

# Genetic evaluation of some safflower genotypes under salinity stress

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**Abstract:** In the context of the increasing demand for cultivation of marginal lands affected by salinity, it became necessary to assess the most important crop traits that can be relied upon as well as to identify genotypes that have the potential to withstand adverse environmental conditions. In this study, ten genotypes of safflower were evaluated under two salinity levels during two successive agricultural seasons. According to correlation and path coefficient analysis and positive direct effect of studied traits, 100 seed weight followed by number of capitulum per plant strongly related to seed yield. Also, the results confirmed based on tolerance indices to salt stress, that line 6 was followed by local cultivar recorded the highest values as an indicator of tolerance. Protein analyses were conducted to identify protein markers associated with salinity tolerance for selection of promising lines tolerant to salt stress. This investigation revealed substantial polymorphism in protein markers that could be useful tools to assist breeders in the selecting and breeding of safflower lines tolerant to salinity stress.

**Keywords:** safflower, salinity, path coefficient, tolerance indices, protein marker.

## Introduction

The cultivated area of arable land is decreasing as a result of unsustainable agriculture, climate change, and soil degradation (Lobell et al., 2008). Both salinity and drought are the most dangerous abiotic stresses that threaten the productivity of diverse crops around the world (Guo et al., 2014). Based on these environmental changes, plant breeders work to develop and identify the genotypes that are more capable of tolerating salinity and water stress (Janmohammadi et al., 2008). In general, it is possible to benefit from the use of the lands affected by the high salt content by cultivating crops that tolerate salinity. However, it is necessary to assess the variation within and between genotypes available for tolerance to salinity. For example, this has been done in different types of crops such as cotton, *Brassica napus* (Akram and Jamil, 2007) and Safflower (Siddiqi et al., 2007).

Safflower (*Carthamus tinctorius* L.) is a diploid ( $2n = 24$ ) and belonging to oilseed crops because its seeds contain an oil percentage, ranging from 32 to 40% (Weiss, 1983). Due to the tolerance of safflower to salt stress compared to commonly cultivated oilseed crops, it is usually grown in dry and semi-arid regions where soil salinity is one of the most important areas a threat to agriculture (Kaya, 2009). Simple correlation analysis is not sufficient to give an accurate indication of the relative importance of the crop, so path analysis is an important tool to assist plant breeders in dividing the correlation coefficient into direct and indirect contributions, which gives a better insight about character and its relationship with the yield. Biochemical markers like proteins are considered a powerful tool for assessment of crop germplasm, characterization of genetic diversity and determination of differentiation among crop plants (Jan et al., 2017; Ibrar et al., 2018). Assessments of genetic diversity based on biochemical markers of different crops have been studied by different researchers. Akbar et al. (2012) used SDS-PAGE technique to study genetic diversity of *Sesamum indicum* for total seed protein and got satisfactory results. Likewise, Zada et al. (2013) utilized this method to study protein based genetic diversity in *Brassica carinata* (Ethiopian mustard) germplasm.

This study aims to select the best traits in the selection process for the yield as well as the

selection of the best genotypes to tolerate salt stress through some indications represented by path coefficient analysis, tolerance indices, and genetic expression of the tested genotypes of safflower.

## Materials and Methods

### Experimental conditions

The field experiments were carried out during the two growing seasons 2017/2018 and 2018/2019 in the experimental farm of Ras-Sudr Research Station Desert Research Center, South Saini Governorate. Nine lines of safflower (*C. tinctorius* L.) were obtained from International Center for Bio-saline agriculture (ICBA), and a local cultivar obtained from the Agriculture Research Center (ARC), Egypt (Table 1). The genotypes were grown under two levels of salinity (75 and 150 mM NaCl). The experiments were carried out in a split-plot design where salinity levels in main plots and safflower genotypes in sub-plots with three replicates. The t-test and least significant difference (LSD) test were used to compare salinity levels and genotype means at 0.05% level respectively. The soil sample and irrigation water were collected from the experiment wells and analyzed as shown in Table 2. With regard to meteorology, the lowest temperature (9.46 C°) was recorded during January, while the highest temperature was recorded during November and April (29.23 and 30.80 C°, respectively), and the relative humidity percentage ranged from 66.87 in December to 88.01% in April. On the other hand, the lowest wind speed was in January (19.58), while the highest wind speed was recorded in March (35.52 km/h). The average rainfall was monitored during the two growing seasons and ranged from 0 to 5 mm.

### Measurements

At harvesting, a number of measurements were recorded as follow: plant height (cm), capitulum length (cm), capitulum diameter (cm), No. of seeds/capitulum, No. of capitulum / plant, 100 seed / plant and seed yield/plant (g).

### Parameters

Correlation and path coefficient analysis using SPSS/AMOS program. Tolerance indices were used as follow:

Salinity tolerance index (STI);  $STI = (Y_n) \times (Y_s) / (\bar{Y}_n)^2$  (Fernandez 1992).

**Table 1.** The describe of tested genotype based on pedigree, institution and origin.

Name	Pedigree	Institution offered	Origin
Line 1	(PI 167390)	ICBA	UAE
Line 2	(PI 173885)	ICBA	UAE
Line 3	(PI 181866)	ICBA	UAE
Line 4	(PI 199892)	ICBA	UAE
Line 5	(PI 239707)	ICBA	UAE
Line 6	(PI 248836)	ICBA	UAE
Line 7	(PI 250714)	ICBA	UAE
Line 8	(PI 250840)	ICBA	UAE
Line 9	(PI 251267)	ICBA	UAE
Giza 1	(local cultivar)	Agricultural Research Center	Egypt

UAE: United Arab Emirates, International Center for BiosLine Agriculture: ICBA

**Table 2.** Chemical analysis of soil and irrigation water in two wells.

Well	Irrigation water analysis									
	pH	Salt mM	Cations ( meq/L)				Anions ( meq/L)			
			Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
First well	7.81	75	10.8	7.15	53.6	0.35	-	5.30	39.1	26.8
Second well	7.66	150	19.3	13.8	105.1	0.90	-	7.50	93.1	38.7
Soil	Soil analysis									
	7.77	106	4.6	3.2	88.3	0.67	-	4.95	65.7	26.1

Yield injury % (YI);  $YI = (Y_n - Y_s) / Y_n \times 100$  (Blum et al., 1983). Superiority measure (SM);  $SM = Y_s / Y_n$  (Lin & Binns 1988). Relative performance (RP);  $RP = (Y_s / Y_n) / (\bar{Y}_s / \bar{Y}_n)$  (Abo-Elwafa and Bakheit, 1999). Where,  $Y_n$  = yield of genotype under normal conditions;  $Y_s$  = yield of genotype under salinity stress conditions;  $\bar{Y}_n$  = mean yield of all genotypes under normal conditions;  $\bar{Y}_s$  = mean yield of all genotypes under salinity stress.

Protein pattern was studied using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). It was washed till such time till the color of background disappeared and bands could be observed. The protein bands were scored as 0 for absence or 1 for presence for polymorphism. The similarity matrix was calculated using NTSYS-pc version 2.02e and this similarity matrix was used in cluster analysis using an unweighted pair group method with arithmetic averages (UPGMA) to obtain a dendrogram.

## Results

t test in Table 3 showed that there were significant differences between the two salinity levels under this study, as well as clear differences between the genotypes tested under the conditions of the experiment for all the traits that were monitored depending on the values of least significant difference (L.S.D) at 0.05% level.

### Correlation and path coefficient analysis

The estimation of correlation coefficient between the studied traits and the yield is one of the important implications in plant breeding for direct or indirect selection. Therefore, it is of great value in guiding the procedures of the breeding program. The results of the correlation between the traits in Table 4 showed that both the weight of 100 seeds and the number of capitulum / plant had the highest correlation with the seed yield / plant (0.667 and 0.521, respectively).

Path coefficient analysis can be used to divide the correlation into direct and indirect effects of the traits used in contributing to the seed yield as a

useful criterion in selection through the different traits of the safflower plant. Path coefficient analysis was performed by looking at the traits associated with the seed yield as shown in Table 5. The results indicated that the highest positive direct effect for the seed yield was recorded by the weight of 100 seeds (0.537). While, both number of seeds/capitulum and plant height were negative direct effects however these traits can be benefited from it using indirect positive selection, passing through of the weight of 100 seed followed by

number of capitulum / plant and as showed in Table 5.

#### *Tolerance indices*

The evaluation of salinity tolerance index in Table 6 confirms that the genotypes can be divided into two groups based on the general mean (0.631), the first group is tolerant to salinity, and genotype 10 ranked the first place in as tolerant to stress followed by genotype 9 while, the second sensitive group, genotype 4 occupies the highest place of sensitivity to salinity (0.351).

**Table 3.** Means of some yield-related traits for two salinity levels and ten genotypes across two seasons.

	Plant height (cm)	Capitulum length (cm)	Capitulum Diameter (cm)	No. of seeds/capitulum	No. of capitulum / plant	100 seed weight (g)	Seed yield/ plant (g)
<b>Salinity levels</b>							
Low salinity	73.017	2.266	2.436	31.858	12.602	5.406	46.490
High salinity	63.683	1.907	2.070	21.850	5.686	4.584	34.836
t-test (0.5)	*	*	*	*	*	*	*
<b>Genotypes</b>							
Line 1	64.17	2.158	2.200	16.63	6.822	4.861	38.51
Line 2	75.08	2.417	2.283	24.08	8.220	4.812	40.48
Line 3	67.92	2.158	2.117	27.75	8.211	4.269	38.55
Line 4	67.08	1.858	2.217	29.67	9.872	5.185	47.02
Line 5	61.08	2.425	2.092	25.50	8.687	5.264	42.51
Line 6	74.08	2.000	2.100	19.75	8.669	5.418	37.66
Line 7	70.08	2.183	2.483	36.67	9.142	4.666	33.08
Line 8	67.17	2.117	2.258	20.67	7.807	4.683	44.29
Line 9	67.17	2.117	2.483	41.00	7.955	5.114	31.68
Giza 1	69.67	1.908	2.300	26.83	10.87	5.679	52.86

**Table 4.** Correlation coefficient among studied traits under overall treatments and seasons.

Trait	Plant height	Capitulum length	Capitulum Diameter	No. of seeds/capitulum	No. of capitulum / plant	100 seed weight
Capitulum length	0.360*					
Capitulum diameter	0.400*	0.476*				
No. of seeds/capitulum	0.185 <sup>ns</sup>	0.288*	0.632*			
No. of capitulum/ plant	0.466*	0.488*	0.603*	0.493*		
100 seed weight	0.243*	0.335*	0.390*	0.311*	0.532*	
Seed yield/ plant	0.241*	0.424*	0.323*	0.136 <sup>ns</sup>	0.521*	0.667*

<sup>ns</sup>: not significant; \* significant at the 0.05 level of probability, respectively.

**Table 5.** Partition of correlation coefficients into direct and indirect effect for mean seed yield under.

Trait	Direct effect	Indirect effect					Correlation with	
		Plant height	Capitulum length	Capitulum diameter	No. of seeds/capitulum	No. of capitulum / plant		100 seed weight
Plant height	-0.047		0.067	0.011	-0.041	0.121	0.130	0.241
Capitulum length	0.186	-0.017		0.013	-0.063	0.126	0.180	0.424
Capitulum diameter	0.027	-0.019	0.088		-0.139	0.156	0.209	0.323
No. of seeds/capitulum	-0.220	-0.009	0.054	0.017		0.128	0.167	0.136
No. of capitulum / plant	0.259	-0.022	0.091	0.016	-0.108		0.285	0.521
100 seed weight	0.537	-0.011	0.062	0.010	-0.068	0.138		0.667

With regard to the percentage of yield injury, we note that there is a large variation between the tested genotypes and the least percentage of yield injury was in the genotype 6 followed by genotype 10 while both genotypes 1 and 7 recorded the highest of yield injury (73.92, and 51.62 %, respectively) under the high salinity level compared to low salinity level. It is evident from the data in Table 6 for the tolerance indices that the results of superiority measure (SM) and relative performance (RP) are completely in harmony with the results of the yield injury (%) for all genotypes tested under conditions of salt stress.

#### SDS-PAGE for Protein electrophoresis

A total of 45 protein bands were detected as a result of SDS-PAGE technique. On the basis of these proteins ten safflower Lines (Table 7) Figure

1 were evaluated under salinity conditions. Out of these, 5 protein bands were consistently present throughout the genotypes and considered as monomorphic while 40 (89%) protein subunits reflected variations and were polymorphic. Size of the protein bands ranged from 14.787 to 450.871kDa. Lines with minimum proteins bands were Giza 1, Line8, Line5 and Line1. They have 6, 9, 10, and 10 protein bands, respectively. Some lines showed maximum protein bands, such as line9 and line2 showed 14 and 18 bands, respectively. Genotypes of line10 and line9, regarded as salt tolerant, exhibited a high number of specific bands with different molecular weights, which appeared in the salt treatment. These bands could be considered as a positive biochemical marker for salt tolerance.

**Table 6.** Salinity tolerance indices of tested genotypes grown under low salinity and high salinity levels for seed yield during two seasons.

Genotype	Salinity tolerance index (STI)			Yield injury (YI) (%)			Superiority measure (SM)			Relative performance (RP)		
	2107	2018	Mean	2107	2018	Mean	2107	2018	Mean	2107	2018	Mean
Line 1	0.638	0.610	0.624	72.77	75.06	73.92	0.272	0.249	0.261	0.421	0.398	0.410
Line 2	0.628	0.579	0.604	30.18	29.65	29.92	0.698	0.704	0.701	1.078	1.122	1.100
Line 3	0.603	0.557	0.580	37.01	44.29	40.65	0.630	0.557	0.594	0.973	0.888	0.931
Line 4	0.880	0.909	0.895	42.70	43.14	42.92	0.573	0.569	0.571	0.885	0.907	0.896
Line 5	0.676	0.607	0.642	27.41	21.21	24.31	0.726	0.788	0.757	1.121	1.256	1.189
Line 6	0.480	0.463	0.472	04.76	9.20	06.98	0.952	0.908	0.930	1.471	1.448	1.460
Line 7	0.456	0.458	0.457	50.39	52.85	51.62	0.496	0.471	0.484	0.766	0.752	0.759
Line 8	0.695	0.704	0.700	16.34	19.87	18.11	0.837	0.801	0.819	1.292	1.278	1.285
Line 9	0.369	0.332	0.351	19.62	21.36	20.49	0.804	0.786	0.795	1.241	1.254	1.248
Giza 1	0.985	0.992	0.989	15.72	18.12	16.92	0.843	0.819	0.831	1.302	1.305	1.304
Mean	0.641	0.621	0.631	31.69	33.47	32.58	0.683	0.665	0.674	1.055	1.061	1.058

Table 7. SDS-PAGE pattern of total proteins of ten safflower Lines.

Parameters	Number
Monomorphic bands	5
Polymorphic (without Unique)	8
Unique bands	32
Polymorphic (with Unique)	40
Total number of bands	45
Polymorphism (%)	89%

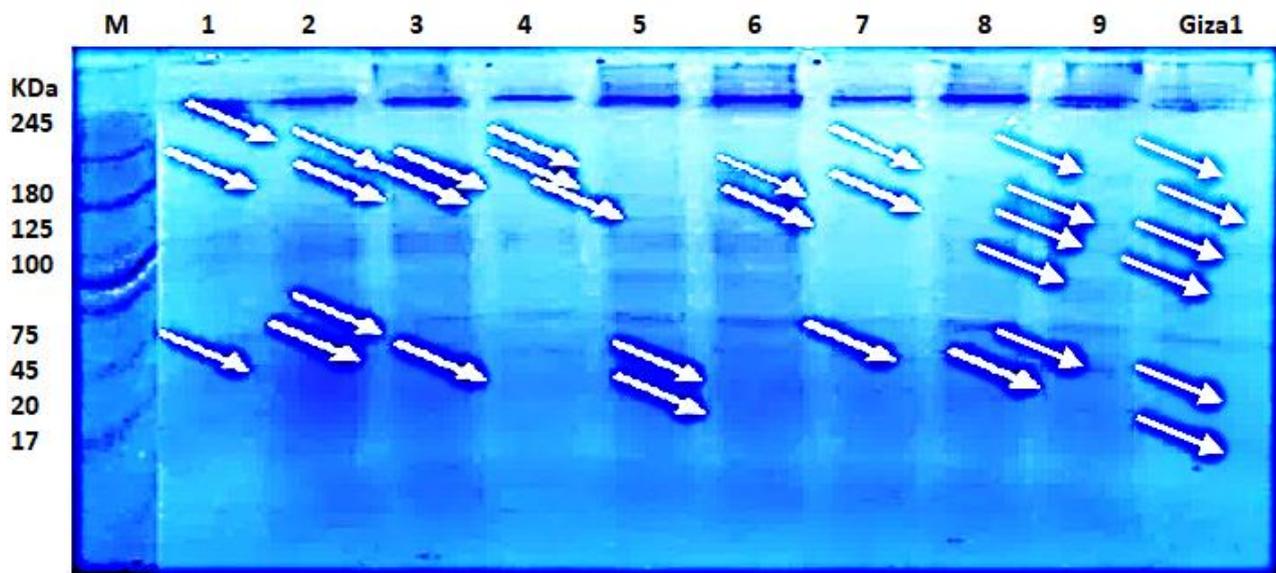


Figure 1. Protein profile of ten safflower lines using SDS-PAGE technique.

A dendrogram, representing the relationships among the genotypes of ten lines of safflower, indicated that lines 10 and line 9 were the most diverse among the studied lines with tolerance to salinity (Figure 2). The dendrogram separated the ten lines of safflower into two main clusters, first cluster included line 1 by itself and the second cluster included the remain lines.

## Discussion

The context of increasing the demand for the cultivation of salt-affected lands, it has become necessary to focus on the most important plant traits that can be relied upon for selection for high yield, directly or indirectly, depending on the correlation and path coefficient analysis. It is also necessary to determine the genotypes of crop that have the ability to resist harsh environmental changes, depending on tolerance indices and gene expression.

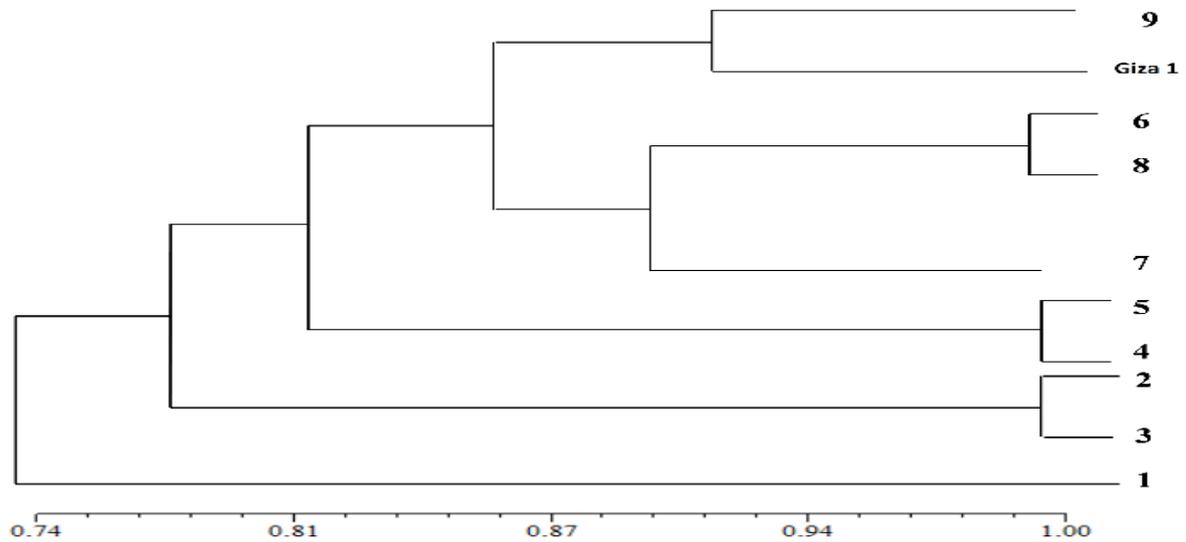
## Correlation and path coefficient analysis

Dewey and Lu (1959) asserted that simple correlation coefficient analysis to elucidate the relationship between a single variable and grain yield may not provide a complete understanding of the selection process. Also, Badran et al., 2015 stated that reliance on the direct selection of the grain yield is very difficult because it depends on a large number of genetic factors, so it is necessary to rely on the components of the yield because these variables are controlled through limit genetic factors and this facilitates direct or indirect selection of grain yield through it.

By discussing the results in Table 5, it is evident that the direct effect of 100 seed weight followed by the number of capitulum / plant contributes to the seed yield / plant is greater than the direct effect of other traits indicating that direct selection through them may be good traits for improving the seed yield / plant. It is also possible to depend

on the other traits through indirect selection using 100 seed weight / plant to improve the seed yield/ plant. The previous discussion is in harmony with those by Singh and Chaudhary (1977), who

reported that if the correlation between a causal trait and the effect is almost equal to its direct effect, then correlation explains that a direct selection through this trait will be effective.



**Figure 2.** Dendrogram representing the relationships among ten Lines of safflower based on similarity matrix derived from protein profile.

#### *Tolerance and sensitive indices*

This investigation separated the tested safflower genotypes to tolerant and sensitive under low salinity and high salinity levels based on seed yield. Assessment based on the salinity tolerance index (STI and means productivity (MP) are a reliable predictor for determining high-yielding genotype of crops under high stress conditions compared to low stress or normal conditions according to previous report (Pourdad, 2008) on safflower. While, Ramirez-Vallejo and Kelly (1998) on common bean and Guttieri et al. (2001) on wheat relied on the stress sensitivity index (SSI) to identify high yield genotypes to improve resistance to abiotic stresses in crops. Therefore, I believe that it is better to rely on more than one of the environmental stress tolerance indices for classifying genotypes tested under stress conditions compared to optimal conditions or less stress. These previous findings are in harmony with Fernandez (1992) and Badran (2015) who reported this, genotypes are classified into four groups according to their performance under non-stress and water stress conditions.

#### *Protein analysis under salinity stress*

Plants have different types of proteins on the basis of which they are diverse from each other and this could be a source of identifying diversity at protein level using SDS-PAGE (Shah, 1999). SDS-PAGE technique is mostly thought as a reliable mean and has proved to be an important way of revealing the differences and relations between and within taxa (Iqbal et al., 2005). Few investigations showed diversity in the germplasm of safflower (Ramirez-Vallejo and Kelly, 1998; Mukhlesur and Hirata, 2004; Ali et al., 2007). Žilić et al. (2010) also reported a very similar proteins profiling pattern in sunflower protein bands. One possible explanation for appearance of some proteins under salt stress is that the gene(s) responsible for certain proteins had been completely enhanced as a result of stress (Harb et al., 2010). Our results are parallel with those of several authors, who used some total protein pattern electrophoresis variations to evaluate some cultivars under different salt stress to be used as biochemical genetic markers for early evaluation (Sayed and Gabr, 2013; Mahgoub et al.,

2016). The present results indicates that SDS-PAGE technique succeed in presenting a means for studying genotypes discrimination based on genetic variation in proteins as useful methods to detect specific markers for salinity tolerance, in the genotypes of safflower under salinity conditions.

### Conclusion

Our research work will provide valuable information on safflower breeding and genetic variation to researchers and genetic resources managers working with this crop. Considering these facts, correlation and path coefficient analysis as well as tolerance indices of ten lines of safflower, demonstrate a strong correlation with biochemical proteins markers indicating significant polymorphism. This offers a promising avenue for identifying specific markers related to salinity tolerance and constructing adaptive strategies for salt stress. Identification of tolerance indices and specific positive markers could greatly benefit breeding programs by enabling the prediction of the most tolerant cultivars.

### Supplementary Materials:

No supplementary material is available for this article.

### Author Contributions:

AEB wrote the initial draft of this manuscript and conceived the original ideas. AEB and RMK designed the research and AEB and EM conducted the experiment and analysis. AEB, RMK and EM contributed to idea refinement, writing, and revising of the manuscript. AEB supervised the work and AEB and RMK revised and finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

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### Conflicts of Interest:

The authors have no conflict of interest.

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# ارزیابی ژنتیکی برخی از ژنوتیپ‌های گلرنگ تحت تنش شوری

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**چکیده:** با توجه به افزایش تقاضا برای کشت در اراضی حاشیه‌ای تحت تأثیر شوری، ضروری است تا مهمترین صفات زراعی موثر و همچنین ژنوتیپ‌هایی با پتانسیل مقاومت بالا در برابر شرایط نامساعد محیطی شناسایی شوند. در این مطالعه ۱۰ ژنوتیپ گلرنگ در دو سطح شوری در دو فصل زراعی مورد ارزیابی قرار گرفتند. با توجه به تجزیه همبستگی و ضریب مسیر و اثرات مستقیم مثبت صفات مورد مطالعه، وزن صد دانه و بعد از آن تعداد طبق در بوته، با عملکرد دانه در ارتباط بودند. همچنین نتایج شاخص‌های تحمل به تنش شوری نشان داد که لاین شماره ۶ و بعد از آن رقم محلی بالاترین شاخص‌های تحمل را به خود اختصاص داده بودند. ارزیابی پروتئین‌ها برای شناسایی نشانگرهای پروتئینی مرتبط با تحمل به شوری جهت انتخاب لاین‌های امید بخش مقاوم به تنش شوری نیز انجام شد. چند شکلی بالایی در نشانگرهای پروتئینی مشاهده شد که می‌توان از آن به عنوان ابزار مولکولی کارا جهت انتخاب لاین‌های متحمل به تنش شوری در گلرنگ استفاده نمود. از نتایج این تحقیق می‌توان در برنامه‌های اصلاحی شناسایی و انتخاب ژنوتیپ‌های متحمل به تنش شوری بهره‌مند شد.

## تاریخ

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**کلمات کلیدی:** گلرنگ، شوری، ضریب مسیر، شاخص‌های تحمل، نشانگر پروتئینی.

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