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Genetic Evaluation of Several Physiological Traits for Screening of Suitable Spring Safflower (*Carthamus tinctorius* L.) Genotypes under Stress and Non-Stress Irrigation Regimes

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Abstract: Seven cultivars and one line of spring safflower (*Carthamus tinctorius* L.) were used to estimate genetic variation, heritability, genetic gain and genetic factor analysis for several physiological traits. Each experiment was conducted in a randomized complete block design with three replications. Factor loadings in first factor were used for determination of important physiological traits for suitable genotype screening under each irrigation regimes. Under non-stress conditions, factor analysis technique extracted six factors which exploited about 93% of the total genetic variation, while 30% of the total genetic variance was associated by the first factor. Under stress conditions factor analysis extracted four factors and they totally explained 100% of the total genetic variation, while, the first factor accounted for 38% of the total genetic variation. Ultimate, leaf area index (at stem-elongation and flowering), leaf osmotic potential (at stem-elongation) and rate of water loss from excised leaves (at flowering) under non-stress conditions and also leaf area index (at flowering and grain filling) and rate of water loss from excised leaves (at grain filling) under stress conditions were the best criteria for screening of suitable genotype under explicated conditions.

Key words: Genetic evaluation, physiological traits, genetic factor analysis, irrigation regimes

INTRODUCTION

Drought is a multidimensional stress, which causes various physiological effects on plants (Altinkut *et al*., 2001). During the last few years there has been an increasing interest in identifying attributes which contribute to drought resistance and which can be used as selection criteria in crop breeding programs (Johnson *et al*., 1983; Morgan *et al*., 1986; Blum, 1989; Blum *et al*., 1989; Richards and Passioura, 1990).

Several physiological traits for screening drought resistance cultivars have been proposed. Water status measurements of excised leaves has been related to drought resistance (Dedio, 1975; Clarke and McCaig, 1982; Schonfeld *et al*., 1988; Winter *et al*., 1988; Clarke *et al*., 1989; McCaig and Romagosa, 1991). Furthermore several researchers have shown that canopy temperature (Singh and Kanemasu, 1983; Pinter *et al*., 1990; Mustafa *et al*., 1996; Alza and Fernandez-Martinez, 1997; David and Duniway, 1997), Leaf osmotic potential (Chimenti and Hall, 1994; David and Duniway, 1997) have been considered to be effective for drought resistance screening in several plants. Maintained techniques have been used separately to screen drought resistance genotypes in different crops and there is not enough information about their interrelationships.

Knowledge of the physiological and functional relationships among traits would be beneficial to plant breeders in choosing traits for selection in a breeding program. Factor analysis can be used successfully for analysis of large amount of multivariate data, but it is impeded by genotype through environmental interaction. Whereas, knowledge of parameters such as genetic coefficient of variation, heritability, expected genetic advance and genetic gain is a prerequisite for the genetic improvement of a crop (Pandya *et al*., 1996).

The objectives of this research are to evaluate several physiological traits and their relationships for determination of the best criteria for suitable genotypes screening under stress and non-stress irrigation regimes using genetic variation and genetic factor analysis.

MATERIALS AND METHODS

Seven cultivars (Arak, Esfahan and Poshtkooh from Iran, Gila, Nebraska 10 and UC 10 from USA and RH 8018 from FAO) and one line (RH 410118 from FAO) of spring safflower were grown in two separate experiments under stress and non-stress irrigation regimes at the Experimental Station of Agriculture College, Shiraz University in Badjgah, Iran (29°50′ N, 52°46′ E) in 2001. The soil texture was clay loam (Fine, mixed, mesic,

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Table 1: A synopsis of weather information of Badjgah and irrigation regimes during the experiment

	Temperature (°C)			Mean			Irrigation (mm)	
Month	Max	Min	Mean	Relative humidity (%)	Evaporation (mm)	Precipitation (mm)	Stress	Non-stress
April	25	-3.0	11.6	48	6.0	3.0	150	150
May	32	2.5	17.3	44	7.9	1.5	100	170
June	31	5.0	20.7	36	8.5	0.5	120	210
July	37	10.5	24.4	34	8.7	-	140	250
August	35	6.5	23.3	34	7.4	-	90	120
Total	-	-	-	-	-	5.0	600	900

calcixerollic xerochrepts). The stress and non-stress experiments received water when 80±5 and 160±5 mm evaporation occurred from a Standard Class Evaporation Pan, respectively. Applied water was measured in each experiment. Soil moisture status was measured in gravimetric basis. Each experiment was conducted in a randomized complete block design with three replications. Each plot consisted of six 4 m long rows spaced 60 cm apart. The four middle rows were used for sampling and the two remaining rows were considered as border effects. The sowing date was April 15 and each genotype was harvested at its full maturity (early to mid August). A synopsis of weather information for the growth season at the experimental station is provided in Table 1.

The canopy maximum daily temperature at stem elongation, flowering and grain filling stages was measured for each plot in both experiments by an infrared thermometer (Kane-May Model Infratrace 800). The instrument was pointed down at three random points in each plot from a distance of 1m and held at an oblique angle to the canopy to minimize the influences of soil exposure (Golestani Araghi and Assad, 1998). Leaf water potential (LWP) was measured at flowering and grain filling stages, by pressure chamber (PMS Model) technique (Sivakumar and Virmani, 1979). Leaf osmotic potential (LOP) was measured at stem elongation, flowering and grain filling stages, after sap extraction by Cryoscopy method and digital thermometer ETI-2001 Model. Osmotic potential was determined as:

$$\Psi_s = (\frac{T}{1.86}) \times 2.27$$

Ψ_S= osmotic potential (Mpa) and T = freezing point of sap (°C).

The rate of water loss (RWL) from excised leaf and initial water content (IWC) were measured at three developmental stages (stem elongation, flowering and grain filling stages) (Clarke and McCaig, 1982). RWL and IWC were determined as:

$$RWL = \frac{(W_0 - W_2) + (W_2 - W_4) + (W_4 - W_6)}{3 \times W_d \times (T_2 - T_1)}$$

$$IWC = \frac{W_0 - W_d}{W_d}$$

Where:

T_1-T_2 = time interval between two subsequent measurements (2 h),
W_0 = fresh weight (g),
(W_2, W_4, W_6) = weight after 2, 4 and 6 h in a controlled chamber at 25°C and
W_d = oven-dry at 50°C for 24 h.

The Leaf Area Index (LAI) was measured at stemelongation, flowering and grain- filling stages using a portable leaf area meter (ΔT devices).

Data analyses: Data obtained were subjected to variance components and genetic advance was calculated as suggested by Johnson *et al.* (1955) and Vogel *et al.* (1981). Genetic correlation coefficients were computed according to the formula of Miller *et al.* (1958) for using in factor analysis. Principal Factor Analysis (PFA) was performed according to the procedures outlined by Cattell (1965) and Guertin and Bailey (1982). Prior to the PFA, the data were subjected to Principal Component Analysis (PCA) (Harman, 1976) also varimax rotation method (an orthogonal rotation) suggested by Kaiser (1958) was used. Factor loading used to recognize the physiological criteria associated to adaptation under each irrigation conditions on the basis of the magnitude signs in the first factor. Data of each experiment were analyzed, separately, using SAS Statistical Program Package (2000).

RESULTS

Genetic variation: The phenotypic and genetic coefficient of variation (PCV and GCV), estimates of the components of variance, broad-sense heritability, genetic advance and genetic gain under stress and non-stress conditions are shown in Table 2 and 3. PCV was generally higher than GCV for all the characters, but in many cases, the two values differed slightly (Table 2 and 3).

Under non-stress conditions heritability estimates ranged from 93 to 98%, while rate of water loss from excised leaves (stem-elongation), leaf area index (at stem-elongation and flowering), leaf water potential (at grain filling), initial water content (at stem-elongation and flowering), leaf osmotic potential (at stem-elongation and grain filling) and also canopy temperature (at

Table 2: Phenotypic and genetic coefficient of variation, components of variance, Heritability (H) and Genetic Advance (GA), Genetic Gain (GG) of characters in spring safflower under non-stress conditions

Character	PCV (%)	GCV (%)	Estimate of components of variance* σ^2_{ph}	σ^2_g	σ^r_e / r	H (%)	GA** as (%) of mean	GG** as (%) of mean
Stem-elongation stage								
Leaf area index (LAI)	33	32	0.22	0.21	0.01	98	66	65
Rate of water loss (RWL) from excised leaf	38	37	0.00	0.00	0.00	93	74	72
Initial water content (IWC)	14	14	0.67	0.66	0.28	98	28	27
Leaf osmotic potential (LOP)	50	50	0.32	0.31	0.02	98	100	99
Canopy temperature	4	4	1.23	1.20	0.31	97	7	7
Flowering stage								
Leaf area index (LAI)	33	33	0.25	0.24	0.01	98	67	66
Rate of water loss (RWL) from excised leaf	30	29	0.01	0.01	0.00	93	59	57
Initial water content (IWC)	20	20	3.54	3.46	1.29	98	40	39
Leaf osmotic potential (LOP)	30	29	0.32	0.31	0.02	97	60	59
Leaf water potential (LWP)	10	9	0.09	0.09	0.02	97	20	19
Canopy temperature	4	4	2.14	2.09	0.38	98	8	8
Grain filling stage								
Leaf area index (LAI)	35	34	0.26	0.25	0.01	97	71	70
Rate of water loss (RWL) from excised leaf	40	39	0.00	0.00	0.00	95	78	76
Initial water content (IWC)	19	18	0.33	0.12	0.21	97	38	37
Leaf osmotic potential (LOP)	29	29	0.77	0.74	0.03	98	59	58
Leaf water potential (LWP)	7	7	0.07	0.06	0.01	98	14	13
Canopy temperature	13	13	2.14	1.58	20.80	98	25	24

*σ^2_{ph}, σ^2_g and σ^r_e / r are phenotypic, genetic and error variances of genotype means, respectively,** The selection differential used was 2.06 at 5% selection intensity

Table 3: Phenotypic and genetic coefficient of variation, components of variance, Heritability (H) and Genetic Advance (GA), Genetic Gain (GG) of characters in spring safflower under stress conditions

Character	PCV (%)	GCV (%)	Estimate of components of variance* σ^2_{ph}	σ^2_g	σ^r_e / r	H (%)	GA** as (%) of mean	GG** as (%) of mean
Stem-elongation stage								
Leaf area index (LAI)	49	48	0.38	0.36	0.02	95	97	94
Rate of water loss (RWL) from excised leaf	53	34	0.00	0.00	0.00	41	45	29
Initial water content (IWC)	19	13	1.46	0.75	0.71	51	20	14
Leaf osmotic potential (LOP)	26	20	0.16	0.09	0.07	60	33	25
Canopy temperature	4	3	1.24	0.89	0.35	72	5	4
Flowering stage								
Leaf area index (LAI)	34	30	0.06	0.05	0.01	79	56	50
Rate of water loss (RWL) from excised leaf	102	96	0.00	0.00	0.00	88	185	173
Initial water content (IWC)	50	48	12.32	11.30	1.02	92	94	90
Leaf osmotic potential (LOP)	25	23	0.39	0.32	0.07	83	44	40
Leaf water potential (LWP)	9	7	0.13	0.07	0.06	58	10	8
Canopy temperature	3	3	2.85	2.43	0.42	85	5	5
Grain filling stage								
Leaf area index (LAI)	44	40	0.03	0.02	0.01	83	75	69
Rate of water loss (RWL) from excised leaf	92	72	0.00	0.00	0.00	61	116	91
Initial water content (IWC)	32	23	0.53	0.29	0.23	56	36	27
Leaf osmotic potential (LOP)	18	10	0.46	0.13	0.33	28	11	6
Leaf water potential (LWP)	15	15	0.55	0.52	0.03	96	30	30
Canopy temperature	3	3	2.85	2.43	0.42	85	6	6

*σ^2_{ph}, σ^2_g and σ^r_e / r are phenotypic, genetic and error variances of genotype means, respectively, ** The selection differential used was 2.06 at 5% selection intensity

flowering and grain filling) had a very high heritability estimates (98%).

The expected genetic advance, expressed as percentage of the mean, varied from 7% for canopy temperature (at stem-elongation) to about 100% for leaf osmotic potential (at stem-elongation). High expected genetic advance was also observed for leaf area index and rate of water loss from excised leaves (at all stages) and also leaf osmotic potential (at flowering and grain filling) (Table 2).

The genetic gain, expressed as percentage of the mean, also revealed a high value for leaf osmotic potential (at stem-elongation) and a low value for canopy temperature (at stem-elongation). The values in this parameter were the same for expected genetic advances in character ranks, but in all cases genetic gain were lower than genetic advance, beside canopy temperature (at stem-elongation) (Table 2).

Under stress conditions, PCV was generally higher than GCV for all of the traits, expect for canopy

Table 4: Loading of the first six most important Principal Factors (PF) from a factor analysis of 17 physiological traits under non-stress conditions in spring safflower

	Factor (matrix of factor coefficients)						
Variables	1	2	3	4	5	6	Communality
Stem-elongation stage							
Leaf area index (LAI)	0.87	0.12	-0.09	0.35	0.13	0.19	0.91
Rate of water loss (RWL) from excised leaf	0.26	-0.12	0.14	-0.10	-0.34	0.81	0.97
Initial water content (IWC)	-0.40	-0.84	0.17	-0.33	0.29	-0.01	0.91
Leaf osmotic potential (LOP)	0.74	0.26	-0.45	-0.03	-0.30	0.27	0.98
Canopy temperature	-0.27	0.13	-0.03	-0.09	0.90	-0.00	0.94
Flowering stage							
Leaf area index (LAI)	0.91	0.001	-0.29	-0.02	0.05	-0.17	0.98
Rate of water loss (RWL) from excised leaf	-0.69	0.48	0.13	-0.44	0.21	0.18	1.00
Initial water content (IWC)	0.09	0.93	-0.16	-0.27	-0.00	-0.14	0.98
Leaf osmotic potential (LOP)	0.24	-0.06	-0.18	0.90	0.01	0.18	0.89
Leaf water potential (LWP)	-0.06	-0.28	0.88	-0.12	-0.04	0.36	0.99
Canopy temperature	-0.44	0.36	0.72	-0.19	-0.30	0.00	0.48
Grain filling stage							
Leaf area index (LAI)	0.14	-0.17	-0.80	-0.13	-0.37	0.36	0.99
Rate of water loss (RWL) from excised leaf	-0.16	0.04	-0.07	0.36	0.03	0.56	0.99
Initial water content (IWC)	0.15	-0.18	0.09	-0.18	0.91	-0.25	0.98
Leaf osmotic potential (LOP)	-0.03	-0.04	0.11	0.95	-0.25	-0.04	0.99
Leaf water potential (LWP)	0.89	0.28	0.17	-0.15	-0.24	0.05	0.95
Canopy temperature	-0.02	0.86	0.34	0.15	0.18	0.03	0.99
Proportion of total variation (%)	30.00	19.00	15.00	12.00	10.00	7.00	
Cumulative variance (%)	30.00	49.00	64.00	76.00	86.00	93.00	

Coefficients larger than 0.60 have been bolded

temperature (at flowering and grain filling) (Table 3). The highest PCV and GCV were associated to rate of water loss from excised leaves (at flowering), whereas, least values were associated to canopy temperature (at all stages) (Table 3).

The heritability estimates ranged from 28 to 96% for leaf osmotic potential and leaf water potential (at grain filling), respectively. Other traits had heritability of higher than 40% (Table 3). The expected genetic advance expressed as percentage of the mean, varied from 5% for canopy temperature (at stem-elongation and flowering) to 185% for rate of water loss from excised leaves (at flowering). In this condition, rate of water loss from excised leaves (at flowering and grain filling) was higher than 100% of genetic advance. The high expected genetic advance was also observed for leaf area index (at all stages) and initial water content (at flowering).

The genetic gain expressed as percentage of the mean, revealed the largest value for rate of water loss from excised leaves (at flowering) (173%) and the lowest value was related to canopy temperature (at stem-elongation) (4%). The values in this parameter were the same for values in expected genetic advance in character ranks. In all cases genetic advance was higher than genetic gain.

Factor analysis: Factor analysis was used for determination of high genetic variance traits as a benefitical criterion for use in suitable genotypes screening under each irrigation regimes. Since no test of significance was performed for factor loadings, the decision was rather arbitrary as to how many factors should be extracted from the data set and what magnitude of loading coefficient a variable should possess to be considered meaningful. Factors whose eigenvalues were greater than 1.0 were retained. Traits with loading greater than 0.6 in a factor were deemed major (Acquaah *et al*., 1992).

Under non-stress conditions factor analysis technique divided the 17 variables into six factors. The six factors explained 93% of the total genetic variation in the dependence structure (Table 4). Factor I was strongly associated with leaf area index (at stem-elongation and flowering), leaf osmotic potential (at stem-elongation), rates of water loss from excised leaves (at flowering) and leaf water potential (at grain filling), showed that explained 30% of the total genetic variation.

In this factor leaf area index (at stem-elongation and flowering), leaf osmotic potential (at stem-elongation) and leaf water potential (at grain filling) loaded with positive signs (0.87, 0.91, 0.74 and 0.99, respectively), whereas, rate of water loss from excised leaves (at flowering) loaded with opposite sign (-0.69).

The sign of the loading indicates the direction of the relationship between the factor and the variable. Thus, two variables with high magnitude of loading in the same factor would be expected to exhibit a high correlation (Seiler and Stafford, 1985). Thus, these traits may be influenced by the same gene or genes and may be beneficial for suitable safflower genotypes screening under non-stress conditions. Other factors (I, II, III, IV, V

Table 5: Loading of the first six most important Principal Factors (PF) from a factor analysis of 14 physiological traits under stress conditions in spring safflower

	Factor (matrix of factor coefficients)				
Variables	1	2	3	4	Communality
Stem-elongation stage					
Leaf area index (LAI)	0.35	0.55	-0.70	-0.15	1.00
Rate of water loss (RWL) from excised leaf	-0.15	0.00	-0.80	0.39	1.00
Initial water content (IWC)	0.22	0.83	0.03	-0.17	0.97
Canopy temperature	0.99	0.02	0.12	0.06	0.98
Flowering stage					
Leaf area index (LAI)	-0.75	-0.40	-0.32	0.45	1.00
Rate of water loss (RWL) from excised leaf	-0.40	0.79	-0.80	-0.40	0.92
Initial water content (IWC)	-0.53	-0.35	0.52	-0.37	1.00
Leaf osmotic potential (LOP)	0.35	0.52	-0.70	-0.17	1.00
Leaf water potential (LWP)	0.34	-0.23	0.18	0.93	0.96
Canopy temperature	0.07	-0.99	0.12	-0.13	0.79
Grain filling stage					
Leaf area index (LAI)	0.97	-0.03	0.11	-0.01	0.99
Rate of water loss (RWL) from excised leaf	0.63	0.23	0.70	0.32	1.00
Initial water content (IWC)	0.42	0.04	0.90	0.20	1.00
Leaf water potential (LWP)	-0.25	0.16	-0.11	0.86	0.85
Proportion of total variation (%)	38.00	29.00	19.00	13.00	
Cumulative variance (%)	38.00	68.00	87.00	100.00	

Coefficients larger than 0.60 have been bolded

and VI) explained 19, 15, 12, 10 and 7% of the total genetic variation, respectively and may be not important in safflower improvement programs.

Under stress conditions, canopy temperature (at grain filling) and leaf osmotic potential (at stem-elongation and grain filling) were not included in the analyses because these traits had genetic correlation coefficients higher than 1.00 in relation to other traits. Factor analysis technique extracted four factors, which explained 100% of the total genetic variation. Variable compositions of the four factors with loadings are given in Table 5.

Factor I accounted 38% of the total genetic variability in dependence structure (Table 5). In this factor canopy temperature (at stem-elongation), leaf area index and rate of water loss from excised leaves (at grain filling) loaded with positive sign (0.99, 0.97 and 0.63, respectively), whereas, leaf area index (at flowering) loaded with negative sign (-0.75). Thus, these criteria are benefitical for suitable safflower genotypes screening under stress conditions. Other factors (II, III and IV) explained 29, 19 and 13% of the total genetic variation, respectively and supposed to be not important in safflower improvement programs under stress conditions.

DISCUSSION

The genetic variation exhibited by the safflower genotypes for several physiological traits indicates that which trait may be benefitical for screening of suitable safflower genotypes under each irrigation regimes. Whereas, selection efficiency is related to magnitude of heritability and genetic advance (Johnson *et al*., 1955).

Under non-stress conditions factor analysis showed that selection based on leaf area index (at stem-elongation and flowering), leaf osmotic potential (at stem-elongation), rate of water loss from excised leaves (at flowering) and leaf water potential (at grain filling) (Table 4) may be more efficient for suitable safflower genotype screening. Whereas, leaf water potential (at grain filling) has low genetic advance in this condition (14%), thus selection based on leaf water potential (at grain filling) can not be efficient in safflower improvement programs under non-stress conditions.

Under stress conditions factor analysis indicated that selection based on canopy temperature (at stem-elongation), leaf area index (at flowering and grain filling) and rate of water loss from excised leaves (at grain filling) may be more efficient for suitable safflower genotype screening (Table 5). Whereas, canopy temperature (at stem-elongation) have very low genetic advance (5%) and can not be efficient in screening of suitable genotypes in safflower improvement programs under stress conditions. Recent research indicat that leaf area index and rate of water loss from excised leaves are drought indipendent criteria. The same result is reported by Shaw and Laing 1966, Sobrado (1990), Clarke and McCaige (1982) and Winter *et al*. (1988) in other crops. Finally, our results indicated that selection based on leaf area index (at stem-elongation and flowering), leaf osmotic potential (at stem-elongation) and rate of water loss from excised

leaves (at flowering) might be desirable criteria for suitable safflower genotype screening under non-stress conditions. Whereas, selection based on leaf area index (at flowering and grain filling) and rate of water loss from excised leaves (at grain filling) are more efficient criteria for genotype screening under non-stress conditions in safflower.

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