INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 6, p. 190-197, 2013

RESEARCH PAPER OPEN ACCESS

The effect of kinetin treatment on indices of germination and activity of canola seed enzymes under salt stress

Rasool Mohammadi Echi[1*], Davood Eradatmand Asli[2], Sied Javad Vajedi[3], Zahra Fakharian Kashani[4]

[1,3]Research scholar in Agronomy, Department of Agronomy, Saveh Branch, Islamic Azad University, Saveh, Iran

[2]Department of Agriculture, Payame Noor University, Iran

[4]Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Key words: Peroxides, germination, salinity, kinetin, canola.

doi: http://dx.doi.org/10.12692/ijb/3.6.190-197 Article published on June 22, 2013

Abstract

An experiment was conducted in agricultural plants physiologic laboratory in agriculture faculty of Islamic Azad university of Saveh branch. Improved canola seeds of Isfahan 14 cultivar were treated by chemical substance of kinetin in four levels of 5(control), 10, 15 and 20 ppm. NaCl was used in four levels of 0(control), 70,140 and 210 mg/lit for applying salt stress. During germination process traits like germination percentage, length of seedlings, length of stem, seedling fresh weight, seedling dry weight, and activity of catalase and peroxides enzymes were assessed. After statistical analysis of the studied traits in germination and growth of seedling, it was observed that by increase of salt level the percentage of germination, length of root and stem were reduced significantly. Also, kinetin increased length of stem. Enzyme activity increased under salt stress so that lowest and highest activity of catalase and peroxides enzymes was obtained in level 0 and level 4 respectively in this case. Also, the results of mean comparison showed that there is no difference among different levels of kinetin in activity of catalase and peroxides enzymes.

* **Corresponding Author:** Rasool Mohammadi Echi ✉ r.mohammadiechi@yahoo.com

Introduction

Canola (*Carthamus tinctorius* L.) is planted for extraction of oil and its pharmaceutical properties. Canola is a native plant in Iran that it resists on draught, salinity and coldness and it is important resource for production of oil (Tabrizi *et al*., 1999).In other hand, salinity of soil and irrigation water are important limiting factors in enhancement of agriculture products and considerable areas are not used due to lack of plants resistance on salt stress and lack of information about tolerance mechanism under this stress(concerning to selection of resistant cultivars).Canola resists on soil salinity to 7 dc but this level of salinity impacts on seed germination as sensitive period of growth that leads to low establishment and production of seedling and reduction of products (IREC, 2007, Homantarajan,1998).By intake of salt in seed inner tissues, water capacity is reduced and level of intake is increased and germination is reduced(Teb *et al*.,1999).The results of researches on germination of different plants show that by increase of salinity germination, length of root and length of stem and also, seedling dried weight are reduced significantly(Kaya *et al*., 2006 and Okiuo *et al*., 2005).The reason for reduction of length of stem in high concentration of salt is prevention of transferring nutrients from cotyledon to embryo (Bageri *et al*.,1988). Seed germination is determinant step of growth in plants since it assures establishment of plant and final yield. Three differentiating steps of germination are: seed swelling step that seed intakes water, delay step that enzymes are activated and growth activities are begun and finally, growth begins with lengthening of root and stem and leaving them from seed skin. This succession is controlled by water intake from external environment. Level and speed of germination is reduced by reduction of external water potential and there is a special potential for every plant that the seed is not germinated in less than this threshold (Stavir, Gupta and Kaure., 1998). Reduction of growth under stress is result of prevention of cell division, growth and both of them. These preventive effects could be result of change in hormones balance due to stress (Stavir *et al*., 1998).It was found that under unpleasant external conditions phyto hormones endogenous level is changed. Reduction of cytokines under salt and drought stress has been reported in different plants (Tsonev *et al*., 1998).Although information about hormone balance mechanism in plants is limited but it was found that concentration of cytokine and other growth regulators impact on synthesis and metabolism. So, exogenous treatment or external growth regulators as reaction factor on plants affected by stress could be used for elimination of non biologic external stress (Ranjan, Purohit and Prasad, 2003; Fahimi, 1997).This research reports the effect of kinetin on seeds germination and primary growth of seedling of canola under salt stress. This research investigates the role of external treatment of kinetin as growth regulator under salt stress and probability of return of salt stress effects in canola.

Materials and methods

Methods

The experiment was conducted in crop physiology laboratory of Agriculture faculty, Islamic Azad University, Saveh Branch, Saveh, Iran. The experiment was laid out in a completely randomized design (CRD) with three replications. The variety of seeds was Esfahan 14 prepared by seed and plant institute of Esfahan and the seeds were disinfected by sodium hypochloride solution by 5% for five minutes and then washed by distilled water for three times. The petri dishes were disinfected by oven before the experiment conduction. To treat the seeds by kinetin, the seeds were placed in the darkness for six hours at 20 ^{o}C and put in the solutions that their concentration consisted of 5 (control), 10, 15, 20 ppm. The seeds were dried in the room temperature before germination test for 36 hours (for 5 ppm of kinetin used non-treated seeds). For germination test of the treated seeds, the seeds were placed in the petri dishes (30 seeds per petri dish) with Whatman filter paper. For germination, the seeds were placed in the petri dishes in growth chamber at 25±1 ^{o}C for 14 days, irrigated daily with sodium chloride of 0 (control), 70, 140, 210 mg/lit to induce treatment of salinity stress. After that some traits were measured

like germination percentage, length of radicle and coleoptiles, fresh and dry weight of seedling, activity of catalase and proxidase enzymes. The germinated seeds were counted with the intervals of less than 12 hours to calculate the percentage of germination. For counting, the seeds were known as germinated seeds that their radicles had a length of at least 2mm. The counting continued till three consecutive days. The number of the germinated seeds was constant in each sample. Germination percentage is determined by the following formula:

$$\text{Germination percentage} = \frac{\text{number of the germinated seeds till final day}}{\text{number of whole of the seeds}} \times 100.$$

The caliper was used to determine the length of radicle and coleoptiles. Also, the samples were measured by digital scales to determine the seedling weight, as well as to determine the dry weight of seedling; the samples were placed in the oven for two days at 70 ºC, and then measured by digital scales. To measure the enzymes activity, the seedlings were maintained in frozen liquid nitrogen in freezer until the bio chemical analysis was performed.

Measurement of the catalase enzyme activity

It was carried out by using Cakmak and Horst`s (1991) method. In brief, 0.2 g of new frozen tissue was attrited and chafed in liquid Nitrogen of 3 ml buffer with 25 mm sodium phosphate, pH=6.8 at 0-4 ºC. The obtained homogeneous was centrifuged in 15000 rpm for 15 minutes at 4 ºC and the obtained solution was used to determine the activity of catalase enzyme. Decomposition of hydrogen peroxide by reduce absorption was followed in 240 nm of wavelength and for per mg of protein was expressed in enzyme extract.

Measurement of the proxidase enzyme activity

It was done by Ghanati`s (2002) method. In brief, 0.2 g of new freezed tissue in liquid Nitrogen in 0.02 M of phosphate potassium buffer, pH=6.8 at 0-4 ºC was attrited and chafed. The obtained homogeneous was centrifuged in 12000 rpm at 0-4 ºC for 15 minutes and obtained solution was used to determine activity of peroxides enzyme. Enzymatic activity was read by adding the proper amount of enzyme extract, buffer, Grayacul with finally concentration of 28 Mm and hydrogen peroxide with finally concentration of 5 mm in 470 nm of wavelength by the spectrophotometer (Cintra 6 GBS) and enzyme activity was expressed for per absorption variation by mg of protein in per minute.

The experimental data were collected and they were saved after calculating their mean in EXCEL software and analysis of variance of the traits was done. Then the data were normalized and experimental errors and also homogeneity of the experimental treatment variance were tested .In case of necessity the power of scores was converted by SAS software.

Results

The results of analysis of variance showed that different levels of NaCl affect significantly on percentage of germination inn confidence level of %1 (Table 1).

Also, the results of mean comparison of data showed that there is difference in different levels of chloride sodium on germination percentage so that highest germination level was reported in zero level (control) and lowest level was obtained in level four (table 2). Probably the reason for reduction of germination percentage due to application of NaCl is reduction of physiologic processes. So, abundance of available nutrients leads to problem and decrease of germination. The results of mean comparison of data showed that there is a difference between different levels of kinetin and germination percentage so that highest percentage was achieved in level 2 and lowest one was reported in level 4(table 2).Also, the results of mean comparison of reciprocal effect of different levels of salinity and kinetin on germination percentage show significant difference (table 2).Highest germination percentage obtained in salt level 1(control) and kinetin level 1. Probably the reason for reduction of germination percentage due to utilization of kinetin was change in membrane permissively to this substance. Also, the results of analysis of variance of this research showed that the

effect of different levels of chloride sodium on length of root and stem and canola seed was significant in %1 (Table 1).The results of mean comparison of data indicate that there is a difference in different levels of chloride sodium application and length of canola seeds. So that highest percentage of rot and stem length was achieved in salt zero level (control) and lowest one was reported in level 4 (Table 2). The results of mean comparison showed that there is a difference between different levels of kinetin and growth of stem highest stem growth obtained in kinetin level 4 and the lowest one was achieved in level 1 (table 2). Probably the reason for reduction of stem and root growth due to utilization of NaCl was reduction or lack of transfer of nutrients from cotyledon to embryo. In addition, reduction of water intake in seed under salt stress reduces hormone excretion and enzyme activities and as a result disorder in seedling growth. The results of mean comparison showed that there is difference between different levels of kinetin and growth of root. Highest stem growth obtained in kinetin level 4 and the lowest one was achieved in level 1 (table 2). Probably the reason for reduction of root growth due to utilization kinetin was reduction or lack of transfer of nutrients from cotyledon to embryo and the reason for increase of stem due to application of kinetin is increase of nutrient transfer from cotyledon to embryo.

Table 1. Analysis of variance of salinity stress and kinetin on germination indices, Catalase and Peroxidase activity of safflower.

S.O.V	df	Germination percentage	Fresh weight of seedlings	Dry weight Seedlings	Shoot length	Root length	Catalase activity	Peroxidase activity
Stress	3	**2193.5	**35746.7	**821.83	**3585.54	**4136.53	*597.91	**2249.37
kinetin	3	**2746.75	**7192.8	**704.12	**16.05	**561.76	*1194.21	**2575.31
Error	9	2.96	1.23	4.48	0.34	0.31	3.31	4.2

*, **, ns: significant at 5%, 1% level and not significant, respectively.

The results of analysis of variance of this research showed that the effect of different levels of NaCl on seedling weight and seedling dried weight of canola seeds is significant in %1(table 1).The results of mean comparison showed that there is a difference between different levels of NaCl on seedling weight and seedling dried weight so that highest weight seedling weight and seedling dried weight weight and seedling weight and seedling dried weight dried weight was achieved in salt level 1(control) and lowest weight was obtained in level 4(table 2).Probably the reason for reduction of seedling weight and seedling dried weight due to application of NaCl was reduction or lack of transfer of nutrients from cotyledon to embryo. In addition, reduction of water intake by seed under salt stress reduces cell division. Elements like cadmium and sodium reduce growth by effect of proton bombardment and disorder in system due to decrease of cell division and lengthening of cell. The results of mean comparison of data show that there is a difference between the effects of different kinetin levels on seedling weight and seedling dried weight fresh and dried weight. So that the highest seedling weight and seedling dried weight fresh and dried weight was observed in kinetin level 1 and lowest weight was reported in level 4(table 2).Also, the results of mean comparison of interactional effect of different levels of salinity and kinetin on seedling weight and seedling dried weight fresh and dried weight showed significant difference(table 2).So that highest fresh and dried weight was obtained in salinity level 1 and kinetin level 1(table 2).Probably the reason for highest fresh and dried weight of seedling weight and seedling dried weight due to application of kinetin is reduction of root length. The results of analysis of variance of this research show that the effect of different levels of NaCl on catalase and peroxides activity in canola seed was significant

in %1(table).Also; there is no significant difference between applications of kinetin in different levels in %1. The results of mean comparison indicate that there is a difference in different levels of catalase and peroxides so that the lowest and highest activity was observed in salt level 1 (control) and kinetin level 4 respectively (Table 2).The reason for increase of enzymes activity due to application of NaCl is accumulation of active oxygen in cell and damaging membrane lipid, proteins and nucleonic acids. The results of mean comparison show that there is no difference between catalase and peroxides enzyme activities in seeds(table 2).Also the results of mean comparison of reciprocal effects showed that there is no difference between different levels of salinity and kinetin on activity of catalase and peroxides (Table 2).
Table 2: mean comparison of evaluated traits in experiment of the effect of kinetin on germination and growth of canola under salt stress.

Table 2. mean comparison of evaluated traits in experiment of the effect of kinetin on germination and growth of canola under salt stress.

Treatments	stress	kinetin	Germination Percentage	Fresh weight of seedlings (mg)	Dry weight Seedlings (mg)	Shoot length (mm)	Root length (mm)	Catalase activity (1M H2 O2 min)	Peroxidase activity (OD.g^{-1} FW.min^{-1})
Stress * kinetin	0	5	92.67a	140.02a	22.66a	27.47d	52.77a	12.530d	12.39d
	0	10	88.95b	107.67c	21.81a	34.43c	49.41b	12.630d	12.39d
	0	15	85.45c	110.51b	21.49a	39.42b	47.78c	12.570d	12.26d
	0	20	75.26hi	103.91d	21.26a	45.15a	45.63d	12.390d	12.39d
	70	5	80.05f	111.57b	17.46cd	19.76f	43.88e	15.51c	19.08c
	70	10	82.20e	106.48c	18.33c	23.77e	44.38de	15.46c	19.15c
	70	15	81.69e	99.77e	18.39c	28.38d	40.56g	15.32c	19.12c
	70	20	84.04d	88.94f	18.04c	34.88c	42.3f	15.52c	19.08c
	140	5	76.09hi	87.45g	16.15de	16.43g	38.28g	18.33b	24.57b
	140	10	74.80i	85.14h	15.76e	19.42f	35.92i	18.33b	24.51b
	140	15	77.73g	81.54i	17.46cd	24.66e	35.2i	18.24b	24.68b
	140	20	76.45gh	78.62j	19.71b	28.80d	32.6j	18.21b	24.57b
	210	5	66.20kl	67.73k	11.36f	7.640i	30.46k	20.75a	28.15a
	210	10	71.00j	53.07l	11.88f	13.17h	28.39l	20.66a	28.09a
	210	15	69.90jk	45.51m	11.15f	19.21f	27.01m	20.61a	28.02a
	210	20	68.16l	43.37m	11.01f	24.32e	23.75n	20.75a	28.15a

Discussion

Decrease of cytokine indigenous levels in plants under stress refers to this possibility that reduction of cytokine limits growth in plants under stress and external application of kinetin could lead to increase seedling weight and seedling dried weight under stress (Hare *et al.*, 1997).Thus it is necessary to investigate indigenous levels of different plant hormones under different stresses in order to reach to rational conclusion. Increase of seedling weight and seedling dried weight and shoot weight under stress by kinetin could be related to increase of water intake due to permissively of membrane and osmotic active minerals inner concentration (Stavir, Gupta and Kaure, 1998).In addition to primary effects of stress, seedling weight and seedling dried weight growth is decreased because of reduction of starch movement under stress. This condition is due to reduction of amylase activity and high content of starch in cotyledon of plant under stress. Decrease in amylase

activity in seeds under stress causes to reduction of formation of glucose from starch and decreases in sucrose synthesis. This conditions lead to limitation of growth and reduction of seedling weight and seedling dried weight under stress. Kinetin increases amylases activity in seeds of plants under stress(Stavir, Gupta and Kaure, 1998).Also, these researchers found that the harmful effects of stress on seedling weight and seedling dried weight and amylase activity are returned by adding kinetin growth regulator exogenous in culture of pea seeds. These substances neutralized stress conditions and by improvement of starch metabolism and amylase activity in cotyledon increased seedling weight and seedling dried weight growth .In addition under environmental stresses like salinity, oxidative stress is applied resulted from oxygen free radicals that affect on plant growth (Smirnoff, 1993).In this experiment salt stress caused to increase of catalase enzyme while, pretreatment of seeds with kinetin prevented this enzyme. Although, high concentration hydrogen peroxide is armful and it is eliminated by enzyme catalase and ascorbic peroxide of anti oxidant ascorbic galantine cycle but in low concentration it could transfer message in message transferring processes and activities resistance genes (Foyer *et al*., 1997).Also, antioxidant enzymes patterns are changed under stress of heavy elements and other stresses by treatment of salicylic acid (Matewally, Finkemeir, Georgi and Dietz, 2003).This procedure shows that salicylic acid reduces its activity in tobacco and other plants by bounding to catalase enzyme (Chen *et al*.,1993,Sanchez-Cassas and Klessing,1994). Bor *et al*., (2003) showed that salt stress increases lipid per oxidation . In Beta vulgaris L. leaves malon di aldehid was increased in seeds under pretreatment and salt increased malon concentration (Bor, Zdemir and Tu rkan, 2003). Other researchers reported reduction of protein; enhancement of nitrate, ammonium and free amino acid under salt stress (Yonis *et al*., 1993).Decrease in protein content could be due to reduction of nitrate reductase, glutamine syntase and glutamine exgoaloglotarat amino transferase under salt stress. The studied have shown that there is reaction between kinetin endogenous levels and other herbal hormones and in some cases these reactions affect on plant growth as accelerator of physiologic substance. So it is recommended to investigate this hormone reaction as external treatment. In this experiment different growth regulator concentrations were used in different treatment concentrations then combined with different levels of NaCl as solution for irrigation. It is recommended to uses kinetin in concentration less than 10 mg/li.

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