# The Evaluation and Relationships of some Physiological Traits in Spring Safflower (*Carthamus tinctorius* L.) under Stress and Non-stress Water Regimes

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#### **ABSTRACT**

Eight genotypes of spring safflower (Carthamus tinctorius L.) were evaluated for several physiological traits under stress and non-stress water regimes. Data were analyzed using principal factor analysis. The factor analysis technique extracted six factors under non-stress conditions. Six factors explained about 80% of the total variation, and only 40% of the variance was accounted for by the first two factors. Factors I and II were identified as water consumption, and water balance capacity, respectively. Similarly, seven factors were extracted under stress conditions, and 34% of the total variation was accounted for by the first two factors. Factors I and II were described as water conservation, and water holding capacity, respectively. Important physiological criteria were recognized by Factors I and II in two experiments. Ultimately, initial water content (at stem elongation and grain filling stages), canopy temperature (at stem elongation and flowering stages), and leaf water potential (at flowering stage) under non-stress conditions, and canopy temperature (at all stages), leaf area index (at stem elongation), and rate of water loss from excised leaf (at grain filling) were the best criteria for screening suitable genotypes under the afore mentioned conditions.

Keywords: Drought resistance, Physiological traits, Principal factor analysis (PFA), Safflower.

# INTRODUCTION

Water deficit is a common phenomenon in plants. It is accentuated when drought or lack of sufficient water in the rhizosphere occurs and the rate of evapotranspiration is high. Drought may occur in any type of crop, irrigated or rainfed, and may have a special impact in association with the prevalent farming system and environment. This fact has prompted agronomists, breeders, physiologists, and physical scientists to study the nature of development and yield, management practices that would alleviate drought and to search for "drought resistant" genotypes [1]. Hence, it seems necessary to

use appropriate criteria for selecting drought resistant genotypes for breeding programs. Several physiological criteria for selecting resistant genotypes have been proposed.

Canopy temperature has already been considered to be effective for drought resistance screening in wheat (*Triticum aestivum* L.) [19, 20], pearl millet (*Pennisetum glaucum*) [24], and sunflower (*Helianthus annuus* L.) [3]. Leaf water potential (LWP) is another criterion used by several researchers in safflower, wheat [9] and sunflower [18]. Leaf osmotic potential has been used for drought resistance screening in sunflower [6], safflower and wheat [9]. Correlations between physiological traits and drought tolerance

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indexes have been determined in safflower [29].

Several researchers have shown that a high initial water content (IWC), and low rate of water loss (RWL) from excised leaves are related to drought resistance, and may be used as screening criteria in breeding programs [7, 27]. Leaf area index (LAI) is a drought-sensitive criterion and it decreases under drought conditions [23, 26].

Although the criteria mentioned above have been used separately to screen drought resistance genotypes in different crops, there is not enough information about their interrelationships. Greater knowledge of the physiological and functional relationships among traits would be beneficial to plant breeders in choosing traits for selection in a breeding program.

The factor analysis can be used successfully to analyse large amounts of multivariate data, and describes the interrelationships among all traits on the basis of overall pattern of the data, whereas the correlation coefficient only describes the relationships between two traits. Thus, using factor analysis by plant breeders has the advantage of increasing the understanding of the causal relationships of variables in breeding programs [22]. Also, this technique has previously been used successfully for detecting yield-related characteristics for screening high yield performance genotypes under stress and non-stress conditions, [4].

The objectives of this study were to use the factor analysis to extract factors from the measured traits and to assign to them a meaningful physiological interpretation, as well as to identify specific traits which could be used directly to predict the improvement of plant physiological traits under stress and non-stress conditions.

## **MATERIALS AND METHODS**

#### General

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 at a 20m distance under stress and non-stress water regimes at the Experimental Station of the Agriculture College of Shiraz University in Badjgah, Iran (29°50′ N, 52°46′ E) during 2001. The soil texture was clay loam (fine, mixed, mesic and calcixerollic xerochrepts). The stress and nonstress experiments received water once 160±5 and 80±5 mm evaporation had occurred from pan class A, respectively. The water applied was measured for each experiment. Soil moisture status was measured using a weighing method. 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 with a 15cm plant distance in the rows. The four middle rows were used for sampling. The sowing date was April 15 (2001), and each genotype was at full maturity from beginning to mid-August. Climatic data recorded during the growth season at the experimental location are shown in Table 1.

# **Physiological Crop Parameters**

The Canopy temperature at the stem elongation, flowering, and grain filling stages at the daily peak temperature (15.30-16.00 hour) was measured for each plot in both experiments using 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 [11]. Leaf water potential (LWP) was measured at the flowering and grain filling stages using a pressure chamber (PMS Model) technique [25]. Leaf osmotic potential (LOP) was measured at the stem elongation, flowering, and grain filling stages, after sap extraction using the Cryoscopy method and a digital thermometer ETI-2001 Model. Osmotic potential [11, 16] was determined as:

Table 1. Maximum, minimum and average daily air temperature, relative humidity, evaporation
from pan class A, precipitation distribution, and total irrigation water for each experiment.

Month	Tem	peratur	e (°C)	M	ean	Precipitation (mm)	Irrigatio	on (mm)
	Max	Min	Mean	Relative humidity (%)	Evaporation from pan class A (mm)	, ,	Stress	Non- stress
April	25	-3	11.6	48	6	3	150	150
May	32	2.5	17.3	44	7.9	1.5	100	170
June	31	5	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	600	900

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

 $\Psi_{S}$ = osmotic potential (Mpa), and T= freezing point of sap.

The rate of water loss (RWL) from excised leaves and initial water content (IWC) were measured at three developmental stages (stem elongation, flowering, and grain filling stages) [8]. RWL and IWC were determined

as:  

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

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

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

Where,  $T_1$ - $T_2$ = time interval between two subsequent measurements (2 h),  $W_0$ = fresh weight, (W2, W4, W6)= weight after 2, 4, and 6 hours in a controlled chamber at 25°C, and  $W_d$ = oven-dry at 50°C for 24 hours.

The Leaf Area Index (LAI) was measured at the stem elongation, flowering, and grain filling stages, with an electronic leaf area meter ( $\Delta T$  devices).

#### **Statistics**

The data were subjected to Principal Factor Analysis (PFA) according to the procedures outlined by Cattell [5] and Guertin and Bailey [12], using the PFA Package of SAS [21]. Prior to the PFA, the data were subjected to Principal Component Analysis (PCA) [13]. The varimax rotation method (an orthogonal rotation) suggested by Kaiser [14] was used. Data from each experiment was analyzed, separately. The correlation between identical pairs of factors (e. g. Factor I versus Factor I) under the two irrigation conditions were calculated. Factor loadings were used to interpret the results and to recognize the physiological criterion associated with adaptation under each irrigation conditions on the basis of the magnitude signs in the two first factors. Traits with identical signs in a factor were positively interrelated. Biplot (Factor I versus Factor II) were used graphical interrelationships among physiological traits, and visual recognition of important traits for screening suitable genotypes and the best criteria associated with screening suitable genotypes under each experiment were identified.

## RESULTS AND DISCUSSION

For general evaluation, yield, yield components and drought stress indexes of different genotypes under stress ands non-stress conditions are presented in Table 2.

The results of factor analysis for total physiological traits under non-stress and stress conditions are shown in Tables 3 and 4. 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



Table 2. Yield, yield components and drought resistance indexes for safflower genotypes under non-stress and stress irrigation regimes.

Irrigation treatment	Genotype	Seed yield (Kg ha <sup>-1</sup> )	Biologica I yield	Harvest index	No. of bolls per	No. of seeds per	1000 seeds weight (gr)	Biol harvest index	No. of days to maturity	Seed	Seed oil (%)	Drough resistance
6			(Kg ha')		plant	llod				(%)		티
Non-stress	Poshtkooh	2123.7	10825.5	16.5	14.9	56.9	32	0.71	115.7	42	32	
	Arak	2663.2	9326.6	9.21	9.61	50.9	30	0.73	116.7	20	33	
	Esphahan	2052.06	8643.3	21.5	17.4	48.4	30	0.72	114	19	35	
	RH 8018	2289.3	6788.3	25.7	14.4	52.7	34	0.85	114.7	20	36	
	UC 10	3253.7	10080.5	25.4	14.7	45.2	37	0.78	114.3	19	32	_
	Gila	2780.9	10675.5	20.6	17.2	99	35	0.75	116.3	19	34	0
	RH 410118	1724.8	8062.2	17.6	14.2	51.7	33	0.75	118	19	37	-
	Nebraska 10	1986.5	11050.5	15.5	20.8	9.99	34	0.73	115	19	31	-0.298
Significant		*	NS	SN	NS	SN	SN	Ns	NS	SN	NS	
OST 27		835-95	t	ē		е	Ü	í.	r	i	E:	
•												
Stressed	Poshtkooh	7.806	4016.6	18.5	8.7	43.9	31	0.47	114.3	20	31	
	Arak	804.3	3399.5	19.1	8.1	39.3	32	0.44	109	21	32	
	Esphahan	899.5	3692.3	19.5	6.7	48.9	29	0.50	112.3	22	32	
	RH 8018	909.2	3381.2	21.1	7.3	39.7	31	0.48	112	20	36	
	UC 10	1083.5	4019.3	20.9	8.9	35.7	39	0.50	109.7	21	32	
	Gila	1315.2	4306.3	23.5	8.8	54.2	29	0.49	112	21	33	
	RH 410118	768.9	3224	19.5	8.5	23.7	29	0.43	106	20	40	
	Nebraska 10	944.6	3481.6	21.3	6.5	46.6	28	0.49	109	21	32	
Significance		*	SN	Ns	Ns	*	*	*	*	NS	*	
TSD		274.26	·			8.63	3.2	0.03	2.68	ı	0.2	
Non-stressed vs stressed	ve etracead	*	*	SN	*	*	*	**	*	NC	NC	

NS: non-Significant.
\* Significant at 5 % probability.
\*\* Significant at 1 % probability.

greater than 1.0 were retained. Traits with a loading of greater than 0.6 in any factor were deemed major [2].

#### **Non-stress Conditions**

The factor analysis technique divided the 17 variables into six groups or factors. Six factors explained about 80% of the total variability in the dependent structure. Variable compositions of the six factors with loadings are shown in Table 3. In this analysis, only 40% of the variance was accounted for by the first two factors.

Factor I accounted for about 25% of the total variability present in the dependent structure (Table 3). Factor I was identified as growth and development or water consumption and in this factor, initial water content (at stem elongation stage) was loaded with a

positive sign, whereas canopy temperature (at stem elongation stage) was loaded with a negative sign. This factor indicated that plant growth depends on water adsorption by the roots, and its transpiration from the leaves [15].

Factor II accounted for about 16% of the total variability present in the dependent structure and was described as the water balance capacity factor. In this factor canopy temperature and leaf water potential (at flowering stage) were loaded with positive signs (0.82 and 0.89, respectively), whereas the initial water content (at grain filling stage) was loaded with the opposite sign (-0.77). This may be due to the hydrolabile nature of safflower which can tolerate water potential influences, and temporary wilt [17].

Factors I and II identified the important physiological traits for screening suitable

**Table 3**. 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.

Variables	F						
v ai lables	1	2	3	4	5	6	Communality
Stem elongation stage							
Leaf area index (LAI)	-0.06	-0.09	-0.13	0.04	$0.70^{a}$	0.34	0.63
Rate of water loss (RWL) from excised leaf	-0.34	-0.08	-0.13	0.04	0.14	$0.76^{a}$	0.73
Initial water content (IWC)	$0.88^{a}$	-0.03	-0.06	0.02	-0.01	-0.10	0.80
Leaf osmotic potential (LOP)	0.15	0.16	0.15	0.30	$0.81^{a}$	0.11	0.83
Canopy temperature	$-0.80^{a}$	-0.27	-0.10	-0.01	-0.17	0.01	0.75
Flowering stage							
Leaf area index (LAI)	0.09	-0.42	-0.16	-0.36	$0.66^{a}$	-0.19	0.82
Rate of water loss (RWL) from excised leaf	0.39	0.17	$0.75^{a}$	0.18	-0.12	-0.18	0.83
Initial water content (IWC)	0.53	0.10	$0.72^{a}$	-0.12	0.26	-0.20	0.93
Leaf osmotic potential (LOP)	-0.04	-0.03	0.15	$0.93^{a}$	0.19	-0.01	0.93
Leaf water potential (LWP)	-0.16	$0.89^{a}$	0.01	0.14	-0.02	-0.12	0.85
Canopy temperature	0.25	$0.82^{a}$	0.31	0.22	-0.08	-0.20	0.93
Grain filling stage							
Leaf area index (LAI)	0.32	-0.09	-0.20	-0.16	0.40	0.54	0.63
Rate of water loss (RWL) from excised leaf	0.55	-0.26	0.29	0.21	-0.15	0.48	0.76
Initial water content (IWC)	-0.25	$-0.77^{a}$	-0.15	0.10	-0.05	-0.35	0.81
Leaf osmotic potential (LOP)	0.10	0.24	-0.07	0.91 a	-0.03	0.01	0.90
Leaf water potential (LWP)	-0.30	0.35	0.41	0.28	0.48	-0.21	0.65
Canopy temperature	-0.33	0.15	0.81	0.01	-0.09	0.10	0.81
Proportion of total variation (%)	24.6	15.9	13.5	9.6	9.1	7.2	
Cumulative variance (%)0	24.6	40.5	54	63.6	72.7	79.9	

<sup>&</sup>lt;sup>a</sup> Coefficients larger than 0.60.



genotypes in crop improvement programs, under non-stress conditions, for safflower. These criteria consisted of initial water content (at stem elongation and grain filling stages), canopy temperature (at stem elongation and flowering stages), and leaf water potential (at the flowering stage). Other factors (III, IV, V, and VI) explained 13, 9, 9, and 78% of the total variation, respectively (Table 3), and indicated that loaded variables in these factors are not important in safflower improvement programs. Factors I and II which showed the highest variations were considered and the other factors explaining lower variations were discarded.

#### **Stress Conditions**

The factor analysis technique extracted seven factors, which explained 80% of the

total variation. Variable compositions of the seven factors with loadings are given in Table 4. The first two factors accounted for 34% of the total variability present in the dependence structure (Table 4).

Factor I was identified as canopy temperature, and accounted for about 19% of the total variation. In this factor canopy temperature (at flowering and grain filling stages) was loaded with a positive sign, whereas leaf area index (at stem elongation stage) was loaded with a negative sign. This factor indicated moisture stress during vegetative growth reduction in leaf size and leaf area index after flowering, closure of the stomata, and increase of the canopy temperature due to severe stress [10].

Factor II accounted for 15% of total variation (Table 4), and was described as the water holding capacity factor. In this factor, all variables (canopy temperature at stem elon-

**Table 4.** Loading of the first six most important principal factors (PF) from a factor analysis of 17 physiological traits under stress conditions in spring safflower.

Variables	Factor	(matrix o	f factor c	oefficient	s)			Commu-
	1	2	3	4	5	6	7	nality
Stem elongation stage								
(LAI) Leaf area index	$-0.74^{a}$	0.04	-0.28	0.36	0.11	-0.04	-0.01	0.77
Rate of water loss (RWL) from excised leaf	-0.06	-0.07	$0.88^{a}$	0.24	0.15	0.01	0.11	0.87
Initial water content (IWC)	-0.13	-0.06	-0.89 a	0.13	0.01	-0.01	0.23	0.89
Leaf osmotic potential	0.05	0.26	-0.12	0.07	0.02	-0.05	$0.84^{a}$	0.80
Canopy temperature	-0.40	$0.63^{a}$	0.15	0.15	-0.16	-0.02	-0.12	0.65
Flowering stage								
(LAI) Leaf area index	0.04	0.02	0.22	0.01	0.24	$0.86^{a}$	0.11	0.86
Rate of water loss (RWL) from excised leaf	-0.38	-0.20	0.27	0.34	-0.36	-0.24	0.37	0.69
Initial water content (IWC)	-0.03	0.16	0.16	$-0.72^{a}$	-0.40	0.10	1	0.78
Leaf osmotic potential	-0.05	-0.06	0.31	0.25	0.24	$-0.77^{a}$	0.25	0.88
Leaf water potential (LWP)	0.28	-0.16	0.00	0.03	0.71	-0.22	-0.34	0.77
Canopy temperature	$0.87^{a}$	-0.03	0.04	0.27	-0.07	-0.06	0.03	0.84
Grain filling stage								
(LAI) Leaf area index	-0.48	-0.16	0.04	-0.46	-0.02	-0.44	0.31	0.77
Rate of water loss (RWL) from excised leaf	0.00	$0.89^{a}$	-0.01	-0.00	0.06	0.04	0.13	0.81
Initial water content (IWC)	0.07	$0.90^{a}$	-0.01	-0.19	-0.22	0.08	0.19	0.96
Leaf osmotic potential	0.21	0.02	0.25	$0.84^{a}$	-0.21	-0.09	0.00	0.87
Leaf water potential (LWP)	-0.12	-0.10	0.19	0.00	0.79	0.27	0.22	0.81
Canopy temperature	$0.81^{a}$	-0.09	-0.11	0.23	0.25	-0.02	-0.02	0.80
Proportion of total variations (%)	18.9	15.3	12.8	11.3	9.6	7.6	5.9	
Cumulative variance (%)	18.9	34.2	47.1	58.3	67.9	75.5	81.4	

<sup>&</sup>lt;sup>a</sup> Coefficients larger than 0.60.

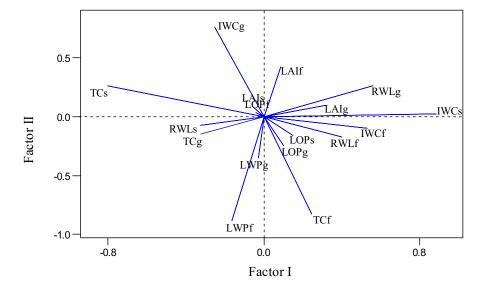
gation stage, and RWL and IWC at grain filling stage) had positive loadings. The sign of the loadings indicates the direction of the relationship between the factor and the variable. Therefore, three variables with high loadings in the same factor with the same sign would be expected to exhibit a positive correlation. This factor indicated that soil water storage capacity is related to transpiration reduction during vegetative growth.

Important physiological traits identified by Factors I and II consisted of canopy temperature (at stem elongation, flowering and grain filling stages), leaf area index (at stem elongation stage), rate of water loss (RWL) from excised leaves and IWC (at grain filling stage). These criteria screen suitable genotypes in safflower improvement programs under stress conditions. Other factors (III, IV, V, VI, and VII) explained about 13, 11, 10, 8, and 6% of the total variations, respectively (Table 4), and indicated that loaded variables in these factors are not important in safflower improvement programs under non-stress conditions.

# Physiological Traits Biplot

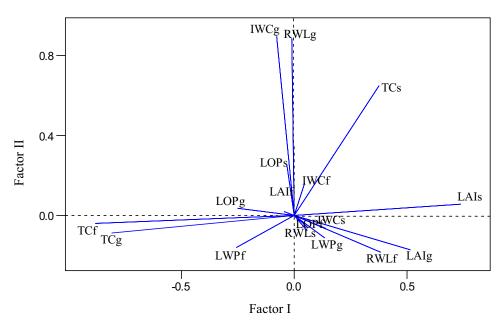
The biplot (Factor I versus Factor II) for each of the experiments shown in Figures 1 and 2, describes the interrelationships among all traits on the basis of the overall pattern of the data. In the biplot, a vector is drawn from the biplot origin to each marker of the traits to facilitate visualization of the relationships between and among the traits. The correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. Thus,  $r = \cos 180^\circ = -1$ ,  $\cos 0^\circ = 1$ , and  $\cos 90^\circ = 0$  [28].

Under non-stress conditions, the largest variation resulted from initial water content (at stem elongation and grain filling stages), canopy temperature (at stem elongation and flowering stages), and leaf water potential (at flowering stage) (Figure 1). Under stress conditions, the largest variation was explained by canopy temperature (at stem elongation, flowering and grain filling stages), leaf area index (at stem elongation stage), IWC and rate of water loss (RWL)



**Figure 1**. Biplot (Factor I versus Factor II) for 17 physiological traits of spring safflower under stress conditions. s: Stem elongation stage; f: Flowering stage; g: Grain filling stage; IWC: Initial water content; RWL: Rate of water loss from excised leaf; LOP: Leaf osmotic potential; TC: Canopy temperature; LWP: Leaf water potential, and LAI: Leaf area index.





**Figure 2**. Biplot (Factor I versus Factor II) for 17 physiological traits of spring safflower under non-stress conditions. s: Stem elongation stage; f: Flowering stage; g: grain filling stage; IWC: Initial water content; RWL: Rate of water loss from excised leaf; LOP: Leaf osmotic potential; TC: Canopy temperature; LWP: Leaf water potential, and LAI: Leaf area index.

from excised leaf (at grain filling stage) (Figure 2).

The most prominent relations revealed by these are: (i) a strong negative association between initial water content and canopy temperature (at stem elongation stage), in Factor I, and between IWC (at grain filling stage), and LWP and canopy temperature (at flowering stage) under non-stress conditions (Figure 1), as well as under stress conditions; (ii) a strong negative association was observed between leaf area index (at stem elongation stage) and canopy temperature (at flowering and grain filling stages), and a strong positive association was observed between canopy temperature at flowering and grain filling stages in Factor I, and a strongly positive correlation between the rate of water loss (RWL) from excised leaves and IWC (at grain filling stage) in Factor II. Interrelationships revealed by these physiological traits can be informative in safflower breeding programs under stress

and non-stress conditions.

# The Correlation between Factor Loadings of Two Experiments

The correlation coefficients between principal factors in under both stress and nonstress conditions are shown in Table 5. The negative correlation between Factor II (nonstress) and II (stress) and also between Factor IV (non-stress) and VI (stress) is attributed to the negative loadings in Factor II (non-stress) and VI (stress). The positive correlation between Factor IV (non-stress) and IV (stress) was related to the same loadings in Factor IV under non-stress and stress conditions. Thus, in the two experiments Factor IV, behaved in the same manner whereas the other factors, especially Factor II (non-stress) and VI (stress) behaved differently in the stressed and non-stressed experiments.

<b>Table 5</b> . Linear correlations among loading coefficients in the principal factors extracted from data
consisting of 17 traits on eight spring safflower cultivars grown in two experiments.

Non-stress				Stress condition	ons		
conditions	Factor I	Factor II	Factor III	Factor IV	Factor V	Factor VI	Factor VII
Factor I	-0.04	-0.16	-0.43	-0.23	-0.25	0.01	0.27
Factor II	0.45	-0.61 <sup>a</sup>	0.08	0.23	0.36	-0.10	-0.22
Factor III	0.36	-0.17	0.05	-0.12	-0.09	-0.04	-0.3
Factor IV	0.16	-0.04	0.17	$0.59^{a}$	0.03	$-0.55^{a}$	0.16
Factor V	-0.36	-0.24	-0.01	-0.21	0.26	0.26	0.42
Factor VI	-0.30	-0.20	0.28	0.05	0.04	-0.25	0.12

<sup>&</sup>lt;sup>a</sup> Significant coefficients p<0.05.

This paper demonstrates the good performance of factor analysis for considering variations among physiological traits in contrasting conditions. We advise such experiments be conducted in several places and over several years to give more reliable results.

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# ارزیابی برخی معیارهای فیزیولوژیک و روابط آنها در گلرنگ بهاره (.*Carthamus tinctorius* L.) تحت رژیمهای تنش و بدون تنش آبی

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## چکیده