol / 1: acetic acid) for 24 hours at room temperature and stock up in 70 % ethanol at 4°C until making the microscopic slides. *A. cepa* rootlets were hydrolysed for 15 minutes in 1 N HCl at 60°C, dyed with Feulgen for 1-1.5 hours and lysed with a drop of 45 % CH_3COOH. Squashes have prepared as suggested by Sharma and Gupta [10]. At the end of 24 hours, microscopic preparations were made permanent by means of balsame. With a digital camera (Olympus C-5060) mounted on the Olympus CX41 microscope has photographed mitotic phases, micronuclei and mitotic aberrations (500X).

The cell division densities of these preparations were analyzed by calculating the mitotic index (%) (MI) assessed by analyzing at least 30000 cells per sample (about 10000 per preparation). Chromosomal abnormalities were calculated as the percentage of 2000 dividing cells counted for each concentration. The latter was determined as a percentage between the number of dividing cells (N') and the total number of cells analyzed (N) according to formula: MI (%) = (N' / N) x 100 [11]. The statistical analysis was carried out using SPSS program according to DMRT. Statistically, all values mentioned in this study are highly significant ($P<0.05$).

2.4. Micronucleus (MN) assay

For micronucleus analyses, 1000 cells per slide were scored. MN was examined with the help of a binocular light microscope. For the scoring of micronucleated cells, Fenech et al. [12] used the protocol they followed. These: (i) the diameter of the micronucleus should be a tenth of

the main nucleus, (ii) Micronucleus should be separated from or marginally overlapped from the main nucleus, provided that the nucleus boundary is clearly defined, (iii) the micronucleus staining should be similar to that of the main nucleus.

III. RESULTS

3.1. Effects of arginine on the seedling growth and seed germination

The results from Table 1 clearly demonstrate that while the radicle length, fresh weight and germination percentage of group III germinated in alone Arg medium showed statistically the same values as group I (control) germinated in distilled water medium, their radicle number partly decreased according to the control seeds.

NaCl exhibited an inhibitory effects on all growth parameters examined. For instance, the control (group I) seeds germinated in distilled water medium after 7 days showed 100 % germination, whereas this value was 23 % in group II seeds germinated at 0.175 M salinity. That is to say, NaCl prevented 77 % germination of *Allium cepa* seeds. The inhibitive effect of salt stress on the seed germination was markedly mitigated by Arg application. Group IV seeds treated with Arg in this salt level demonstrated 82 % germination. In addition, Arg continued its success on the seedling growth parameters such as the fresh weight and radicle number. However, this amino acid has been ineffective in attenuating the negative effect of NaCl inhibition on the radicle length. The number of radicle and fresh weight of group II seedlings grown in 0.175 M salinity were 12.7 and 7.0 g, respectively while these values became 18.2 and 11.1 g in group IV seedlings treated with Arg (Table 1, Fig. 1).

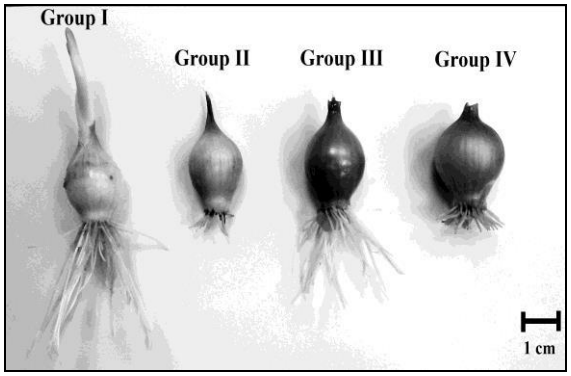

Fig. 1: Allium cepa root tip cells showing germination situations at the end of the seventh day. Control (Group I) seeds were treated with distilled water; Group II seeds were treated with 0.175 M NaCl alone; Group III seeds were treated with a 10 mg L^{-1} dose of L-arginine; Group IV seeds were treated with a 10 mg L^{-1} dose of L-arginine+0.175 M NaCl. Scale bar = 1 cm

Table 1: Effects of L-arginine 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^{b}	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}	63.4 ± 1.0^{b}	56.0 ± 0.5^{c}	14.3 ± 0.3^{c}
Group IV	82 ± 2.8^{b}	11.1 ± 0.9^{a}	18.2 ± 0.5^{b}	11.1 ± 0.6^{b}

*The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Control (Group I) seeds were treated with distilled water; Group II seeds were treated with 0.175 M NaCl alone; Group III seeds were treated with a 10 mg L^{-1} dose of L-arginine; Group IV seeds were treated with a 10 mg L^{-1} dose of L-arginine+0.175 M NaCl.

3.2. Effects of arginine on the micronucleus formation, chromosomal aberrations and mitotic activity

The mitotic index of group III seeds germinated in only Arg medium reduced 20 % compared to group I (control) seeds germinated in distilled water medium. In addition, Arg application increased the chromosomal aberrations and frequency of the micronucleus according to the control (Table 2). Exposure to 0.175 M salinity resulted in a significant inhibition in the mitotic index. In an other words, the mitotic index in the root tip meristems of group II seeds germinated in 0.175 M salt media compared to the group I seeds (distilled water, control) decreased by 89 % and increased significantly the chromosome aberrations (17 %) and the frequency of micronucleus (13 %). On the other hand, Arg treatment became successful in improving

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the adverse effects of salinity on the micronucleus formation, mitotic activity and chromosomal aberrations. These values became 9.6 % (MN), 9.1 % (MI) and 13.6 % (CAs) in group IV seeds treated with Arg (Table 2).

Figure 2 shows the abnormal mitotic phases observed in course of microscopic examination in meristem cells of *A. cepa* root tip. Double nuclear lesion and micronucleus were the most frequent abnormalities induced by Arg and its salt constituents. Some other aberrations were also observed in cells with the frequency of occurrence as: nucleus disintegration > polar deviation in telophase > anaphase with vagrant chromosome > scattering at metaphase > telophase with vagrant chromosomes > condansed nuclei > uncoiling chromosomes > alignment anaphase > laggards at anaphase > notched nuclei > ring chromosome > nuclear bud > giant cell > prophase with chromosome loss > anaphase with chromosome loss > disturbed in telophase.

Table 2: Effect of L-arginine on some cytogenetic parameters of Allium cepa L.

Groups	Mitotic index (%)	Micronucleus frequency (%)	Chromosome aberration (%)
Group I	*****11.6 ± 1.0^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Group II	1.2 ± 0.2^{a}	13.0 ± 1.0^{d}	17.0 ± 0.4^{c}
Group III	9.2 ± 0.5^{b}	1.3 ± 0.5^{b}	14.3 ± 0.5^{b}
Group IV	9.1 ± 0.3^{b}	9.6 ± 0.5^{c}	13.6 ± 1.3^{b}

*The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Control (Group I) seeds were treated with distilled water; Group II seeds were treated with 0.175 M NaCl alone; Group III seeds were treated with a 10 mg L^{-1} dose of L-arginine; Group IV seeds were treated with a 10 mg L^{-1} dose of L-arginine+0.175 M NaCl.

IV. DISCUSSIONS

4.1. Cytogenetical and physiological effects of exogenous arginine under normal conditions

If stress conditions are not present in the environment, any plant growth regulator should be added as exogenous in the germination process. The addition of a plant growth regulator exogenously under stress-free conditions can have negative or positive effect on the seedling growth and seed germination [13, 14]. However, there are few studies on the effects of Arg on the seed germination and seedling growth under normal conditions. Therefore, in the laboratory study, the effects of Arg application on the mitotic activity, seed germination, micronucleus frequency, chromosomal aberrations and seedling growth under normal conditions requested to be tested. The seed germination depending on the used concentration, application method and plant species.

laboratory study's results revealed that the germination percentage, fresh weight and radicle length of the seeds germinated in the only Arg medium statistically showed the same values as the control seeds germinated in distilled water medium, whereas their radicle number decreased slightly compared to the control (Table 1). El-Bassiouny et al. [15] reported that 0.6, 1.25, 2.5 and 5 mM Arg applications increased significantly the fresh weights of wheat seedlings grown in normal conditions. Samia and Rania [16] determinated that 2.5 mM Arg resulted in obvious enhancement in the radicle length and fresh weights of lupine seedlings under normal conditions. These results are not consistent with the present research findings, so Arg may have different effects on the seedling growth and

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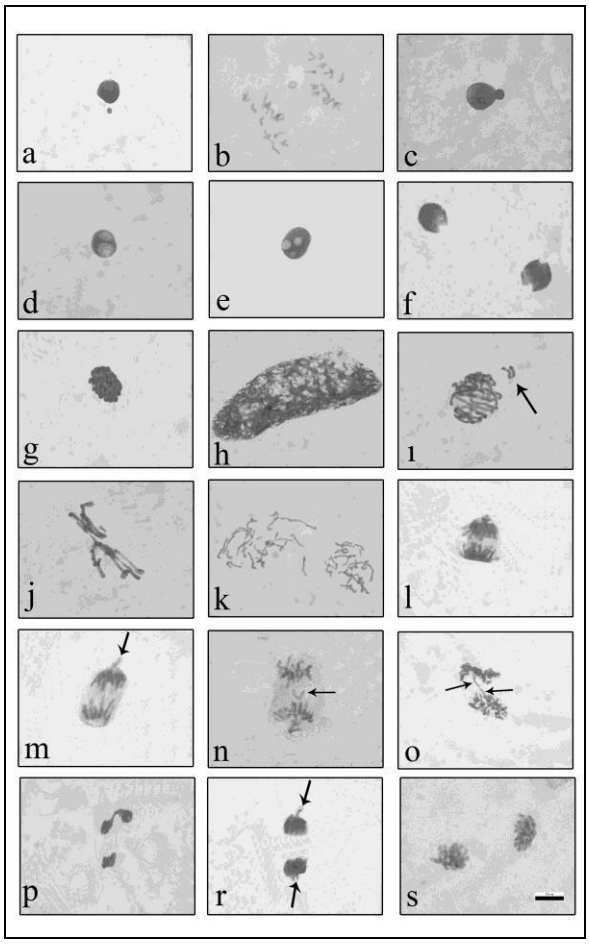

Fig. 2: Chromosomal aberrations; a-micronucleus; b-ring chromosome; c-nuclear bud; d-double nuclear lesion; e-nucleus disintegration; f-notched nuclei; g-condansed nucleus; h-giant cell; ı-prophase with chromosome loss=arrow; j-uncoiling chromosomes; k-scattering at metaphase; l-alignment anaphase; m-anaphase with vagrant chromosome=arrow; n-anaphase with chromosome loss=arrow; o-laggards at anaphase=arrows; p-disturbed in telophase; r-telophase with vagrant chromosomes=arrows; s-polar deviation in telophase. Scale bar = 10 µm

The effects on the chromosomal aberrations, mitotic activity and micronucleus frequency of Arg application under normal conditions are still unknown. The findings of this study showed that the MI in root meristems of *A. cepa* seeds subjected to Arg application under normal conditions decreased about 20 % compared to the control seeds germinated in distilled water medium. Thus, administration of 10 mg L^{-1} Arg demonstrated an effect repressive on the mitotic activity by slowing cell division. This dose of Arg application increased markedly CAs and MN frequency according to the control. In this case, some abnormalities can be said to be caused by this stimulator (Table 2). In this study, micronucleus and double nuclear lesion were the most frequent abnormalities. Various abnormalities have been observed in all stages of mitotic division as a result of structural deviations in chromosomes. A part of the chromosomes can be connected to each other instead of separating to the poles forming fragments and bridges. Laggards observed occured during the chromosomal migration to the poles. Vagrant chromosome in anaphase may also be mainly the result of non-polar mitotic spindles.

4.2. Cytogenetical and physiological effects of exogenous arginine under saline conditions

Salt stress like many other abiotic stresses inhibits plant growth. Because the high salt concentration in the soil solution prevents from being absorbed the nutrient ions in a balanced manner by the plants. NaCl stress affects plant physiology in all plants and also affects cellular levels through osmotic and ionic adjustments resulting in a reduction in biomass production. The negative impact of salt stress is possible to see at all plant levels in almost all growth stages including, seedling, germination, maturity and herbal stages. Although salinity causes ionic and osmotic stress, it causes ionic imbalances that may induce potassium deficiency and may impair the selectivity of root membranes [17]. The results from Table 1 clearly demonstrated that as expected the seedling growth and germination of *A. cepa* seeds were inhibited under saline conditions. In agriculture, soil salinity indicates the presence of high concentrations of soluble salts in the soil moisture of the root zone. Due to their high osmotic pressures, the concentrations of these soluble salts affect plant growth by limiting the water uptake of the roots [17]. Results of these statements are consistent with the results of the present study in terms of showing the decrease in the water content and fresh weight of the seedlings in salted conditions. The inhibitive effect of NaCl on the radicle number and radicle length may result from reducing protein synthesis, nucleic acid and cell division [18].

On the other hand, by application of the amino acid Arg, the inhibitory effect of saltinity stress on the seed germination, fresh weight and radicle number was significantly eliminated (Table 1). To date, there have been several studies investigating the effects of Arg on the seedling growth and seed germination in saline conditions. Abd El-Monem [19] found that the optimum concentration of Arg was 2.5 mM in alleviating the harmful effects of salt stress in wheat. Zeid [20] observed that 4 mM Arg pretreatment promoted the growth parameters and germination percentage of bean seedlings under salinity stress. Nasibi et al. [21] also showed that pre-treatment with three concentrations of Arg (0, 5 and 10µM) could

reduce the harmful effect of salinity on the fresh weight of canola seedlings. Nejadalimoradi et al. [22] observed that 1 and 5 mM Arg pretreatment increased to the radicle length of sunflower plants under salinity stress. In addition, Samia and Rania [16] determinated that spraying 2.5 mM Arg attenuated the retarder effects of salt stress in lupine plants. All of these results are consistent with the amino acid arginine's findings. As can be seen in Table 1, Arg can be understood from the decrease in the osmotic effects of the salt, which relieves the salt stress on the seedling growth and seed germination. For example, in 0.175 M NaCl medium, it is observed that the fresh weight of seedlings is significantly increased by Arg application compared to Group I indicates this probability. Additionally, Arg may have been successful in reducing the inhibitive effects of salinity stress on the seedling growth and seed germination by increasing antioxidant enzyme activities [21].

Mitotic index is a reliable parameter that reflects the frequency of cell division in the root growth area and is used to identify cytotoxicity [8]. Cytotoxicity levels can be determined by a decrease or an increase in the mitotic index [23]. The mitotic index can be used to determine root growth rate and as a reflection of cell proliferation. More interestingly, this study results showed that the salt caused a decrease in the mitotic activity and this decrease was achieved by decreasing the number of cells entering mitotic division. The decrease in the number of divided cells suggests that the salt may have mitodepressive effects on *A. cepa* L. cell division. Mitodepression blocks nucleus proteins and DNA synthesis [24]. With this study, it should be noted that the salinity adversely affects chromosome behaviors and the mitotic activity of *A. cepa* root meristem cells. The results of this study show that salinity decreased MI by 89 % compared to the control group and showed an excessive increase in the number of chromosomal abnormalities and micronucleus. For example, while the MN and CAs in the root tip meristems in group I were 0.0 % and 0.0 %, respectively these values became 13.0 % and 17.0 % in 0.175 M salt. Furthermore, Arg+NaCl became effective in alleviating the harmful effect of salt on the MI. In contrast, administration of simultaneously Arg+NaCl showed a significant success compared to the Arg alone in alleviating the harmful effects of salinity on the frequency of MN and CAs. So, the frequency of CAs with the application of simultaneously Arg+NaCl decreased by 20 %. This result shows Arg repair role against salt injuries during *A. cepa*'s mitosis (Table 2).

Chromosomal abnormalities (CAs), which may occur as a result of both spontaneous and exposure to physical or chemical agents, are characterized by changes in the total chromosome number or chromosomal structure. Chemical and physical agents can induce CAs, which is carried out by different mechanisms including aneugenic and clastogenic actions. While aneugenic effect involves inactivation of a cell structure such as mitotic spindles leading to chromosomal losses, clatogenic effect is characterized by induction of chromosomal break during cell division. Nuclear abnormalities are derived from various types of CAs such as micronucleus, lobed nuclei, mini cells, nuclear buds and polynuclear cells. A number of chromosomal abnormalities are derived from nuclear abnormalities such as micronuclei, lobated nuclei, polinucleated cells, nuclear buds and mini cells. Micronucleus (Fig. 2a) may originate from all chromosomes (clastogenic agent) or from acentric fragments (aneugenic agent) not included in the main nucleus during the cell cycle [25]. Ring chromosomes (Fig. 2b) are the result of chromosome losses in the telomere domain [26]. Nuclear buds (Fig. 2c) associated with the formation of micronucleus are indicative of the initial process to discard nuclear material [23]. According to Akaneme and Iyioke [27], the presence of nuclear lesions (Fig. 2d) indicates cytological evidences for the inhibitory effect on DNA biosynthesis. Giant cells (Fig. 2h) occur due to incomplete cytoplasmic division but they grow up with nuclear division and DNA replication before they die [28]. The presence of vagrant chromosomes (Fig. m, r) means a deviation of mitotic spindle irregularity, an aberration which may result in delayed metaphase and /or prophase [29].

V. CONCLUSION

There are no literature data on the cytogenetic parameters examined in normal and saline conditions. Therefore, the results of this study have been particularly reported for the first time in normal and saline conditions. As a conclusion, this study showed that Arg can significantly increase activations such as the seed germination, seedling growth, mitotic index, MN and CAs under saline or normal conditions.

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