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Estimates of Genetic Parameters for Seed Germination of Safflower in Different Salinity Levels

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Abstract: This study carried out to clear the effects of NaCl salinity on seed germination characteristics of different generation progenies in safflower (*Carthamus tinctorius* L.). Seeds of parents, F₁ and F₂ generations of 3 crosses of Safflower along with their reciprocals were germinated in three salinity levels of 0, 0.5 and 1% of NaCl. The percent and rate of germination and also seedling root length were measured. The effect of genotype, salinity and the genotype × salinity level interaction were highly significant for all of the traits. Estimates of heterosis (based on high parent) and inbreeding for germination percent in all crosses and salinity levels varied from -7.4 to 9.7% and -4.4 to 4.3%, respectively. These estimates ranged from -10.4 to 41.9% and -10.4 to 24.5% for rate of germination and from -17.3 to 37.0% and -27.0 to 29.3% for seedling root length, respectively. For salinity levels of 0.5 and 1% hybrid seeds were superior to their parent's in terms of percent and rate of germination and also length of seedling root.

Key words: Safflower, NaCl, seed germination, heterosis

INTRODUCTION

Safflower, an annual plant adapted to arid and semi-arid climates and also saline soils, produces high quality edible oil that not utilized to its potential. Iran was one of the centers of safflower production in the Old World^[1]. Safflower is grown in many areas of Iran where accumulation of salts in irrigated soils may reduce seed germination and depress seedling growth. Genetic information regarding seed germination and related traits can help improve seedling emergence in saline soils through breeding programs.

Genetic factors influencing seed vigor have been investigated in some small grains^[2], but not in safflower. Li and Rutger^[3] reported that both additive and dominance effects of the genes were important in seedling vigor of rice (*Oryza sativa* L.), although additive effects were significantly larger than dominance in both F₁ and F₂ generations. Non-additive gene actions for seed weight has been reported by Ehdaie and Ghaderi^[4] in safflower. Also, it was shown that seedling emergence was correlated with seedling weight and seed size which affect seedling vigor in Sand Bluestem (*Andropogon ahallii* Hack.)^[5]. Kotecha and Zimmerman^[6] showed that overdominance effects of the genes were more important for lower seed germination in wild safflower

(*C. palastinus*). Francois and Bernstein^[7] found that poor quality of seed might have been responsible for the inferior germination and growth of safflower when salty irrigation water was used. Ghorashy *et al.*^[8] found that a local Iranian genotype of safflower had the least reduction of germination compared to the other genotypes at NaCl concentrations of greater than 1%.

Many of the genetic studies in safflower have been directed to yield and its components^[4,9,10], but there is not enough genetic information regarding to the factors which control germination of safflower seed, especially in saline environments. The main objectives of this study were to estimate heterosis and inbreeding depression, investigate the types of gene actions for percent and rate of seed germination, seedling root length and also seed weight in safflower.

MATERIALS AND METHODS

The seeds of crosses IUTK₁₁₅ × IUTM₁₂ (P₁ × P₂), IUTH₁₃ × IUTE₁₄₄₉ (P₃ × P₄), along with their reciprocals and also seeds of the cross IUTH₁₃ × IUTM₁₂ (P₃ × P₂) were used in this study. All the parents were breeding lines that selected from various local populations of safflower in Iran. The crosses were made in Research Farm of Isfahan University of Technology in 2001. Except for

crosses ($P_4 \times P_3$) and ($P_2 \times P_3$), F_2 seeds for all crosses were produced by bagging the F_1 plants during flowering period in 2002.

Effect of different salinity levels 0 (distilled water), 0.5 and 1% NaCl were investigated on both percent and rate of germination and seedling root length of different genotypes including parental, F_1 and F_2 generations (Table 2). One hundred seeds from each genotype were treated with 5% Clorox (5.25% sodium hypochlorite) and were placed on filter paper in sterilized petri dishes (15 cm diameter) as an experimental unit. A Complete Randomized Blocks Design with three replications was used in the experiment. The petri dishes were incubated in dark conditions at of $28 \pm 0.5^\circ\text{C}$ in a vertical incubator. During the experiment, adequate solutions were added to keep the seeds continuously damp. The number of germinated seeds was daily recorded to calculate the rate of germination according to George^[11]. Also, the germinated seeds in each petri dish were counted after 7 days to calculate percent of germination. To measure root length of seedlings, three replicates of 50 seeds from each genotype were placed in a rolled standard weight paper towels and incubated in germination conditions mentioned above. After seven days, root length of the seedlings was measured. Weight of 100 seed for each genotype was calculated based upon weighting of 300 seeds.

The estimates of Heterosis (Ht) based on the High Parent (Hp) and Inbreeding depression (In) for all the traits were calculated using following formulas^[10]:

$$\begin{aligned} \text{Ht} &= 100 [F_1 - \text{Hp}] / \text{Hp} \\ \text{In} &= 100 [F_1 - F_2] / F_1 \end{aligned}$$

The gene actions was studied by estimating genetic effects in an additive-dominance model in which the expectation of generations means in terms of m (mean), a (pooled additive effects) and d (pooled dominance effects), are as follow:

$$\begin{aligned} P_1 &= m+a \\ P_2 &= m-a \\ F_1 &= m+d \\ F_2 &= m+ \frac{1}{2}d \end{aligned}$$

An unweighted least square procedure was used to estimate a and d parameters and also to partition the sum of squares into three components including additive, dominance and interaction (residual) effects^[12]. For determining the effect of genotype on seed weight, the data were analyzed in a Completely Randomized Design with three replicates. The statistical differences

between means of genotypes were determined by LSD test, where the F-test was significant. Pearson correlation coefficients were calculated to find any relationship among the traits. The analysis of data was performed using the Statistical Analysis System program^[13].

RESULTS

The results of analysis of variance (Table 1) showed that the effects of salinity levels, genotypes and their interaction on both percent and rate of germination and also on length of seedling root were highly significant. At salinity level of 0.5%, the percent of germination was significantly lower than that of in control (distilled water) (Table 2). With increasing NaCl concentration, the rate of germination was significantly reduced. At 0.5% salinity level, the mean of seedling root length was significantly higher than the other salinity levels. There were significant differences among the genotypes for all of the traits (Table 2). Averaged over all salinity levels, the F_2 seeds of cross $P_3 \times P_2$ and the seeds of genotype P_2 had the highest and lowest means of germination, respectively (Table 2). Also, the highest and lowest means of germination rate were found to be for F_2 seeds of the cross $P_3 \times P_4$ and seeds of genotype P_2 , respectively. For the length of seedling root, the highest and lowest means belonged to F_2 seeds of cross $P_3 \times P_4$ and seeds of genotype P_4 , respectively. In general, the highest mean of each trait was observed in F_2 seeds and the lowest in the parents.

A significant interaction between genotypes and salinity levels (Table 1) indicated that the response of the genotypes were not consistent across salinity levels in terms of percent and rate of germination and seedling root length. Some genotypes had more variation than others for the traits over the salinity levels (Table 2). Averaged over all salinity levels, maternal effects were observed for percent of germination in F_1 seeds of cross $P_1 \times P_2$, for rate of germination and length of seedling root in F_1 and F_2 seeds of both crosses $P_1 \times P_2$ and $P_3 \times P_4$ (Table 2). For seed weight, there were maternal effects only in F_2 seeds of cross $P_1 \times P_2$.

In general, there was a relatively consistent and positive correlation between percent and rate of germination at all salinity levels (Table 3). Positive and significant correlation between percent of germination and each of root length and seed weight was observed only when distilled water was used. Also, the rate of germination had a significant and positive correlation with length of root in distilled water and salinity level of 1% and it had a positive correlation with seed weight in distilled water (Table 3).

Table 1: Analysis of variance for percent and rate of germination and length of seedling root

SOV	df	Means of square		
		Length of seedling root	Rate of germination(%)	Germination (%)
Block	2	23.49	10.23	0.76
Genotype (G)	12	89.51**	41.30**	3.31**
Salinity levels (E)	2	43.64**	63.18**	167.10**
G × E	24	53.57**	60.93**	1.16**
Error	76	6.38	2.31	0.02

** : significant at 1% level of probability

Table 2: Mean of percent and rate of germination, length of seedling root of the genotypes in different salinity levels along with 100-seed weight

Genotype	Generation	Percent of germination (%)				Rate of germination (%)				Length of root (cm)				100 seed weight (g)
		Salt. 1 ^a	Salt. 2	Salt. 3	Mean	Salt. 1	Salt. 2	Salt. 3	Mean	Salt. 1	Salt. 2	Salt. 3	Mean	
IUTK ₁₁₅	P ₁	99.7	88.3	96.3	94.8	71.6	59.5	58.4	63.2	6.3	6.92	2.16	5.12	2.98
IUTM ₁₂	P ₂	95.7	77.3	91.0	88.0	57.9	45.2	38.5	47.2	4.79	5.44	1.63	3.95	13
P ₁ × P ₂	F ₁	97.3	95.3	92.3	95.0	65.0	68.6	52.3	62.0	5.21	6.1	2.96	4.75	2.93
P ₂ × P ₁	F ₁	92.3	93.7	98.7	94.9	66.0	67.3	66.3	66.5	5.64	6.8	2.46	4.96	3.05
P ₁ × P ₂	F ₂	96.0	91.3	95.0	94.1	65.8	61.4	60.8	62.7	4.95	4.78	2.16	3.96	2.76
P ₂ × P ₁	F ₂	96.3	95.0	94.3	95.2	74.9	68.4	69.1	70.8	6.61	6.88	2.55	5.34	3.29
IUTH ₁₃	P ₃	93.3	89.3	92.7	91.8	64.2	59.1	44.6	56.0	6.05	6.43	2.2	4.89	2.98
IUTE ₁₄₄₉	P ₄	84.7	96.3	89.7	90.2	57.4	66.3	59.6	61.1	5.27	3.18	1.65	3.36	2.55
P ₃ × P ₄	F ₁	96.0	99.7	93.0	96.2	64.0	67.5	56.7	62.7	5.24	6.39	2.64	3.96	3.09
P ₄ × P ₃	F ₁	96.0	95.3	98.3	96.6	67.5	72.3	64.3	68.0	6.35	5.19	2.77	4.76	3.05
P ₃ × P ₄	F ₂	99.0	98.7	91.7	96.4	74.0	77.7	70.6	74.1	6.78	6.69	2.61	5.36	2.89
P ₃ × P ₂	F ₁	96.0	98.0	95.7	96.6	69.4	64.0	63.3	65.6	6.39	7.11	2.46	5.32	2.75
P ₃ × P ₂	F ₂	99.0	95.7	97.3	97.3	72.2	67.0	64.7	67.9	5.52	6.82	2.18	3.83	3.43
Mean	-	95.5 [†]	93.4 ^b	94.3 ^b	94.4	66.9 ^a	64.9 ^b	59.2 ^c	63.7	5.78 ^b	6.06 ^a	2.34 ^c	4.72	2.99
LSD _(0.01)		4.00	6.30	3.70	3.15	4.20	2.10	1.65	1.89	0.34	0.32	0.16	0.17	0.43

a: Salt. 1, 2 and 3 are index of 0.0, 0.5 and 1% NaCl, respectively, LSD (0.01) values for comparing of interaction means (genotype × salinity levels) for percent and rate of germination and also length of seedling root were 3.849, 2.032 and 0.217, respectively, † : Means followed by the same letters are not significantly different at the 0.01 level of probability using the LSD test

Table 3: Correlation coefficient between the characters in different salinity levels

Characters	Salt. 1 ^a	Salt. 2	Salt. 3	In all
Germination % and rate of germ.	0.641**	0.798**	0.766**	0.672**
Germination % and length of root	0.362*	0.072	0.142	0.033
Germination % and seed weight	0.585**	-0.181	-0.163	0.023
Rate of germ. and length of root	0.712**	0.014	0.350*	0.434**
Rate of germ. and seed weight	0.410**	-0.096	-0.044	0.048
Length of root and seed weight	0.079	0.530**	0.180	0.143*

* and **: significant at 0.05 and 0.01 level of probability, respectively, a: Salt. 1, 2 and 3 are index of 0.0, 0.5% and 1% NaCl, respectively

Table 4: Estimates of Heterosis and Inbreeding in different crosses and salinity levels

Cross	Percent of germination			Rate of germination		Length of root		100 seed weight	
	Salinity level ^a	Ht	In	Ht	In	Ht	In	Ht	In
P ₁ × P ₂	1	-2.40	-1.33	-9.21	1.23	-17.30	-4.99	-6.38	-5.80
	2	7.92	-4.19	15.29	-10.49	-11.84	-21.63		
	3	-4.15	2.92	-10.44	16.25	37.03	-27.02		
P ₂ × P ₁	1	-7.42	4.33	-7.82	13.48	-10.47	17.19	-2.55	7.86
	2	6.11	1.38	13.10	1.63	-1.73	1.17		
	3	2.49	-4.45	13.52	4.22	13.88	3.65		
P ₃ × P ₄	1	2.89	3.12	-0.31	15.62	-13.38	29.38	3.69	-6.47
	2	3.53	-1.00	1.80	15.11	-0.62	4.69		
	3	0.32	-1.39	-4.86	24.51	20.00	-1.13		
P ₃ × P ₂	1	0.31	3.12	8.09	4.03	5.61	-13.61	-12.14	24.72
	2	9.74	-2.34	8.29	4.68	10.57	-4.07		
	3	3.23	1.67	41.92	2.21	11.81	-11.38		

Ht: Estimates of Heterosis (% from high parent); In: Estimates of Inbreeding depression (%), a: Salinity levels 1, 2 and 3 are index of 0.0, 0.5% and 1% NaCl, respectively

For percent of germination, the heterosis values were considerably higher at 0.5% salinity in comparison to other salinity levels in all crosses (Table 4). The maximum heterosis (9.74%) for this trait was observed in cross

P₃ × P₂ in which their parents had lower means of germination than the others. Heterosis values for rate of germination varied from -10.4 to 41.9% in different crosses and salinity levels. The minimum and maximum

Table 5: Mean squares for the model and estimates of additive and dominant parameters for the crosses in different salinity levels

Cross		$P_1 \times P_2$			$P_2 \times P_1$			$P_3 \times P_4$			$P_3 \times P_2$		
Salinity level†	Character	1	2	3	1	2	3	1	2	3	1	2	3
Percent of germination	a	0.02	0.05*	0.04*	0.02*	0.05*	0.04*	0.04*	-0.03*	-0.04*	-0.01	0.06**	0.02
	d	-0.01	0.13**	0.01	-0.01	0.09*	0.081	0.08*	0.07*	0.00	0.02	0.15**	0.08*
	Model	0.001	0.02**	0.005	0.01	0.01*	0.01*	0.01**	0.01**	0.005*	0.001	0.03**	0.009**
Rate of germination	a	0.06**	0.07**	0.09*	0.06**	0.07*	0.09**	0.03	-0.03	-0.07*	0.03*	0.06**	0.03
	d	0.0	0.17**	0.06	0.02	0.11*	0.14*	0.07	0.07	0.07	0.09**	0.13**	0.23**
	Model	0.01**	0.04**	0.03*	0.01*	0.02**	0.05**	0.01	0.01	0.02*	0.01**	0.03**	0.06**
Length of root	a	0.75**	0.73**	0.26*	0.75**	0.73**	0.26*	0.39	1.62**	0.27*	0.63**	0.49**	0.28**
	d	-0.41*	-0.32	1.01**	0.28	0.69**	0.62**	-0.17	1.78**	0.77*	0.90**	1.22**	0.53**
	Model	1.89**	1.72*	1.26**	1.80*	2.11**	0.61**	0.48	11.1**	0.84**	2.05**	2.28**	0.53**
100 seed weight	a		-0.07			-0.07			-0.21**			-0.07	
	d		-0.07			-0.07			-0.21**			-0.07	
	Model		0.04			0.01			0.24**			0.05	

†: Salinity level 1, 2 and 3 are index of 0.0, 0.5% and 1% NaCl, respectively, * and **: significant at 5% and 1% levels of probability, respectively

heterosis for this trait belonged to cross $P_1 \times P_2$ and $P_3 \times P_2$ (at salinity level of 1%), respectively. Almost, cross $P_3 \times P_2$ showed a higher heterosis for rate of germination than others in all salinity levels (Table 4). For seedling root length, the highest variation in heterosis (-17.3 and 37.0%) was observed in cross $P_1 \times P_2$ at distilled water and salinity level of 1%, respectively.

The cross $P_3 \times P_4$ had the highest heterosis for seed weight (3.6%). However, the parental lines P_3 and P_4 were moderate in terms of seed weight (Table 2). The lowest value of heterosis for this trait was observed in cross $P_3 \times P_2$ (-12.1%).

Inbreeding depression for percent of germination in different crosses and salinity levels had a variation from -4.4% (for cross $P_2 \times P_1$ at salinity level of 1%) to 4.3% (for cross $P_2 \times P_1$ at 0% of salinity) (Table 4). For the rate of germination and seedling root length, inbreeding values varied from -10.4% (in cross $P_1 \times P_2$ at salinity level of 0.5%) to 24.5% (in cross $P_3 \times P_4$ at salinity level 1%) and from -27.0% (in cross $P_1 \times P_2$ at salinity level 1%) to 29.3% (in cross $P_3 \times P_4$ at salinity level of 0%), respectively. All the inbreeding values for the rate of germination were positive, except in cross $P_1 \times P_2$ at salinity level of 0.5%. For seed weight, inbreeding values was varied from -6.4% (in cross $P_3 \times P_4$) to 24.7% (in cross $P_3 \times P_2$) (Table 4).

The results of generations means analysis (Table 5) showed that for percent of germination in non-saline condition (distilled water), an additive-dominance model was significant only in cross $P_3 \times P_4$. However, with the exception of cross $P_1 \times P_2$ in salinity level of 1%, an additive-dominance model was suitable for percent of germination in all salinity levels and crosses (Table 5). Almost, in all of the crosses, additive effects of the genes played a significant role in variation of percent of germination. However, different degrees of additive and dominance effects were involved in percent of germination at salinity level of 0.5%. At salinity level of

1%, only additive effects had the major contribution in variation of germination (Table 5).

The additive-dominance model of gene actions for the rate of germination was significant in all crosses and salinity levels, except for cross $P_3 \times P_4$ in salinity levels of 0 and 0.5% (Table 5). Almost in all of the crosses and salinity levels, the additive effects of the genes had significant effects on rate of germination and the dominance effects of the genes played a significant role on this trait in cross $P_3 \times P_2$ (Table 5).

Also an additive-dominance model explained the variation of length of root and additive and dominance effects of the genes were important in expression of the trait (Table 4).

For 100 seed weight, the model of additive-dominance of the gene actions was significant only in cross $P_3 \times P_4$ in which both additive and dominance effects were important in explaining the variation of the trait (Table 5).

DISCUSSION

The significant effects of salinity levels on percent and rate of germination and also seedling root length in this study were similar to those results which previously reported in safflower^[3] indicating that seed germination was reduced as salinity increased from 0 to 1% NaCl. These findings also are in agreement with the results of Gulzar^[14] in *Urochondra setulosa* (Trin). Over all salinity levels, the highest and lowest means of germination were belonged to F_2 and parents seeds, respectively. This could be attributed to genetic segregation in F_2 population and it can be explained by gathering of favorable genes for germination, similar to what was found in winter wheat (*Triticum aestivum* L.)^[15]. Greater growth of seedling root at salinity level of 0.5% compared to 0% was not surprising as it was previously reported by Mayaki *et al.*^[16] in soybean (*Glycine max* L.). They found

that roots of soybean in osmotic conditions were more penetrated in soil to absorb more water.

There were significant differences for percent and rate of germination and also length of seedling root among the salinity levels in some genotypes. This means that, the effect of salinity was not consistent in all genotypes. Maftoun and Sepaskhah^[17] also have previously reported this type of interaction in safflower. Almost all inconsistent genotypes were parental lines (P_1 , P_2 and P_4 in Table 2) and F_1 generations. It was possible that greater genetic variation in F_2 seeds caused greater stability of germination over different salinity levels in comparing to the parental and F_1 seeds.

The means of seed weight for F_2 seeds of cross $P_3 \times P_4$ was greater than those of their parental lines (Table 2). This might be due to transgressive segregation for seed weight. Cho and Scott^[18] reported similar findings for seed vigor in soybean. There were no maternal effects for seed weight. Similarly, Yazdi-samadi *et al.*^[19] reported that cytoplasmic effects had little or no effects on the expression of seed weight in safflower.

The results of this study showed that there was a considerable influence of maternal effects almost in all of the traits in different salinity levels. Therefore selection of special parents, as pollen recipient is important to obtain vigorous hybrid seeds for seeding in saline conditions on the traits.

Significant correlation coefficient between each of percent and rate of germination with 100 seed weight in non-saline condition (Table 3) were indicated that larger seeds germinated faster and more vigorous. Soltani *et al.*^[20] also reported that use of larger seeds would have an advantage in producing more vigorous seedlings of chickpea (*Cicer arietinum* L.) only in non-saline condition and this was related to lower water absorption of small seeds to initiate germination.

The considerable amount of heterosis for rate of germination and seedling root length, particularly in saline conditions (Table 4) indicated the superiority of F_1 seeds in these conditions. In this study there was a relatively small heterosis for percent of germination and seed weight. However Ehdaie and Ghaderi^[4] obtained relatively large heterosis for seed weight in safflower. Such heterosis effects could be arisen from partial to complete dominance and overdominance effects of the genes and also interallelic interaction^[12].

A considerable inbreeding depression was observed for rate of germination, length of seedling root and seed weight (Table 4). However Yazdi-samadi *et al.*^[19] found a small magnitude of inbreeding depression for seed weight in safflower. This inconsistency could be raised from differences in genetic backgrounds of the parental lines

that were used. For some of the traits positive heterosis in F_1 generation was followed by a negative inbreeding in F_2 (Table 4) that indicated most of the responsible alleles for higher value of the trait were dominant^[12]. For seed weight in cross $P_1 \times P_2$, the direction of heterosis and inbreeding depression were identical and negative. This could be attributes to decrease of seed weight from parents to F_1 and then F_2 generation.

The significant differences among genotypes for all traits arise from genetic variation among the parents and their progenies in different generations. Almost in 75% of the cases, additive-dominance models significantly explained the observed variations of the traits (Table 5). In those cases that additive-dominance genetic model failed to be fitted suitably, the variation of the trait significantly could be affected by epistatic effects^[12]. Since, additive effects of genes were important in controlling percent of germination in all salinity levels, selection should be effective in improving of seed germination in both saline and non-saline conditions. Cho and Scott^[18], reported similar findings for seed vigor in soybean.

The significant role of dominance effects in controlling the rate of germination in some crosses at saline conditions indicates that selection for improvement of this trait should be delayed to later generations. Also, in those crosses that heterosis for the trait was considerably high, breeding programs could be designed towards producing hybrid seeds. Snoad and Arthur^[21] also reported that dominance effects were important in genetic variation of seedling characters in pea (*Pisum sativum* L.).

Similar to findings of Li and Rutger^[3] for seedling vigor in rice, both additive and dominance effects were important in controlling of length of seedling root in this study (Table 5). Importance of the additive and dominance effects of genes for seed weight in cross $P_3 \times P_4$ was similar to the results obtained by Ehdaie and Ghaderi^[4]. However, in other crosses, interallelic interaction could be more involved in controlling the seed weight.

Changes in genes action that controls percent and rate of germination and length of seedling root over different salinity levels (Table 5) indicates that selection for improvement of seed vigor in non-saline conditions would not result in genotype with vigorous seeds in saline environments.

The results of this study suggested that for improvement of seed vigor of safflower for growing in saline conditions, breeding efforts should be performed in the same conditions. Because of existing hybrid vigor for germination percent and rate, length of seedling root and

seed weight, it seems that hybrid (F₁) seeds of safflower can have better performance in saline environmental conditions.

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