Annual Research & Review in Biology
4(1): 337-346, 2014

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Genetic Analysis of Salt Tolerance in Safflower (*Carthamus tinctorius* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

Received 9th July 2013
Accepted 28th August 2013
Published 16th October 2013

ABSTRACT

Aim: The mode of agronomic traits inheritance was investigated in safflower (*Carthamus tinctorius* L.) in salt stress as a new report in this study.
Place and Duration of Study: This experiment was carried out in Agricultural Research Farm of Shahid Bahonar University at Ekhtiarabad, Kerman, Iran, in 2011 -2012.
Methodology: Five generation including P_1, P_2, F_1, F_2 and F_3 that derived from the cross of IL.111 (salt sensitive) × Mex.22-191 (salt tolerance) were used in a randomized complete block design with two replications.
Results: According to generation mean analysis, different types of gene action was obtained for studied traits**.** The additive model [d] was fitted for branches/plant, seeds/capsule and seed yield/plant. The simple additive - dominance model [d, h] was fitted for number of seeds/plant. Also, dominance× dominance epistasis [l] was added to fit the model as [d, h, l] for capsule/plant and dry weight/plant. The genetic model of [d,h,i] and [d, i] was fitted for genetic control of plant height and seed weight, respectively.
Conclusion: Obtained results could be suitable for designing of breeding strategies to

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improve seed yield of safflower in salt stress.

Keywords: Action; additive; action; dominance; effect; safflower.

1. INTRODUCTION

Salinity is a major limitation in plant growth and leads to lower agricultural production in arid and semi-arid regions [1] Salinity has a very pronounced effect on almost all crops production. Salinity affects on all growth stages of plant from germination to maturity but sensitivity varies from one growth stage to the other [2, 3]. It is estimated that 20% of the irrigated land in the world is presently affected by salinity [4]. So, tolerance to salinity stress is a key topic to consider for crop improvement [5].

Safflower (*Carthamus tinctorius* L.) is a moderately salt tolerance [6,7] that cultivated in mostly arid and semi-arid climates [5,7] and it could produce profitable crops on saline soils [8]. Improvement of salt tolerance is a major objective in plant breeding programs for arid regions [9]. The expression of salt tolerance in crop species is a complex trait which is manifested by many plant characters, both physiological and morphological [3]. Future research on genetic control mechanisms and heritability of plant responses to salinity stress should lead to development of new crop cultivars specifically bred for adaptation to saline soils.

Improvement of salt tolerance in plants is still not very much fruitful due to lack of understanding of the complex nature of tolerance, its interaction with environments and their genetic basis involved.

Distinguish the mode of inheritance, magnitude of gene effects and their mode of action is essential to understand an efficient breeding program for developing salt tolerance [10,11]. Identification of plant mechanisms for salt tolerance and production of new cultivars, are the best strategies for reduction of hazardous effects of salt toxicity [12]. Studies on salt tolerance suggest that this tolerance is determined by a number of genes with heterosis, dominance and additive effects [11]. The identification of genes whose expression enables plants adaptation to salt stress is essential for breeding programs of this important oil crop. Available data for salt tolerance in important cereals including [wheat [5,13]; barley [14], rice [15], maize [16] and oil seeds including brassica [17] and cotton [18] suggested that both additive and non-additive gene effects are important in controlling tolerance.

The nature of variation for salt tolerance in safflower has been the subject of previous studies [7,19] but literature review showed that there is no any report about salt tolerance of safflower in reproductive stages. The objective of present investigation was genetic analysis of salt tolerance criteria in safflower to detect non-allelic interactions for evaluated traits to estimate the components of genetic variance (additive and dominance) and inter-genic interactions (epistasis). Also, this study provides information on narrow and broad sense heritability associated with the studied characters.

Generation mean analysis (GMA) was used to estimate genetic parameters in this study. It provides an opportunity to estimate the presence or absence of epistasis (by scaling test).

The present study was designed to estimate the genetic parameters for some agronomic traits in salt stress via generation mean analysis.

2. MATERIALS AND METHODS

This experiment was carried out in Agricultural Research Farm of Shahid Bahonar University at Ekhtiarabad (56º58' longitude and 30º15', 2044 m asl), Kerman, Iran. Two parental genotypes including Mex.22-191 (P_1) a salt tolerant and IL.111 (P_2) a salt sensitive genotype of safflower, were crossed to produce F_1 generation. Generations of F_2 and F_3 produced via selfing single plants of F_1 and F_2 generations, respectively. These five generations (P_1, P_2, F_1, F_2 and F_3) were evaluated in a Completely Randomized Block Design (CRBD) with two replication in saline stress point Electrical conductivity (EC) was adjusted in 12 (ds/m^2) for saline field condition. Each replication consist of 100 rows of F_3 families and 6 rows (2 m length for each row) for P_1, P_2, F_1 and F_2 generations that spaced 50 (cm) and 5 (cm) between and within rows, respectively.

Different agronomic traits were measured including: Plant height, Branches/plant, Capsules / plant, total dry weight/ plant, seed yield/ plant, 1000-seed weight, seeds/ plant and seeds/ capsule. Five randomly plant was selected in each row for data measurement.

2.1 Statistical Analysis

Analysis of variance and generation mean analysis was done by using SAS [20] and MSTAT-C software. Generation mean analysis was performed using the Mather and Jinks method [21]. In this method the mean of each character is indicated as follows:

$Y= m+ \alpha[d]+ \beta[h]+\alpha^2[i]+ 2\alpha\beta[j]+\beta^2[l]$

This formulae estimate the mean (m), additive effect (d), dominance effect(h), additive ×additive (i), additive× dominance (j) and dominance× dominance (l) parameters as genetic parameters and α, β, 2αβ and β^2 are the coefficient of genetic parameters. The best model was selected by using non-significant Chi-square test (χ^2) [21]. Genetic variance details (D and H) and environmental effects variance (E_1 and E_2) were calculated by using four normal equations based on least square method [21].

$$V_{F2} = \frac{1}{2}D + \frac{1}{4}H + E_1, \qquad V_{\bar{F}_3} = \frac{1}{2}D + \frac{1}{16}H + E_2$$

$$\overline{V}_{F3} = \frac{1}{4}D + \frac{1}{8}H + E_1, \qquad W_{F_2.F_3}\frac{1}{4}D + \frac{1}{8}H + E_1$$

The significance of parameters [m, d, h, i, j and l] were tested with t-test at 1% and 5% of probabilities [21]. Broad-sense (h^2_b) and Narrow-sense (h^2_n) heritability was estimated by Following formulae:

$$h^2_b = \frac{V_{F_2} - \sqrt{V_{p_1} \times V_{p_2}}}{V_{F2}}$$ [22], $$h^2_n \frac{1/2D}{V_{F2}}$$ [21].

3. RESULTS

The mean comparison for studied traits in different generations is shown in Table 1. The Mex.22-191 (P_1) mean was greater than IL.111 (P_2) mean for all of the studied traits, except for branches/ plant (Table 1). F_1 means showed superiority than parental means for branches/ plant and seeds/ capsule (Table 1). This result implied that heterotic effects could be effective for improvement of these traits. The means of F_2 generation for studied traits were in the range of parent means, except for plant height, capsules/ plant and dry weight/ plant (g) (Table 1). According to generation mean analysis, the simple dominance-additive model [d and h] was adequate for seeds/ plant in salt stress, but the contribution of dominance gene action was more than additive in salt stress (Table 2).

The additive model [d] was fitted for branches/ plant, seed yield/ plant (g) and seeds/ capsule (Table 2) in saline (Table 2). This result implied on the importance of selection for improvement of these traits in salt stress.

Simple additive-dominance model was insufficient to explain the differences among generation means for plant height, capsules/plan, dry weight (g), and 1000-seed weight that implied on the importance of epistasis on genetic control of these traits (Table 2). Plant height was controlled by additive [d], dominance [h] main effects and additive× additive [i] interaction (Table 2). The number of capsules per plant and dry weight (g) were controlled by [d], [h] and [l] effects (Table 2). Seed weight was controlled by additive[d] and additive × additive [i] epistasis (Table 2).

Variance analysis was carried out to obtain different variance components in different generations (Table 3). Different variance estimates (D, H, E_1 and E_2) is presented in Table 3 according to Mather and Jinks method [21]. The sum of F_2 plants variance (V_{F2}), F_3 progeny variances average ($\bar{V}_{F3}$), F_3 progeny average variance $V_{\overline{F3}}$ is calculated. The dominance variance component (H) was higher than additive variance component the (D) for all of the studied traits.

Heritability (broad and narrow) of studied traits is presented in Table 4. Broad-sense heritability ranged from 80% seed yield/plant) to 32% (seeds/ plant). Narrow-sense heritability ranged from 37% in number of seeds/plant to 15% for 1000-seed weight. The highest value (%) of narrow-sense heritability was devoted to dry weigh/ plant (Table 4).

The average of dominance ratio ($\sqrt{H / D}$) was more than unity for plant height, number branches/plant, capsules/plant, dry weight/plant, seed yield/plant, 1000-seed weight and the number of seeds/capsule. Degree of dominance explains the ratio of additive to dominance effects. This ratio ($\sqrt{H / D}$) is compromised from [D] and [H] components of variance generation analysis. The inconsistency between genetic effects for genetic parameters could be resulted from gene dispersion and ambidirectional effects [21].

Table 1. Mean and standard errors of safflower generations in salt stress

Character	P_1	P_2	F_1	F_2	F_3
Plant height	6.39± 36.75	1.41± 25	6.62±30.37	11.97±37	12.02±39.38
Branches/ plant	1.82± 6	0.7± 6.5	1±6.75	2.98±6.25	2.28±6.19
Capsules/ plant	6.55± 14.5	1.41± 9	5.94±12	10.81±16.25	8.56±15.01
Dry weight/ plant	20.3± 38.72	2.82± 15	24.58±33.75	22.12±40.50	24.65±35.19
Seed yield /plant	6.88± 12.52	0.14±0.45	8.38±11.5	5.8±9.9	7.27±8.71
1000-seed weight	5.53± 40.91	1.40±10.36	19.08±29.46	22.12±40.50	13.34±33.60
Seeds/ plant	205.5± 325.25	8.48±38	174.90±279.25	155.08±256.75	184.80±230.25
Seeds/ capsule	5.03± 20.98	0.28±4.20	4.88±22.13	4.17±15.49	7.34±14

Table 2. Estimation of gene effects and their standard error for different traits in generations of IL.111×Mex.22-191 cross in salt stress

Character	[m]	[d]	[h]	[i]	[l]	χ^2
Plant height	1.35**±42.37	1.67*±5.87	6.41**±-11.93	-11.49±2.13*	-	0.010
Branches /plant	0.512**±6.24	0.25*±-0.51	2.61^{ns}±-0.38	-	2.22±0.635	0.0005
Capsules/ plant	1.69**±11.74	1.69*±2.74	8.75**±17.33	-	8.13**±-17.07	0.0004
Dry weight /plant	5.05**±26.61	5.07*±11.62	26.27**±43.38	-	26.98**±-36	0.012
Seed yield/ plant	1.34**±7.74	1.72*±6.06	5.07^{ns}±3.98	2.18^{ns}±-1.28	-	0.0006
1000-seed weight	1.98**±32.44	1.47**±15.27	7.26^{ns}±4.61	2.46*±-6.79	-	0.13
Seeds/ plant	48.97**±182.75	49.01**±144.74	250.37*±221.09	-	235.79^{ns}±-125.68	0.005
Seeds / capsule	1.21**±12.68	1.21*±8.48	6.31^{ns}±3.71	-	6.15^{ns}±5.46	0.064

**, ** significant at 5% and 1% level of probability, respectively.*
df = 1; χ^2=6.63 and df = 1; χ^2=3.84.

Table 3. Estimation of additive (D), dominance (H) and Environment variances (E_1 and E_2) for different traits of safflower in salt stress

Characters	D	H	$\bar{V}_{F3}$	$V_{\overline{F3}}$	E_1	E_2
Plant height	116.29	399.25	75.91	71.58	24.65	46.25
Branches /plant	2.47	5.91	1.61	1.36	2.75	1.95
Capsules/ plant	49.62	182.60	44.5	39.38	24.50	29.12
Dry weight /plant	449.61	856.53	278.94	219.82	159.37	264.24
Seed yield/ plant	35.60	111.69	24.8	22.83	20.89	21.7
1000-seed weight	56.85	244.32	17.54	13.82	35.89	12.52
Seeds/ plant	10850.47	12415.96	6215.25	4272.35	18721.25	12730.98
Seeds / capsule	28.27	53.83	17.54	13.82	35.89	12.52

Table 4. Estimation of broad-sense and narrow-sense heritability of studied traits in IL.111×Mex.22-191 cross in safflower in salinity stress

character	Broad-sense h^2 (%)	Narrow-sense h^2 (%)	$\sqrt{H/D}$
Plant height	86	31	1.7
Branches /plant	49	22	1.5
Capsules/ plant	74	25	1.9
Dry weight /plant	73	37	1.3
Seed yield/ plant	80	31	1.7
1000-seed weight	49	15	2
Seeds/ plant	32	19	1
Seeds/ capsule	43	22	1.3

4. DISCUSSION

Obtained information about types of gene action in salt stress would be helpful to design breeding programs for improvement of salt tolerance in safflower.

Generation mean analysis fitted different genetic models for studied traits of safflower under salt stress. In this study, genetic control of plant height was under the control of additive, dominance and additive× additive epistasis, that is quiet different to previous reports [23]; Shahbazi and Saeidi [24] and Kotecha and Zimmerman [25] but similar with the results of Golkar [7] for seedling plant height in salt stress. It is the first report that implied on the importance of dominance and [i] epistasis. Hence, these novel finding proposed the accuracy of hybrid production for improvement of plant height in salt condition.

Number of branches per plant was under the control of additive gene effects. This finding was similar to the results of Golkar et al. [23] and Gupta and Singh [26]. Narkhede and Patil [27] reported that epistasis effects had a significant role in controlling branches/ plant that is different to this result. The practical utilization of information regarding epistasis in breeding is a challenging issue that needs to be fully addressed by the scientists in the field of biometrics [23]. Obtained results of this study were quite similar to the reports of Mandal and

Banerjee [28], Golkar et al. [23] and Singh et al. [29] for genetic control of seeds / capsule, but their report was denoted to normal condition. This discrepancy could be related to different genetic backgrounds and environments (normal and saline condition).

Literature reviews showed that there is no any report about the genetic control of dry weight/ plant and seeds/ plant in normal or stress condition in adult plants. So, these novel finding could be important for improvement of these traits in safflower breeding. The genetic control of dry weight/ plant in reproductive stage was different with the result of dry weight/ plant in seedling stage [7]. This result could be resulted from different action of involved genes for salt tolerance in different growth stages. Findings for genetic control of seed yield/plant confirm the results of Shahbazi and Saeidi [24] and Golkar et al. [23]. Previous reports with variance components analysis pointed at over dominance for genetic control of seed yield/plant that was inconsistence with the reports of [26, 28, 29] in normal condition. This discrepancy could be related to different estimates of gene effects with two different method (variance and means) and different environmental conditions (normal and stress).

The importance of additive gene action and additive× additive interaction in genetic control of 1000-seed weight was previously reported by Golkar et al. [23] and Shahbazi and Saeidi [24] in normal condition, that is similar to our results. Kotecha and Zimmerman [25] reported the partial or over dominance gene action for genetic control of seed weight in different crosses of safflower. The efficiency of any selection program is mainly dependent on additive genetic variance which is due to the breeding value of the genotype [30]. Therefore, selection through selfing will be effective for 1000-seed weight improvement. For plant height, the additive (d), dominance (h) and additive × additive (i) effects played an important role in genetic control of these traits. In these traits, the sign of [d] and [i] is opposite; hence duplicate epistasis is involved [21]. So, both methods of selection and hybridization could be used for improvement of mentioned traits. For capsules/plant the additive (d), dominance (h) and dominance × dominance (l) effects played an important role in genetic control of these traits. Golkar et al [23] and Mandal and Banerjee [28] reported the significant importance of dominance gene effects for genetic control of capsule/plant that was similar to our results, quietly. This is the first report on the significance of dominance × dominance interaction on genetic control of capsule/plant. Negative sign of [h] capsules/plant and dry weight/plant showed that reductive alleles were involved in dominant phenotype [21].

In generation mean analysis, additive gene effect might be little because of gene dispersion and also dominance gene effect can be little because of ambidirectional dominant. Genetic variances are mean squares of each locus effects and are not affected by gene dispersion and dominance effect. Thus, the data of generation variances can be used to complete genetic information [31].

The selection efficiency is related to the magnitude of heritability [32]. High percents of broad-sense heritability (>70%) suggested that environmental effects constitute a major portion of the total phenotypic variation of included traits. Golkar *et al*. [23] reported a high value for broad-sense heritability of seeds/capsule (99%) that was different to our results. Kotecha and Zimmerman [25] reported high broad-sense heritability (86%) for 100-seed weight in normal condition that was different to our result. This difference could be compromised from epistasis effect of additive × additive in genetic control of 1000- seed weight in salt stress.

The medium value for broad- sense heritability for branches/plant (49%) in our study was similar with the reports of Camas and Esendal [33]. Results of narrow-sense heritability

indicated that selection for number of plant height and dry weight/plant could be successful quietly, because of the higher proportion of additive variance rather than dominance gene action in total genetic variance. Other studied traits had medium and low narrow-sense heritability that implied on most of the genetic variances is due to dominance or epistasis gene action. The most discrepancy is related to estimation of traits heritability because the heritability is not a property of a trait itself, but it is related to the population, environmental conditions, method of evaluation of genotype and parameter estimation [30].

5. CONCLUSION

This study enables breeders to select suitable breeding method that leading to improvement of certain characters breeding populations under salt condition in safflower.

This study has presented new findings about the genetic control of seed yield and its components of safflower in salt environment**.** Selection in early generations for 1000-seed weight, branches/plant, seed yield/plant, seeds /capsule could be desirable for seed yield improvement in salt stress. On the other hand, those characters which were mostly controlled by additive effects and have high narrow-sense heritability can be improved by selection and inbred lines could be used as commercial cultivars. But for those traits that mainly controlled by dominance interaction effects, heterosis breeding might be effective for development of superior hybrid cultivars [34]. For improving those traits that both additive and non-additive effects of genes were contributed in their inheritance (plant height, capsules/plant, dry weight/plant, seeds/plant) the reciprocal recurrent selection could be suggested, since this breeding procedure will concentrate additive effects of genes, but will not allow dissipating non-additive gene effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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