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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2022 19(2): 1293-1300. OPEN ACCESS

Identification of moderate and drastic salinity stress-tolerant genotypes in canola cultivars using stress tolerance indicators

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Canola (*Brassica napus* L) is one of the most important oilseeds in the world. Then again, saline soils and the salinity of irrigation water are of the most important factors of ambient stress in canola production. Thus, to evaluate and identify moderate and drastic salinity tolerant genotypes in canola cultivars using experimental stress tolerance indicators on 7 canola cultivars named (Talaieh, Hayola 50, Hayola 420, Reg & cob, Opera, H6729, and T98007) in different salinities of irrigation water including (normal water (control), 5 and 10 Desi Siemens per meter) as a split-plot experiment in the format of a randomized complete blocks design with four iterations in the crop year of 2020-21 in Astara city was performed. Salinity was considered as the main factor and cultivars as the sub-factor. In this research, the highest grain yield under normal conditions was related to Hayola 420 and H6729 cultivars with an average of 2.68 and 2.44 tons per hectare, and the lowest grain yield was related to Hayola 50 with an average of 1.88 tons per hectare. The highest grain yield under moderate and drastic salinity stress was the mean (2.33 and 2.3 ton per hectare for Hayola 420) and (2.01 ton per hectare for Hayola 420, respectively), respectively and the lowest yield of the grain was related to Reg & cob under moderate salinity stress conditions with an average of 1.6 tons per hectare and T98007 under drastic salinity conditions with an average of 1.5 tons per hectare. In this study, among the cultivars evaluated, Hayola 420 and H6729 cultivars had the highest values of stress tolerance in both conditions. While cultivar T98007 showed the lowest amount of stress tolerance. In both moderate and drastic salinity stress conditions (salinity 5 and 10 Desi Siemens), very high values of correlation between STI and GMP indicators with MP indicator cause the MP indicator to be hidden in these two indicators, and that's why the figures selected based on Both STI and GMP indicators mostly use high values of MP indicator.

Keywords: Salinity stress, Canola, Tolerance indicators

INTRODUCTION

Canola (Brassica napus L.) is an annual plant of the Brassicaceae family and is called Canola in England, Canola in Canada, Rape in Germany, and Colza in France. It is known as Colza in Iran. (Aran, 2015). Canola oil has a high nutritional quality compared to the oil of other oilseed plants due to its significant content of unsaturated fatty acids and is the second most abundant oilseed in the world with a production of 56.5 million tons (Alizadeh 2021, Karasakal 2021, Karasakal 2021, Mohammadzadeh 2021, Radmanesh 2021) and because of the compatibility of this plant with the climatic conditions of most parts of Iran, the development of its cultivation is promising to supply the crude oil needed by the country and being free from dependence; So that now, canola is the gravity center of programs to increase the production of oilseeds (Arshadi Khamseh et al. 2012). The world's population is growing significantly and is expected to reach 8 billion by 2025, indicating that the world's population increasing by nearly 80 million each year (Gholamin and Khayatnezhad 2020, Karasakal, Khayatnezhad et al. 2020, Bi, Chen et al. 2021, Chen, Khayatnezhad et al. 2021, Hou, Li et al. 2021). It's anticipated that global population increase often occurs in developing countries while food problems are currently a serious problem and on the other hand, population pressure on farmlands for providing food is great (Zhu, 2001; Chinnusamy et al. , 2005 and Bybordi, and Tabatabaei, 2012). Salinity is one of the most important limiting stresses on crop production, which its domain is expanding with the increasing of the cultivated area of Faryab (Gholamin and Khayatnezhad 2020, Cheng, Hong et al. 2021, Huang, Wang et al. 2021, Liu, Wang et al. 2021, Ma, Ji et al. 2021, Ma, Khayatnezhad et al. 2021). Annually, between 250,000 and 500,000 hectares of the world's farmlands are removed from the production cycle due to salinity (Skaggs et al., 2006). Based on Momeni studies (Moameni, 2011), the area of lands with different degrees of soil salinity is 6.55 million hectares, equivalent to 34% of the total area of the country (Iran), of which 6.8 million hectares are farmlands (Gholamin and Khayatnezhad 2020, Khayatnezhad and Gholamin 2020, Guo, She et al. 2021, Peng, Khayatnezhad et al. 2021, Sun and Khayatnezhad 2021, Tao, Cui et al. 2022). For appropriate and steady use of these inputs, we have to evaluate plants in the environmental stress conditions. One of the main approaches for salinity stress conditions is to use an extensive variety of cultivars of a crop concerning salinity stress. In this method, more tolerant cultivars to be used for cultivation in areas with salinity restrictions are identified (Gholamin and Khayatnezhad 2021, Yin, Khayatnezhad et al. 2021, Zhang, Khayatnezhad et al. 2021, Zhao, Wang et al. 2021, Zheng, Zhao et al. 2021). Generally, salinity stress affects different stages of growth of crop (Khorsandi and Anagholi, 2009) and the Brassica family and canola are not spared from this rule (Hatami, 2005). Among crops, canola is known as a salt-tolerant plant (Shannon, 1998). This plant is the first crop to be cultivated in the Netherlands ' sea departed farmlands (Enferad et al., 2004). Some researchers have considered the study of adsorption mechanisms and ion accumulation patterns in different parts of canola in identifying genotypes or salt-resistant and sensitive lines so important (Pustini and Si and Semarde, 2001). The most harmful effect of salinity on canola growth is the reduction of plant size, photosynthetic level, and plant function (Gholamin and Khayatnezhad 2020, Jia, Khayatnezhad et al. 2020, Si, Gao et al. 2020, Sun, Lin et al. 2021, Wang, Shang et al. 2021, Wang, Ye et al. 2021). Salinity significantly reduced plant size, number of pods per plant, number of seeds per pod, the weight of 1000 seeds, and oil quality. Root and shoot weight of canola species also decreased due to salinity, however, there were significant differences between disrupted species (Karasakal, Khayatnezhad et al. 2020, Khayatnezhad and Gholamin 2020, Xu, Ouyang et al. 2021, Zhu, Liu et al. 2021, Wang, Ma et al. 2022). Salinity stress reduced the number of seeds in the germination stage, due mainly to the reduction of photosynthesis under the influence of salinity. The main reason for grain weight loss was osmotic and ionic stress due to salinity stress. The percentage of seed oil was not affected by salinity (Hatami, 2005). Mahmoodzadeh (Mahmoodzadeh, 2008) after evaluating two cultivars of canola Okapi and Symbol in salinity conditions stated that vegetative growth and seed function of both cultivars were not affected by salinity up to 3 Desi Siemens, but crossing the salinity threshold, the ratio of wet to dry biomass, pod weight and leaf thickness increased, while the seed weight of secondary and main pods, weight of 1000 seeds, the number of leaves and branches decreased (Alizadeh 2021, Karasakal 2021, Mohammadzadeh 2021, Radmanesh 2021). Per capita, oil consumption in the country (Ian), is more than 17 kg, so more than one million tons of oil is needed annually, of which a maximum of about 15% is supplied through domestic sources and the rest through imports sources and the outflow of large amounts of currency from our country. Canola oilseed is considered one of the important oilseeds that can adapt to different climates due to its different cultivars and types of growth and having about 45% oil and 20-25% protein in the seed can play an important role to increase oil production in Iran (Khayatnezhad and Gholamin 2021, Li, Mu et al. 2021, Shi, Khayatnezhad et al. 2021, Zhu, Saadati et al. 2021). It is essential to study its various quantitative and qualitative aspects to access the desired performance. One of the strategies to confront the problem of salinity is the cultivation of salinity tolerant cultivars. This study was performed aiming to identify moderate and drastic salinity tolerant genotypes in canola cultivars using stress tolerance indicators in Astara city.

MATERIALS AND METHODS

In this experiment, 6 cultivars of canola named (Talaieh, Hayola 50, Hayola 420, Reg & cob, Opera, H6729, and T98007) were tested in different salinity levels of irrigation water including (normal (control), 5 and 10 Desi Siemens per meter) as a split-plot experiment in the format of a randomized complete blocks design with four iterations in the crop year of 2020-21 in Astara city was performed. Salinity was considered as the main factor and cultivars as the sub-factor. After selecting the test site, soil samples were prepared by sampling from two depths of 0-30 and 30-60 cm, and physicochemical properties of soil including N, Ph, K, O.C, pH, ECe, and texture were measured. The required treatment saline water was provided from the saline river and the above source water was qualitatively examined including EC, pH, the number of required anions, and cations. The irrigation time of all plots was according to what is common among the locals. Each experimental plot had 5 lines with a length of 6 m and a row spacing of 30 cm. Also, the distance between iterations was 2.5 meters. Farm preparation processes including tillage, disc, leveler, and fertilization were carried out in early September (fertilizers were consumed based on soil tests and soil and water research department that were 90 kg/ha urea, 150 kg/ha ammonium phosphate, and 90 kg/ha Potash). Planting was done manually. To control weeds, Terflan herbicide was used one week before planting, at a rate of 2 liters per hectare, and disc operation was performed immediately to mix the herbicide and the soil. In the two-leaved stage, thinning and spacing treatments on the rows were done. To increase the plant growth rate, 100 kg/ha of urea fertilizer was used. Other agricultural cares for farms such as weeding and irrigation were done at the right time and based on climatic conditions and local customs.

Drought tolerance indicators

Measurement of stress sensitivity indicators by the performance of the case study genotypes under flood and stress conditions according to Fischer and Maurer

(Fischer and Maurer, 1978), Rosielle and Hamblin (Rosielle and Hamblin, 1981), and Fernandez (Fernandez, 1992)'s proposed relations was calculated by the following equations:

Stress Sensitivity Indicator (SSI)

This indicator was proposed by Fisher and Maurer (1978), such that the changes in potential and actual performance in different ambiances are displayed by this indicator. They offered the following formula to calculate the drought sensitivity indicator.

Stress Sensitivity Indicator SSI = (1- (Y_{Si}/ Y_{pi})) / SI

Wherein:

Y_{Si} = Hergenotype performance under stress conditions Y_{pi} = Hergenotype performance under normal conditions

SI= intensity of the stress which is defined by the following relation:

Stress intensity SI = 1- (Y_S/Y_P)

Here S and P are the average performance of all genotypes under stress and normal conditions, respectively.

According to the aforementioned formula, the smaller the stress sensitivity indicator, the lower the performance changes of a cultivar in stress conditions in comparison with the favorable conditions, and consequently, that cultivar is less sensitive to stress and is more resistant. According to Fernandez (1992), selection based on SSI leads to the choice of stress-resistant and poor performance genotypes.

Stress Tolerance Indicator (STI)

Fernandez (1992) presented the stress tolerance indicator as follows.

Stress tolerance indicator STI = (Y_{Pi}×Y_{Si}) / Y_{p2}

In which Y_{Pi} and Y_{Si} are the yield of each genotype or cultivar under normal and stressful conditions, respectively, and Y_p is the average yield of all genotypes under normal conditions. According to Fernandez (1992), this indicator can identify genotypes with a good performance in both stressful and normal environments. This indicator causes the selection of genotypes with high performance and resistance to stress.

Stress Tolerance Indicator (TOL)

Stress tolerance is calculated by the following equation:

Tolerance indicator TOL = (Y_{Pi}-Y_{Si})

This indicator was reported by Rosielle and Hamblin (Rosielle and Hamblin, 1981), and the smaller the value, the better the genotype or cultivar reacted to stress and thus had more stress tolerance.

Arithmetic mean performance (MP)

The arithmetic mean can be calculated from the following relation:

Mean performance indicator MP = (Y_{Pi} +Y_{Si}) /2

The higher the arithmetic mean of performance, the more resistant the cultivar was to stress conditions (Rosielle and Hamblin, 1981), and selection based on the arithmetic mean led to the selection of genotypes with high performance (Fernandez, 1992).

Geometric Mean Performance (GMP)

The geometric mean performance is calculated from the following formula:

Geometric Mean Indicator GMP = $\sqrt{Y_{Pi} \times Y_{Si}}$

The higher the geometric mean, the more resistant the cultivar or genotype was to stress conditions (Rosielle and Hamblin, 1981). According to Fernandez (1992), this indicator is effective in identifying plants with higher performance in stressful and flooding conditions. To determine the best indicators, the correlation between grain performance under stress and flooding conditions and different indicators was used and the indicator that had a high and significant correlation with grain performance in both conditions, considered as the best indicator.

Information analysis method

Variance analysis of the data obtained from the measurement of the studied properties was performed by MSTAT-C and SPSS-24 software and the comparison of the mean values was performed with Duncan's method at a probability level of 5%. Correlation between the studied properties was performed by SPSS-24 software. Graphs were drawn using Excel software.

RESULTS AND DISCUSSION

The values of total performance and mean grain performance of the evaluated cultivars under normal, moderate, and drastic salinity stress (salinity of 5 and 10 Desi Siemens) conditions Have been mentioned in Table 1. In this research, the highest grain performance under normal conditions, with mean values of 2.68 and 2.44 tons per hectare were related to Hayola 420 and H6729 cultivars, and the lowest grain performance with a mean value of 1.88 tons per hectare belonged to Hayola 50. The highest grain performance under moderate and drastic salinity stress (salinity of 5 and 10 Desi Siemens) belonged to Hayola 420 cultivar and Hayola 420 cultivar respectively with mean values of (2.33 and 2.3 tons per hectare) and (2.01 ton per hectare), and the lowest grain performance in moderate salinity stress condition with a mean value of 1.6 tons per hectare was related to Reg & cob and in drastic conditions with a mean value of 1.5 tons per hectare was belonged to T98007.

Table 1: Grain performance and mean grain performance of cultivars under control, moderate and drastic salinity stress conditions (salinity of 5 and 10 Desi Siemens)

Genotypes	*Salinity 5 dS*				*Salinity 10 dS*			
	Yp	*Ys*	$\bar{Y}_P$	$\bar{Y}_S$	*Yp*	*Ys*	$\bar{Y}_P$	$\bar{Y}_S$
Talayeh	2.13	1.95	2.11	1.93	2.13	1.65	2.11	1.72
Hayola 50	1.88	1.75	2.11	1.93	1.88	1.52	2.11	1.72
Hayola 420	2.68	2.33	2.11	1.93	2.68	2.01	2.11	1.72
Opera	2.15	1.97	2.11	1.93	2.15	1.88	2.11	1.72
Reg&cob	1.75	1.60	2.11	1.93	1.75	1.52	2.11	1.72
T98007	1.72	1.62	2.11	1.93	1.72	1.50	2.11	1.72
H6729	2.44	2.30	2.11	1.93	2.44	1.98	2.11	1.72

Y_p: Grain performance of a cultivar under non-stress conditions (normal),

Y_S: grain performance of a cultivar under moderate and drastic salinity stress conditions (5 and 10 Desi Siemens)

$\bar{Y}_p$: Mean grain performance of all cultivars under non-stress conditions (normal) $\bar{Y}_S$:Mean grain performance of all cultivars under moderate and drastic salinity stress conditions (5 and 10 Desi Siemens)

In this experiment, using of the SSI indicator showed that the highest tolerance to salinity stress was related to the T98007 cultivar with the lowest values of (0.05 and 0.102) in moderate and drastic stress conditions, respectively. Was among the cultivars studied. In this study, the use of the TOL indicator showed that T98007 cultivar with the lowest value of (0.1 and 0.22) and Hayola 420 cultivar with the highest value of (2.33 and 2.01), respectively had the highest and lowest tolerance to salinity stress among cultivars under both moderate and drastic dehydration conditions (Tables 2 and 3). In evaluating cultivars using the TOL indicator according to the formula of this indicator, a high TOL value indicates more changes in grain performance under stress and non-salinity stress conditions and shows the sensitivity of cultivars to salinity stress conditions. Based on the TOL indicator, more relative tolerance belongs to a cultivar that has a smaller TOL. Thus, the selection for stress tolerance is associated with a minimal difference between YS and YP. The higher the MP indicator values, the higher the stress tolerance of that cultivar. In the current study, the highest mean performance indicator in both moderate and drastic conditions (salinity of 5 and 10 Desi Siemens) has belonged to Hayola 420 cultivar and H6729 cultivar with mean values of (2.5 and 2.35), and (2.21 and 2.37), respectively; which showed more tolerance to salinity stress conditions among the other evaluated cultivars. Also, the amount of this stress tolerance in drastic stress is estimated slightly higher than that in moderate stress (Tables 2 and 3). Among the cultivars studied in both conditions, Hayola 420 and H6729 cultivars had the highest geometric mean productivity. However, the T98007 cultivar with mean values of 1.67 and 1.61 had the smallest geometric mean productivity (Tables 2 and 3).

According to the results of various researchers, the best indicator for selecting cultivars is the stress tolerance indicator; Because it can separate cultivars that have a high performance in both stress and non-stress conditions (group A), from two groups of cultivars that have high performance only in non-stress conditions (group B) or only in stress conditions (group C) (Khayatnezhad and Nasehi 2021, Li, Khayatnezhad et al. 2022, Wang, Khayatnezhad et al. 2022). In this study, among the evaluated cultivars, Hayola 420 and H6729 cultivars had the highest values of stress tolerance in both conditions. While T98007 cultivar showed the lowest amount of stress tolerance (Tables 2 and 3). There was a positive and significant correlation between grain performance under normal conditions and moderate and drastic salinity stress (salinity of 5 and 10 Desi Siemens) and MP, GMP, and STI indicators. However, there was a negative and non-significant correlation between grain performance under moderate and drastic salinity stress (salinity of 5 and 10 Desi Siemens) and stress sensitivity indicator (SSI) (Khayatnezhad and Gholamin 2021, Ren and Khayatnezhad 2021, Zhang, Khayatnezhad et al. 2022). In contrast, a positive and non-significant correlation was observed between SSI and TOL indicators (Table 4-12-4-13). At the same time, in both moderate and drastic salinity stress conditions (salinity of 5 and 10 Desi Siemens), very high values of correlation between STI and GMP indicators with MP indicator cause the MP indicator to be hidden in these two indicators, and therefore the cultivars selected based on STI and GMP indicators, mainly had high values of MP indicator.

Table 2- Different indicators of cultivar stress tolerance under control and moderate salinity stress (salinity of 5 Desi Siemens)

Genotypes	*Yp*	*Ys*	TOL	MP	GMP	STI	SSI
Talayeh	2.13	1.95	0.18	2.04	2.04	0.93	0.077
Hayola 50	1.88	1.75	0.13	1.81	1.82	0.74	0.063
Hayola 420	2.68	2.33	0.35	2.50	2.51	1.40	0.119
Opera	2.15	1.97	0.18	2.06	2.06	0.95	0.077
Reg&cob	1.75	1.60	0.15	1.67	1.68	0.63	0.078
T98007	1.72	1.62	0.10	1.67	1.67	0.63	0.050
H6729	2.44	2.30	0.14	2.37	2.37	1.26	0.052

Ys: Yield Stress; Yp: Yield productivity; SSI: Stress susceptibility Index; TOL: Tolerance; MP: Mean productivity; GMP: Geometric Mean productivity; STI: Stress Tolerance Index

Table 3: Different indicators of cultivar stress tolerance under control and drastic salinity stress (salinity of 10 Desi Siemens)

Genotypes	*Yp*	*Ys*	TOL	MP	GMP	STI	SSI
Talayeh	2.13	1.65	0.48	1.89	1.87	0.79	0.184
Hayola 50	1.88	1.52	0.36	1.70	1.69	0.64	0.156
Hayola 420	2.68	2.01	0.67	2.35	2.32	1.21	0.204
Opera	2.15	1.88	0.27	2.02	2.01	0.91	0.104
Reg&cob	1.75	1.52	0.23	1.64	1.63	0.60	0.107
T98007	1.72	1.50	0.22	1.61	1.61	0.58	0.102
H6729	2.44	1.98	0.46	2.21	2.20	1.09	0.154

Ys: Yield Stress; Yp: Yield productivity; SSI: Stress susceptibility Index; TOL: Tolerance; MP: Mean productivity; GMP: Geometric Mean productivity; STI: Stress Tolerance Index

Table 4: Correlation between different indicators of cultivar stress tolerance under control and moderate salinity stress (salinity of 5 Desi Siemens)

	Ys	**Yp**	**TOL**	**MP**	**GMP**	**STI**
Ys	1					
Yp	0.985**	1				
TOL	0.0.422	0.325	1			
MP	0.986**	0.968**	0.421	1		
GMP	0.992**	0.957**	0.402	0.948**	1	
STI	0.995**	0.975**	0.358	0.965**	0.985**	1
SSI	-0.32	-0.365	0.705	-0.3	-0.21	-0.345

***, ** Significant at the probability level of five and one percent**

Table 5 :Correlation between different indicators of cultivar stress tolerance under control and drastic salinity stress (salinity of 10 Desi Siemens)

	Ys	**Yp**	**SSI**	**TOL**	**MP**	**GMP**
Ys	1					
Yp	0.98**	1				
SSI	0.521	0.52	1			
TOL	0.952**	0.954**	0.61	1		
MP	0.96**	0.945**	0.55	0.95**	1	
GMP	0.985**	0.965*	0.58	0.95**	0.94**	1
STI	0.215	0.115	0.64	0.28	0.31	0.26

***, ** Significant at the probability level of five and one percent**

CONCLUSION

In this study, among the evaluated cultivars, Hayola 420 and H6729 cultivars had the highest values of stress tolerance in both conditions. While T98007 cultivar showed the lowest amount of stress tolerance. In both moderate and drastic salinity stress conditions (salinity of 5 and 10 Desi Siemens), very high values of correlation between STI and GMP indicators with MP indicator cause the MP indicator to be hidden in these two indicators, and therefore the cultivars selected based on Both STI and GMP indicators mainly had high values of MP indicator.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest

ACKNOWLEDGEMENT

This paper was from University project 5849 YBU-5012, 2021.

AUTHOR CONTRIBUTIONS

Arda Karasakal conducted, planned, Analyzed the data, wrote manuscript and interpreted the results and involved in manuscript preparation. All authors read and approved the final version.

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