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Growth, photosynthesis and production of safflower (*Carthamus tinctorius* L.) in response to different levels of salinity and drought

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Abstract. In order to investigate the effects of salinity and drought stress on the photosynthesis, growth and production responses of safflower, two experiments were performed in Isfahan, Iran using four different levels of saline water, four different levels of irrigation water, and their combinations which were applied at three different growth stages (stem elongation, heading, and flowering) in 2016 and 2017. A split-plot experiment based on complete block design was performed with three replicates. The plant height, leaf area index, relative water content, number of heads per plant, number of seeds per head, 1000-seeds weight, seed yield, oil content, net photosynthetic rate, stomatal conductance and transpiration rate decrease under salinity, drought, and simultaneous stresses. The reduction in seed yield caused by the 10 dS m⁻¹ and 40% of irrigation treatment was higher at the heading stage (92.6%) when compared with the stem elongation (71.04%) and flowering (89.9%) stages. In general, the reduction in seed yield caused by salinity-drought stress was higher at the heading stage as compared with stem elongation and the flowering stages.

Keywords: combined stress, net photosynthesis, oil content, plant growth, stomatal conductance, safflower

INTRODUCTION

Plants are mainly subjected to many stresses such as limited water, low/high temperature, salinity, flooding, and heavy metal toxicity (Umar and Siddiqui, 2018). Studying the effects of environmental stresses is an important stage toward increasing our level of understanding of plant behaviour under field conditions (Patil, 2012). Certain abiotic stresses, which pose a threat to crop productivity worldwide, are drought and salinity (Guo *et al.*, 2014). At present, 33% of irrigated agricultural land and 20% of cultivated land is affected by salinity (Shrivastava and Kumar, 2015), with some predictions that salinization could impact 50% of agricultural lands by 2050 (Jamil *et al.*, 2011). Approximately 40% of the world's available land is affected by drought (Zhang *et al.*, 2014).

Increasing our understanding of the mechanisms of how plants respond to salinity and drought stresses is necessary for the adaptation of management strategies for crop plant production under harsh/suboptimal weather conditions, with a view to increasing the yield stability of crop performance under drought and saline conditions (Chaves et al., 2009). Both of the above-mentioned abiotic stresses decrease the water potential in the soil and the ability of plants to absorb water, thereby reducing the rate of cell expansion in growing tissues, the stomatal conductance and hence the net photosynthetic rate (Munns, 2011). The effects of combined stresses on plants are significantly different to their responses to individual stresses (Grzesiak et al., 2016). Sahin et al. (2018) reported that drought and salinity stress have a negative effect on plant height, leaf area, stomatal conductance and also on the net photosynthetic activity of cabbage (Brassica oleracea L.) when

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applied separately, while their combination increases the harmful effects of each stress factor. Ahmed *et al.* (2013) showed that the combination of salinity and drought stress caused the largest reduction in plant height, stomatal conductance and net photosynthesis of barley as compared to the separate application of salinity and drought stresses. Similar to these findings, Umar and Siddiqui (2018) reported that a greater decrease in stomatal conductance in safflower leaves was observed when the plants were treated with a combination of salinity and drought stresses.

Safflower (*Carthamus tinctorius* L.) is a member of the Asteraceae family and one of the prospective and high-quality oilseed crops (Weiss, 1971). The Iranian Agricultural Organization reported that the acreage of safflower is 24855 ha in Iran and that Isfahan province had a 4850 ha planting area in the planting year of 2019. With regard to water and salinity limitations, the average safflower yield in the most cultivated area in Iran is less than world average (0.965 *vs.* 4.9 t ha⁻¹ for California, 2.8 t ha⁻¹ for Australia and 2.5 t ha⁻¹ for Mexico (Gilbert, 2008). It is cultivated in arid and semi-arid areas where salinity stress is one of the major threats to agriculture (Kaya, 2009). Also, due to the growing potential of safflower under drought stress without a substantial reduction in oil and seed yields, it may be considered as an alternate crop (Kar *et al.*, 2007).

Extensive research has been conducted to investigate the effects of separately applied salinity and drought stresses on a variety of plants including safflower, while research concerning this topic is limited to the growth and photosynthetic responses of safflower caused by combined salinity-drought stress at different growth stages. In this respect, the objectives of this research were to evaluate the individual and combined effects of salinity and drought stresses on certain photosynthetic parameters, the growth performance, and the production yield of safflower at different growth stages.

MATERIALS AND METHODS

Two field experiments were conducted in 2016 and 2017 in the Kabotarabad region of Isfahan province, Iran $(32^{\circ} 30' \text{ N}, 51^{\circ} 49' \text{ E}$ and altitude 1541 m). There was a weather station located near the field (about 1000 m from the experimental field) which served to provide a daily recording of the climatic parameters that are shown in Table 1. The soil characteristics of the experimental site are shown in Table 2.

Safflower seeds (the Sofeh variety used were obtained from the Isfahan Agricultural and Natural Resources Research and Education Centre) and were planted on the 3rd and 7th of April 2016 and 2017, respectively.

Each experimental unit had an area of 10 m^2 with five rows (0.4 m spacing). In order to prevent the lateral movement of water between treatments, a distance of 1.5 m was maintained between plots. The phosphorus and potassium fertilizers were mixed with the soil along with the soil

 Table 1. Monthly values of maximum and minimum air temperature and rainfall for the years 2016 and 2017

Vaar	Month	Air temper	rature (°C)	Rainfall
rear	Monui	Max	Min	(mm)
	Apr	23.3	7.41	13.8
	May	28.5	11.8	0
	Jun	35.6	15.4	0
2016	Jul	37.6	18.1	0
	Aug	36.6	17.5	0
	Sep	33.6	12.1	0
	Oct	28.9	8.06	0
	Apr	22.3	6.21	14.3
	May	25.8	10.3	0
	Jun	34.3	16.6	0
2017	Jul	38.7	18.5	0
	Aug	38.6	18.6	0
	Sep	35.9	16.5	0
	Oct	28.2	10.2	12.8

preparation two weeks before planting. Triple superphosphate (46% P_2O_5) and potassium chloride (60% K) were the sources of phosphorus and potassium fertilizers, respectively. Urea was used in 3 split applications (base fertilizer 2: seedling 1: flowering 2). Safflower seeds were hand-planted in each row with a separation of 0.10 m (25 plants m⁻²).

The study was performed in a split plot experiment based on a complete block design with three replications. The experimental factors were salinity and drought stress levels which were applied at three different growth stages (stem elongation, stage 30, heading, stage 50, and flowering, stage 61) (Table 3). The growth stages of safflower were determined according to the extended BBCH scale (Flemmer et al., 2015). For each growth stage, salinity and drought stress treatments were applied, separately (each experimental plot received treatments at only one of these growth stages). Four different salinity treatments were applied as the main plots were irrigated with different saline waters (2.5 dS m⁻¹ for S0, 5 dS m⁻¹ for S1, 10 dS m⁻¹ for S2, and 15 dS m⁻¹ for S3). Sodium chloride (Sigma-Aldrich, purity 100%) was used to provide the required salinity levels (25, 50, 100, and 150 mM NaCl). Drought stress treatments for the subplots were adjusted at a rate of 80% (D1), 60% (D2), and 40% (D3) out of 100% field capacity (D0) of the soil (a total of 11 irrigation treatments were performed with a 7 to 10 day irrigation interval). The volume of full irrigated water (dj) was obtained by Eq. (1) (Babazadeh et al., 2017):

$$dj = (FC - Pv) Z, \tag{1}$$

Year	Soil texture	Nitrogen	Phosphorus	Potassium	рН	EC	Organic matter
			$(mg kg^{-1})$		F	$(dS m^{-1})$	(%)
2016	Sandy-Loam	0.091	8	185	7.3	2.15	0.05
2017	Sandy-Loam	0.093	10	188	7.3	2.11	0.05

Table 2. Soil properties at the beginning of the growing season

where: Pv is the soil moisture at irrigation time obtained using TDR (Field Scout TDR 350, Spectrum, USA), FC is the field capacity of the farm soil and Z is the rooting depth at different growth stages.

When the involucral bracts began to turn yellow (stage 81), four plants were selected from each subplot and the leaf area and plant height were measured. The leaf area was measured with reference to a square metre (WINAREA-UT-11, Iran) and leaf area index (*LAI*) was calculated using the Eq. (2) (Gardner *et al.*, 1985):

$$LAI = \frac{leaf area}{ground \ cover}.$$
 (2)

At maturity in July of 2016 and 2017, the plants were cut at the ground level from three middle rows of subplots and then oven dried at 80°C until a constant weight was achieved. The seeds were separated from the straw and weighed using a balance and then the yields were determined. Yield components number of heads per plant (NHP), number of seeds per head (NSH), and 1000-seeds weight (TSW) were determined after the harvest.

Ten grams of ground seeds was used to extract the oil, petroleum ether was used for 6 h in a Soxhlet system according to the AOCS method (AOCS, 1993), and then the crude fat content as a percentage of the total weight was calculated for each sample.

The net photosynthetic rate (P_n), stomatal conductance (C_{leaf}) and transpiration rate (E) of the plants were measured between 11:00 am and 14:00 pm using a photosynthesis meter (Cl-340, CID Bio-Science, USA, with square leaf chamber; 25 × 25 mm) when the involucral bracts are beginning to turn yellow; stage 81. For this purpose, the fully expanded young leaves of the plants (from the centre row of each subplot) were selected.

The relative water content (RWC) was measured according to Farahbakhsh *et al.* (2017). The following equation was used to calculate RWC:

$$RWC = \frac{Fresh \, weight - dry \, weight}{Turgid \, weight - dry \, weight} \, 100. \tag{3}$$

The relative water content and electrolyte leakage were measured when the involucral bracts are beginning to turn yellow; stage 81. Fresh leaf discs (0.25 cm² of each leaf) were used to determine electrolyte leakage according to Campos *et al.* (2003).

The proline content was measured according to Bates (1973). L-proline (Sigma-Aldrich, purity \geq 99%) with defined concentrations of (0.01-1.5 mM) being used for calibration curve construction.

Bartlett's test was used to evaluate the homogenous mean square error over a period of two years. Due to the non-significant χ^2 value from Bartlett's test for most traits, the data were combined. A Combined Analysis of Variance (PROC ANOVA of SAS ver. 9.2, 2010) was used for each growth stage, separately. The means of treatments were compared using an LSD test procedure at p < 0.05. A linear regression analysis was performed separately for each growth stage using a Stepwise Method (SPSS software, ver. 18.0 was used). The growth stages were compared using SPSS (ver. 18.0, 2010) with an Independent-Samples T-Test.

RESULTS

Plant height and *LAI* were affected by both salinity and irrigation levels at the stem elongation stage (Table 3). The cumulative effects of increasing the salinity and reducing the irrigation water level resulted in greater decreases in plant height and *LAI* (Table 4). The leaf area index in the S0D3 (drought stress), S3D0 (salinity stress), and S3D3 (combined salinity-drought stress) treatments were lower by 24.39, 40.76, and 55.75%, respectively, as compared to the S0D0 (control) value (Table 4).

The means of P_n and C_{leaf} decreased to a significant extent due to salinity stress. Leaf stomatal conductance was decreased under both salinity and irrigation levels, while E was not significantly affected by these experimental factors (Table 3). Salinity and drought interaction did not have a significant effect on the photosynthetic parameters of safflower (Table 4).

The highest *RWC* value of 76% was determined in the S0 and S1 treatments and the S3 treatment had the lowest value, 64% (Table 3). The general trend showed that the REC and proline values increased with increases in the level of salinity and drought (Table 4).

The application of drought and salinity stresses showed a significant reduction in the NHP, NSH, seed yield and oil content of safflower (Table 3). The interaction between salinity and drought did not have a significant effect on the NSH, TSW and oil content (Table 4). Seed yield was reduced by 55.56, 71.40, and 87.13% in the S0D3, S3D0, and S3D3 treatments respectively as compared to the S0D0 treatment (Table 4).

Table 3.	Effects	of different leve	ls of salinity :	and drought or	ı plant growth	, photosynthet	ic parameters, y	ield compone	nts, seed yield	and oil conte	nt of safflower			
Experimer	ıt	Plant height			TION	TSW	Seed yield	Oil con-	P.	Е	C _{leaf}	RWC	REC	Proline
factor		(cm)	T T T	HI	HSN	(g)	(kg ha ⁻¹)	tent (%)	(μmol m ⁻² s ⁻¹	(mm	iol m ⁻² s ⁻¹)	(%)	(%)	$(\mu mol g^{-1} FW)$
Salinity	SO	56.77 ^a	2.45ª	12.44^{a}	18.69^{a}	36.49	2296.8 ^a	34.58 ^a	16.92 ^a	9.22	234.3ª	76.84a	50.45c	0.648c
	S1	56.21^{a}	2.38^{a}	12.13 ^b	$19.24^{\rm a}$	37.39	2306.1^{a}	33.63^{a}	16.36^{ab}	9.41	229.2^{a}	76.37a	50.85c	0.661c
	S2	49.11 ^b	2.04^{b}	9.08°	17.31^{b}	34.80	1508.8^{b}	33.45^{a}	$16.01^{\rm b}$	8.98	217.8^{b}	71.48b	56.21b	1.506b
	S3	41.45°	1.78°	6.81^{d}	16.36^{b}	35.65	1025.2°	31.99^{b}	15.71 ^b	8.97	$219.5^{\rm b}$	63.72c	62.23a	1.873a
L.S.D.	ı	**	**	* *	* *	SU	* *	* *	*	su	*	*	*	* *
Drought	D0	56.92 ^a	2.63 ^a	13.23 ^a	18.74^{a}	40.10^{a}	2531.4^{a}	35.24^{a}	16.87^{a}	9.23	227.6^{a}	82.37a	48.07c	0.594c
	D1	57.77^{a}	2.68^{a}	13.09^{a}	18.61^{a}	38.80^{a}	2560.0^{a}	35.08^{a}	16.52^{ab}	9.46	227.9^{a}	82.35a	48.91c	0.532c
	D2	47.03 ^b	1.86^{b}	9.16°	17.56^{b}	33.84^{b}	1378.9^{b}	32.22 ^b	15.53°	8.81	226.6^{a}	66.29b	54.50b	1.449b
	D3	41.82°	1.48°	4.98°	$16.68^{\rm b}$	31.59^{b}	666.6°	31.11^{b}	$16.07^{\rm bc}$	9.07	$218.7^{\rm b}$	57.39c	68.25a	2.113a
L.S.D.	,	**	* *	*	*	**	* *	**	**	ns	*	* *	* *	* *
Year	2016	50.83	2.17	10.17^{a}	18.01	36.12	1782.7	33.25	16.24	9.17	223.8^{b}	72.33a	55.22a	1.174
	2017	50.93	2.16	10.06^{b}	17.79	36.04	1785.7	33.57	16.26	9.12	226.5^{a}	71.87a	54.65b	1.170
L.S.D.	,	ns	ns	* *	ns	ns	ns	ns	ns	ns	*	ns	**	su
In a colum	n, mean	s with a differe	ant letter dene	oted a statisti	cal difference	between the	treatment group	ps according	to LSD's test	(p < 0.05).	S0: water witl	nout salinity	i, S1: water	with a salinity of
5 ds m ⁻¹ , S2.	: water w	vith a salinity of	'10 ds m ⁻¹ , S3:	: water with a s	alinity of 15 d	s m ⁻¹ , D0: Full	-irrigation, D1:	irrigation witl	h 80% of the E	00, D2: irrigat	on with 60% o	f the D0, D3	: irrigation w	ith 40% of the D0,
L.S.: level c	of signifi	cance, * p < 0.0	15, ** p < 0.01	l, ns: non-sign	ificant, NHP:	number of hea	ds per plant, NS	SH: number o	f seeds per he	ad, TSW: 100	D-seed weight,	LAI: leaf are	ea index, P _n :	net photosynthesis
rate, E: tran	spiration	ו rate, C _{leaf} : stom	natal conducta	nce, RWC: rel	ative water co.	ntent, REC: re	lative electrolyt	e conductivity	y.					

Table 4. Effects of salinity and drought interaction on plant growth, photosynthetic parameters, yield components, seed yield and oil content of safflower at the stem elongation stage

	-	Plant height	17.1	a e	VICIT	TSW	Seed vield	Oil con-	P,	ш	C leaf	RWC	REC	Proline
Salinity	Drought	(cm)	LAI	JHN	HSN	(g)	(kg ha ⁻¹)	tent $(\%)$	(μmol m ⁻² s ⁻¹)	(mmol	. m ⁻² s ⁻¹)	_ (%)	(%)	(µmol g ⁻¹ FW)
SO	D0	66.30^{a}	2.87 ^b	16.49 ^a	19.27	40.85	3158.2 ^a	35.72	18.03	8.96	239.5	85.25	45.58h	0.117h
	DI	64.32^{a}	2.99^{ab}	15.61 ^b	19.59	41.19	3358.6^{a}	36.10	16.34	9.50	233.2	86.97	44.07h	0.124h
	D2	52.77^{bc}	2.51°	12.22°	18.56	39.02	2205.3^{b}	36.07	16.50	9.07	224.1	72.59	49.65fg	0.790g
	D3	44.32 ^d	2.17^{d}	8.61°	17.56	39.36	1403.4^{d}	33.09	16.62	9.40	213.5	62.55	62.50c	1.561e
Sl	D0	68.25^{a}	3.10^{a}	16.03^{b}	19.19	39.28	3328.4^{a}	35.92	16.93	9.33	230.4	83.30	42.99h	0.129h
	DI	67.10^{a}	2.93^{ab}	15.92^{b}	21.47	39.02	3343.9^{a}	34.59	16.54	9.51	235.3	87.62	45.41h	0.129h
	D2	54.89^{b}	2.60°	11.84°	17.05	35.77	2163.0^{b}	34.77	16.54	9.81	211.8	71.64	52.17ef	0.855g
	D3	40.82^{def}	2.10^{d}	8.58°	16.74	41.12	1404.7^{d}	35.01	16.08	9.20	234.0	62.90	62.83c	1.531e
S2	D0	49.15°	2.14^{d}	11.07^{d}	18.41	33.90	1797.7°	34.22	16.06	9.25	240.7	82.79	49.40g	0.890g
	DI	49.75°	2.02^{d}	10.91^{d}	19.11	36.02	1693.8°	32.76	15.92	9.27	227.2	84.10	50.23fg	0.800g
	D2	49.63°	1.66°	8.08^{f}	17.36	33.43	1137.8°	32.05	15.22	8.51	219.6	65.13	55.37d	1.970d
	D3	$39.57^{\rm ef}$	1.61°	6.56^{g}	15.38	32.01	886.18^{f}	29.88	14.94	8.23	218.8	53.88	69.83b	2.363b
S3	D0	43.40^{def}	1.70°	6.16^{gh}	17.88	31.94	$902.93^{\rm ef}$	32.48	16.67	9.35	226.5	78.15	54.32de	1.239f
	DI	43.68^{de}	$1.59^{\rm ef}$	$6.07^{\rm h}$	16.78	33.32	828.01^{f}	31.09	16.65	9.37	221.0	70.70	55.94d	1.075f
	D2	39.13^{f}	1.41^{fg}	4.17^{i}	16.27	30.99	529.16^{g}	30.90	15.76	8.52	215.8	55.80	60.82c	2.181c
	D3	41.08^{def}	1.23^{g}	3.51^{j}	15.78	30.10	406.45^{g}	29.98	15.21	9.05	211.6	50.21	77.85a	2.997a
L.S.		* *	* *	*	su	su	**	su	ns	ns	us	ns	* *	* *
Explanatio	ins as in Table	e 3.												

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Experiment	-	Plant height			TION	TSW	Seed vield	Oil con-	ط	ш	C _{leaf}	RWC (%)	REC	Proline (umol o ⁻¹ FW)
factor	Drought	(cm)	LAI	HN	HSH	(g)	(kg ha ⁻¹)	tent $(\%)$	(µmol m ⁻² s ⁻¹)	(mmol	m ⁻² s ⁻¹)			
Salinity	SO	57.71 ^a	2.65a	13.57^{a}	17.54 ^a	38.57	2425.1 ^a	34.52 ^a	16.49^{a}	9.41 ^a	228.1 ^a	75.74ª	49.49°	0.659°
	S1	58.61^{a}	2.67^{a}	13.28^{b}	16.49^{b}	38.81	2252.7 ^b	35.67^{a}	17.19^{a}	9.12^{a}	219.7^{b}	75.38^{a}	49.85°	0.671°
	S2	46.24^{b}	2.44^{b}	10.82°	14.61°	38.45	1571.4°	31.84^{b}	15.43^{b}	9.06^{a}	220.5^{b}	70.45 ^b	55.33 ^b	1.515^{b}
	S3	41.58°	2.22°	7.41d	12.45d	36.93	921.4^{d}	30.69^{b}	14.86^{b}	8.32 ^b	211.9°	63.78°	61.21 ^a	1.892^{a}
L.S.	ı	* *	* *	* *	* *	su	*	* *	* *	*	* *	* *	*	* *
Drought	D0	55.86^{a}	2.82ª	13.38^{a}	18.45^{a}	38.99^{ab}	2555.9ª	35.45 ^a	16.77^{a}	9.78^{a}	229.4^{a}	81.31 ^a	47.25°	0.592°
	DI	56.99^{a}	2.78ª	13.05^{b}	18.78^{a}	39.06^{a}	2388.7^{b}	35.02^{a}	16.44^{a}	9.06^{b}	223.3^{a}	81.32^{a}	47.89°	0.542°
	D2	$45.92^{\rm b}$	2.34^{b}	11.04°	14.65^{b}	37.34^{b}	1515.9°	31.82^{b}	15.60^{b}	9.02^{b}	217.7^{b}	$65.28^{\rm b}$	53.35 ^b	1.480^{b}
	D3	$45.37^{\rm b}$	2.05°	7.61d	9.20°	37.36^{b}	710.06^{d}	30.43°	15.16^{b}	8.04°	212.8^{b}	57.44°	67.39^{a}	2.123^{a}
L.S.	ı	* *	* *	* *	* *	*	*	* *	* *	*	* *	* *	*	* *
Year	2016	50.89	2.49	11.32^{a}	15.06^{b}	38.14	1782.5^{b}	33.41^{a}	15.87	9.00	219.6	71.30	54.21 ^a	1.188
	2017	51.18	2.50	11.22 ^b	15.48^{a}	38.24	1802.7^{a}	32.94^{b}	16.12	8.96	220.5	71.37	53.73 ^b	1.180
L.S.		su	su	* *	*	ns	*	*	su	su	su	ns	* *	su
Explanations :	as in Table 3													

The effect of salinity and drought stress on the plant height and LAI of safflower plants at the heading stage are presented in Table 5. The plant height and LAI decreased with increasing salinity and drought stress levels. Minimum values of LAI (1.77) and plant height (37.31 cm) were obtained from the S3D3 and S2D3 treatments, respectively (Table 6).

The effects of salinity and drought stress on photosynthetic parameters are presented in Table 5. Increasing the salinity level and reducing the irrigation water level resulted in a decrease in the P_n , E, and C_{leaf} values of safflower (Table 5). The net photosynthesis rate and C_{leaf} values were affected by salinity and drought stress interaction (Table 6). The minimum values of P_n (13.65 µmol m⁻² s⁻¹) and C_{leaf} (196.1 mmol m⁻² s⁻¹) were recorded from the S3D3 treatment.

Salinity and drought stresses notably affected *RWC* (Table 5). The relative water content decreased with increasing salinity and drought stress (Table 5). The highest REC and proline values in safflower plants were found under the conditions of the highest salinity and the most severe drought (Table 5). The cumulative effects of increasing the salinity and reducing the irrigation water levels resulted in the enhanced REC of safflower (Table 6).

The effect of salinity stress on NHP, NSH, seed yield, and oil content were statistically significant (Table 5). The highest values of NHP, NSH and seed yield were produced under the conditions of the zero-salinity stress treatment (S0) (Table 5). Drought stress had a significant effect on NHP (p < 0.01), NSH (p < 0.01), TSW (p < 0.05), seed yield (p < 0.01), and oil content (p < 0.01) of safflower at the heading stage (Table 5). Based on these results, the aforementioned parameters decreased with reducing irrigation water levels (Table 5). The interaction between salinity and drought had a significant effect on NHP, NSH and the seed yield of safflower (Table 6). Seed yield was reduced by 63.77, 71.21, and 92.60% in the S0D3, S3D0 and S3D3 treatments respectively, as compared with the S0D0 treatment (Table 6).

At the flowering stage, the effects of salinity and drought stress on plant height were non-significant but *LAI* was affected significantly by these factors (p < 0.01) (Table 7). Based on the results, increasing the salinity and drought stress levels caused a reduction in *LAI* (Table 7). Salinity and drought interaction had no significant effect on plant height and *LAI* (Table 8).

Salinity and drought stresses affected the P_n , E, and C_{leaf} values to a significant degree, at the flowering stage (Table 7). The changes in the photosynthetic parameters have shown a similar trend, with P_n , E, and C_{leaf} decreasing with increasing salinity and drought stress (Table 7). Salinity and drought interaction did not have any significant effect on the P_n value of safflower (Table 8). Salinity × drought interaction showed a decrease in the C_{leaf} value of safflower with the decrease in water volume (from the

Table 6. Eff.	ects of salin.	ity and drought i	nteraction (on plant grov	עפטוטווע ,וווא	nureuc para	meters, yreiu u	auponones, so				0		
Colimiter	Ducusht	Plant height	1 1 1	NIUD	NSH	TSW	Seed yield	Oil con-	\mathbf{P}_{n}	Ш	$\mathbf{C}_{\mathrm{leaf}}$	RWC	REC	Proline
Samue	DIOUGIII	(cm)	IVT	JUN	LICN	(g)	(kg ha ⁻¹)	tent $(\%)$	(μmol m ⁻² s ⁻¹	omm) (l m ⁻² s ⁻¹)	(0%)	(0%)	(morg rw)
SO	D0	62.69^{a}	3.09^{ab}	16.17^{a}	21.22 ^a	39.65	3541.9ª	35.77	16.48^{bc}	10.00	230.4ª	84.21	44.69 ^g	0.123
	Dl	65.66^{a}	3.13^{a}	16.00^{a}	20.60^{ab}	40.12	3187.0^{b}	37.55	17.77^{ab}	9.93	230.3 ^a	85.83	43.02^{g}	0.133
	D2	52.20^{b}	2.60^{d}	12.60^{d}	16.96°	38.88	2211.7 ^d	34.76	$16.60^{ m bc}$	9.72	230.3 ^a	71.28	48.65^{f}	0.801
	D3	42.91^{de}	2.50^{d}	8.78^{g}	15.04^{de}	37.35	1283.1^{g}	33.75	$16.27^{\rm bc}$	9.49	226.9^{b}	61.65	61.62°	1.581
S1	D0	63.79^{a}	2.84°	15.43^{b}	21.89^{a}	35.14	3139.4^{b}	35.43	15.97°	9.53	235.1 ^a	82.21	42.20^{g}	0.125
	Dl	65.75 ^a	2.91^{bc}	$15.23^{\rm b}$	19.06°	39.93	2957.8°	36.88	18.43^{a}	8.93	209.9^{d}	86.60	44.52^{g}	0.144
	D2	53.00^{b}	2.84°	12.73 ^d	18.88^{b}	42.34	2137.5 ^{de}	34.83	15.94°	9.21	233.9ª	70.65	50.89^{ef}	0.864
	D3	45.42^{cd}	2.54^{d}	8.81^{g}	15.33 ^{cd}	38.86	1320.4^{g}	32.94	$15.44^{\rm cd}$	8.61	214.6°	62.04	61.78°	1.549
S2	D0	53.43^{b}	2.51^{d}	13.24°	15.91 ^{cd}	39.70	1999.5 ^{ef}	34.27	16.78^{bc}	9.26	215.8°	81.72	48.80^{f}	0.873
	DI	53.54^{b}	2.48^{de}	13.03^{cd}	15.21 ^{cde}	37.51	1887.1^{f}	35.40	16.03°	9.07	218.6^{bc}	83.16	49.26^{f}	0.807
	D2	39.43 ^{ef}	2.29 ^{ef}	10.60°	14.18^{de}	36.26	1357.1^{g}	28.70	15.56^{cd}	9.17	214.2°	64.13	54.24^{d}	1.994
	D3	37.31^{f}	2.12^{fg}	7.32^{h}	13.33°	35.90	820.2 ⁱ	28.92	14.06^{de}	8.61	210.3^{d}	52.80	69.02^{b}	2.387
S3	D0	50.94^{b}	2.19^{fg}	9.45^{f}	11.17^{f}	39.81	1019.6^{h}	32.62	16.75^{bc}	8.87	231.4^{a}	77.12	53.32^{de}	1.247
	Dl	$49.53^{\rm bc}$	2.17^{fg}	8.88^{g}	11.12^{f}	37.70	979.2^{hi}	32.86	16.56^{bc}	8.58	220.0^{b}	69.69	54.78^{d}	1.084
	D2	$40.34^{\rm ef}$	2.07^{g}	7.39^{h}	8.44^{g}	36.32	579.5	29.10	13.69°	8.15	203.9°	55.05	59.62°	2.265
	D3	40.70^{ef}	1.77^{h}	4.74	6.11^{h}	35.63	262.0^k	27.17	13.65°	6.59	196.1^{f}	53.27	77.14ª	2.973
L.S.	,	* *	*	* *	*	us	* *	su	*	ns	*	ns	* *	ns
Table 7. Eff	ects of salini	ity and drought c	on plant gro	wth, photos	ynthetic para	meters, yiel	d components,	seed yield an	d oil content of	safflower at tl	he flowering	stage		
Exneriment		Plant heioht				MST	Seed vield	Oil con-	٩	Е	C _{leaf}	RWC	REC	Proline (11mol o ⁻¹ FW)
factor	Drought	(cm)	LAI	HN	HSN	(g)	(kg ha ⁻¹)	tent $(\%)$	$(\mu mol m^{-2} s^{-1})$	(mmol	m ⁻² s ⁻¹)			0
Salinity	SO	65.34	2.88^{a}	15.62	14.61^{a}	32.36^{a}	2110.2 ^a	29.84^{a}	14.01^{a}	7.55 ^a	209.3^{a}	72.09^{a}	52.49°	0.654°
	$\mathbf{S1}$	65.67	2.74^{b}	15.76	15.22 ^a	32.16^{a}	2057.6^{a}	29.00^{a}	13.33^{a}	7.61 ^a	200.3^{b}	72.17^{a}	52.58°	0.672°
	S2	65.23	$2.72^{\rm bc}$	16.01	11.89^{b}	29.03^{b}	1601.8^{b}	27.03^{b}	12.20 ^b	$6.67^{\rm b}$	179.5°	66.47 ^b	58.16^{b}	1.510°
	S3	64.02	2.62°	16.32	10.13°	28.65^{b}	1292.6°	25.01°	11.05°	6.00°	172.6°	58.84°	64.09^{a}	1.886^{a}
L.S.		ns	* *	ns	*	*	**	* *	**	**	* *	* *	* *	**
Drought	D0	66.72	2.89^{a}	16.18	17.42^{a}	38.50^{a}	2718.6^{a}	33.53^{a}	16.19^{a}	8.32^{a}	219.3^{a}	77.54^{a}	50.17°	0.593°
	D1	65.51	2.85^{a}	15.84	17.01^{a}	36.84^{a}	2671.7^{a}	33.11^{a}	15.42 ^b	8.33ª	220.8^{a}	77.96^{a}	50.85°	0.537°
	D2	63.45	2.64^{b}	16.09	11.28^{b}	27.18^{b}	1206.2^{b}	26.47^{b}	10.58°	6.47^{b}	183.8^{b}	61.30^{b}	56.27 ^b	1.465^{b}
	D3	64.58	2.56^{b}	15.60	6.16°	19.69°	465.7°	17.77°	8.40^{d}	4.71°	137.9°	52.78°	70.02^{a}	2.128^{a}
L.S.		ns	*	su	*	*	*	**	**	**	* *	* *	*	**
Year	2016	65.06	2.74	16.01^{a}	13.07^{a}	30.59	1763.7	27.64^{b}	12.65	7.01	189.2^{b}	67.54	57.16	1.181
	2017	65.07	2.74	15.85^{b}	12.86^{b}	30.51	1767.4	27.80^{a}	12.64	6.90	191.7^{a}	67.25	56.50	1.180
L.S.	ı	us	ns	*	*	ns	ns	*	us	ns	*	ns	ns	su

Explanations as in Table 3.

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	Ĺ	Plant height	1 1 1		TIGIT	TSW	Seed yield	Oil con-	P,	Е	$\mathbf{C}_{\mathrm{leaf}}$	RWC	REC	Proline
Salinity	Drought	(cm)	LAI	NHP	HSN	(g)	(kg ha ⁻¹)	tent (%)	$(\mu mol m^{-2} s^{-1})$	(mmol	m ⁻² s ⁻¹)	(%)	(%)	(µmol gʻ FW)
SO	D0	66.30	2.88	16.44 ^a	19.27	40.85	3147.8 ^{ab}	35.73 ^{ab}	18.04	8.97 ^{ab}	239.6 ^a	80.21	47.95 ^f	0.120^{g}
	DI	65.31	2.94	15.62^{bc}	19.50	40.05	3252.3ª	36.10^{a}	16.28	9.40^{a}	231.4^{ab}	82.92	46.01^{fg}	0.129^{g}
	D2	67.36	2.90	16.37^{a}	16.51	37.03	2480.2°	32.99^{cd}	15.59	7.74°	211.1 ^{cd}	67.41	51.53°	0.795^{f}
	D3	67.93	2.88	16.33^{a}	14.40	36.08	1994.2^{d}	29.31^{fg}	14.87	7.20°	$195.2^{\rm ef}$	57.83	64.48°	1.571 ^d
S1	D0	67.94	3.00	$15.73^{\rm bc}$	18.53	38.30	3201.6^{a}	35.73^{ab}	16.49	$8.76^{\rm ab}$	234.2ª	79.26	44.82 ^g	0.127^{g}
	Dl	67.52	2.77	15.53°	20.40	37.19	$3004.7^{\rm b}$	34.02^{bc}	15.76	8.84^{ab}	231.4^{ab}	83.60	47.31^{f}	0.136^{g}
	D2	67.28	2.92	15.76^{bc}	15.28	33.77	2434.7°	31.82^{de}	15.35	$8.66^{\rm b}$	199.6^{def}	66.83	53.79°	0.860^{f}
	D3	59.32	2.76	16.37^{a}	13.84	38.11	2046.1^{d}	30.91^{ef}	14.11	7.10°	218.0^{bc}	59.00	64.40°	1.565^{d}
S2	D0	62.50	2.87	14.93^{d}	13.24	29.05	1507.9°	29.16^{fg}	12.09	7.20°	209.4^{cde}	77.64	$51.57^{\rm e}$	$0.881^{\rm f}$
	D1	65.03	2.66	16.58^{a}	13.91	30.28	1427.6°	27.92^{g}	11.96	7.24°	191.9^{f}	79.39	52.08°	0.804^{f}
	D2	64.29	2.55	16.37^{a}	10.29	26.26	1076.3^{f}	25.79^{h}	10.16	6.12 ^d	176.2^{g}	59.98	57.31 ^d	1.982°
	D3	62.00	2.52	16.51^{a}	7.69	23.13	813.4^{g}	23.03^{i}	8.13	5.34°	$157.8^{\rm h}$	48.88	71.67^{b}	2.376^{b}
S3	D0	64.62	2.79	15.42^{cd}	7.42	21.27	$583.8^{ m h}$	18.76j	9.45	5.29°	$154.0^{\rm h}$	73.04	56.35^{d}	1.243°
	Dl	64.85	2.61	$15.34^{\rm cd}$	7.12	21.12	$546.0^{\rm h}$	17.98j	9.33	$5.00^{\rm ef}$	$146.8^{\rm h}$	65.93	58.00^{d}	1.079°
	D2	62.01	2.51	15.58^{bc}	5.49	19.08	$416.4^{\rm hi}$	17.56j	7.73	4.18^{g}	131.2 ⁱ	50.98	62.47°	2.221^{b}
	D3	66.87	2.35	16.10^{ab}	4.62	17.30	316.9^{i}	16.79j	7.11	4.38^{fg}	119.8^{1}	45.41	79.53^{a}	2.999^{a}
L.S.		ns	su	**	ns	ns	* *	*	ns	*	*	su	* *	* *
Explanations	as in Table 3													

D0 to the D3 level) at each salinity stress level (Table 8). Similarly, the C_{leaf} , transpiration rate (E) of safflower reached its lowest value after exposure to the S3D3 treatment (Table 8).

Drought and salinity stresses exerted a negative effect on the *RWC* as compared to the control (Table 7). The *RWC* value in safflower decreased significantly when the plants were exposed to drought and salinity stresses at the flowering stage (Table 7). The proline content and REC were affected by salinity × drought stress interaction. The maximum values of these parameters (2.999 µmol g⁻¹ FW and 79.53 %, respectively) were obtained from the S3D3 treatment (Table 8).

The highest level of salinity (S3) caused NSH, TSW, seed yield and oil content were reduced to a remarkable extent by about 30.66, 11.46, 38.74, and 16.18%, respectively, whereas the respective values for the highest level of drought stress (D3) were 64.63, 48.85, 82.86, and 47% (Table 7). The number of head per plant, seed yield, and oil content of safflower were affected to a significant extent by salinity and drought interaction (Table 8). The seed yield was reduced by 36.64, 81.45, and 89.93% for the S0D3, S3D0 and S3D3 treatments, respectively, as compared with the S0D0 treatment (Table 8).

The most important variables that significantly affected the seed yield of safflower at the stem elongation stage were NHP ($\beta = 0.343$), *LAI* ($\beta = 0.711$), plant height ($\beta = 0.168$), *RWC* ($\beta = -0.299$) and NSH ($\beta = 0.089$). The adjusted r² value for this model was 99.0 (Table 9).

As the momentous parameters entered into the model, NHP β = 0.586), *LAI* (β = 0.486) and TSW (β = -0.125) significantly affected the seed yield of the safflower plant which was stressed at the heading stage (Table 9).

A linear regression analysis was performed for safflower plant stressed at the flowering stage using the Stepwise Method. The momentous variable that influenced the seed yield of safflower at this stage was NSH ($\beta = 0.978$) (Table 9).

The greatest reduction in plant growth parameters (plant height, *LAI* and NHP) due to salinity-drought stress was obtained at the stem elongation stage as compared with the heading and flowering stages. The reduction in seed yield caused by salinity-drought stress was higher at the heading stage (52.68%) as compared with the stem elongation (46.41%) and flowering stages (46.84%). However, the rate of reduction in NSH (34.87%), TSW (26.88%) and oil content (23.88%) was higher when salinity-drought stress was applied at the flowering stage (Table 10).

DISCUSSION

The harmful impacts of salinity and drought stress are becoming more intense in areas where saline water is used for irrigation (Babazadeh *et al.*, 2016). In this study, plant growth properties were affected by both salinity and irrigation treatments, either applied alone or in combination. The

Growth stage	Model	Model r-Square	Adjusted r-Square	Unstandardized Beta (β)	Standard Error	Standardized Beta (β)	t
Stem elongation	(Constant)	0.998	0.997	-1783.56	335.74		-5.312**
	NHP			82.267	20.30	0.343	4.053*
	LAI			1216.28	153.26	0.711	7.936**
	Plant height			16.763	5.061	0.168	3.312**
	RWC			-24.746	5.00	-0.299	-4.949**
	NSH			57.520	20.365	0.089	2.824*
Heading	(Constant)	0.978	0.972	-875.31	874.33		-1.001ns
	NHP			168.072	31.936	0.586	5.263**
	LAI			1256.35	309.55	0.486	4.059**
	TSW			-62.092	26.721	-0.125	-2.324*
Flowering	(Constant)	0.956	0.953	-810.842	158.925		-5.102**
	NSH			198.678	11.405	0.978	17.420**

Table 9. Summary of linear regression analysis for variables predicting the seed yield of safflower (*Carthamus tinctorius* L.) under salinity-drought stress conditions at different growth stages

Significant at: *5% and **1% and probability level, respectively. Other explanations as in Table 3.

Table 10. Effects of the growth stage of stress application on plant growth parameters, yield components, seed yield and oil content of the safflower

Growth stage	Condition	Plant height (cm)	LAI	NHP	NSH	TSW (g)	Seed yield (kg ha ⁻¹)	Oil content (%)
Stem elongation	Normal	66.30 ^a	2.87^{ab}	16.49 ^a	19.27 ^{ab}	40.85 ^a	3158.30 ^{ab}	35.72 ^{ab}
	Stress	49.86 ^b	2.12 ^d	9.69 ^d	17.81 ^b	35.77°	1692.66°	33.26 ^{bc}
Rate of reduction (%	6)	24.80	26.13	41.24	7.58	12.44	46.41	6.89
Heading	Normal	62.69 ^a	3.09 ^a	16.16 ^{ab}	21.22ª	39.65 ^{ab}	3541.93ª	35.77ª
	Stress	50.26 ^b	2.46°	10.95°	14.88°	38.10 ^b	1676.05°	33.01°
Rate of reduction (%	6)	19.83	20.39	32.24	29.88	3.91	52.68	7.72
Flowering	Normal	66.30 ^a	2.87^{ab}	16.44 ^a	19.27 ^{ab}	40.85 ^a	3147.80 ^b	35.72 ^{ab}
	Stress	64.99ª	2.73 ^{bc}	15.90 ^b	12.55°	29.87 ^d	1673.46°	27.19 ^d
Rate of reduction (%	6)	1.98	4.88	3.28	34.87	26.88	46.84	23.88

In a column, means with a different letter denoted a statistical difference between the treatment groups according to a t-test. * p < 0.05, ** p < 0.01, ns: non-significant, NHP: number of heads per plant, NSH: number of seeds per head, TSW: 1000-seed weight, *LAI*: leaf area index.

combination of salinity and drought has a more negative effect on safflower. It has been reported that simultaneous stresses more severely limit plant growth compared with individual stresses (Ahmed *et al.*, 2013). There are similarities and dissimilarities in the responses of the plant to drought and salinity. It could be pointed out that the occurrence of these responses simultaneously causes the combined stress to have more severe effects. These results were in agreement with the findings of Cho *et al.* (2006), Manuchehri and Salehi (2014) and Jin *et al.* (2020). A decrease in plant height was observed for safflower plants treated under drought stress conditions (Hojati *et al.*, 2011; Bagheri and Sam-Daliri, 2011). This reduction may be attributed to the inhibition of cell elongation because water flow is interrupted from the xylem to the surrounding cells (Nonami, 1998). The inhibition of plant growth under salinity stress was believed to be the result of osmotic and ionic stress components (Munns and Tester, 2008; Siddiqui *et al.*, 2012). However, our results show that the reduction in plant height and *LAI* under combined salinity-drought stress was more pronounced than the same effect caused by salinity and drought stress alone (Tables 4, 6, and 8). Accordingly, Ahmed *et al.* (2013) stated that barley plants treated with the single or combined stress of salinity and drought showed a significant decrease in plant height, with the largest reduction occurring due to combined salinity-drought stress.

Based on plant growth parameters, results indicated that the stem elongation stage was more sensitive than the heading and flowering stages to salinity, drought and salinity-drought stresses. Drought and salinity stresses due to a reduction in the water potential of the soil and the ability of plants to absorb water during the vegetative stage reduce morphological safflower growth components. Iftikhar Hussain *et al.* (2016) reported that the vegetative stage constitutes a growth stage of vital importance to safflower when it is severely affected by water stress. Similar results were found by Shahrokhnia and Sepaskhah (2017) who stated that the sensitive growth phase was stem elongation.

Photosynthesis is one of the most explored mechanisms in plant physiology that largely determines plant functional activity and survival (Ikkonen et al., 2018). Our results found that P_n was affected significantly by both salinity and irrigation treatments at all three different growth stages but it was also found that the effect of combined salinitydrought stress on this parameter was only significant at the heading stage. At each growth stage, Pn decreased with increasing levels of salinity and drought, (Tables 3, 5, 7) and combined salinity-drought stresses (Tables 4, 6, 8). Hernandez-Santana et al. (2017) indicated that stomatal closure, caused by low osmotic potential, is typically the first mechanism involved in photosynthesis reduction. On the other hand, Kalaji et al. (2018) reported that decreasing photosynthesis in salt stressed plants was predominantly caused by photosystem II damage. Furthermore, some studies have reported that P_n reduction under drought stress is considered to be caused by a decrease in CO₂ diffusion from the atmosphere to the site of carboxylation resulting from stomatal closure (Flexas et al., 2004; Grassi and Magnani, 2005).

The results showed that salinity, drought, and salinitydrought stress reduced the Tr and C_{leaf} values of safflower plants. This is consistent with the results of Kong *et al.* (2012) and Chen *et al.* (2019) who found that a reduction in leaf stomatal conductance occurred under salinity stress in cotton and tomato, respectively. Hsiao (1973) pointed out that stomatal closure is the main cause of transpiration reduction under water stress conditions. Stomatal conductance of safflower leaves was reduced due to salinity and drought stresses and when the plants were subjected to a combination of these stresses, a greater reduction in this trait was observed (Umar and Siddiqui, 2018). A steeper decrease in P_n compared to Tr in the present study indicated that P_n is more sensitive to a decreasing irrigation water level than Tr in safflower. These results were in agreement with the findings of Singh *et al.* (2016).

In our study, the *RWC* of the safflower plant was reduced for both single and combined stresses. This decrease may be attributed to the root systems, which are not able to compensate for water loss due to transpiration through a reduction in the absorbing surface (Sreenivasulu *et al.*, 2000). Many reports have revealed that *RWC* is reduced under conditions of drought and salinity (Masoumi *et al.* 2010). It is suggested that reduced shoot height, leaf area and number of leaves in crops under a stressed condition may be due to their leaves having a lower *RWC* (Umar and Siddiqui, 2018).

Elevated REC due to an increase in the intensity of the salinity and drought stresses indicates that an increase in membrane permeability or that a loss in membrane stability might lead to enhanced solute leakage (Farahbakhsh *et al.*, 2017). Enhancing REC under the conditions of salinity and drought stresses may be due to ROS accumulation and lipid peroxidation. Oxidative stress caused by salinity and drought can cause damage to the plasma membrane and increase its permeability and finally, lead to the leakage of electrolytes out of the cells (Amooaghaie and Moghym, 2011). In line with our results, Sajedi *et al.* (2012) and Mahlooji *et al.* (2018) reported that electrolyte leakage gradually increased under drought and salt stress conditions.

The results showed an enhanced proline content in plants subjected to salinity-drought stress. Proline is the key osmolyte providing osmotic adjustment (Valentovič *et al.*, 2006). In response to water deficit and salinity stress, plants accumulate large quantities of proline (Hayat *et al.*, 2012; Krasensky and Jonak, 2012). Our results are consistent with previous studies reporting an increased proline content in response to drought or salinity stress (Parida and Das, 2005; Yan, 2015; Khan *et al.*, 2017).

In the present study, a decrease in seed yield, yield components and oil content in the salinity-drought treated plants was noted. Our results are in accordance with those of Rauf (2008), Pourdad (2008), Johnson *et al.* (2012), Yeilaghi *et al.* (2012), Singh *et al.* (2016) and Shahrokhnia and Sepaskhah (2017).

A yield reduction in drought stress due to low biomass production is associated with decreased photosynthesis in these conditions (Pinheiro and Chaves, 2011). Singh *et al.* (2016) reported that under drought stress, safflower relies more on reducing dry mater and seed yield through a reduction in photosynthesis and transpiration, than in trying to alter biomass partitioning to seed. Johnson *et al.* (2012) stated that a higher seed yield in safflower is relevant to an increase in NHP and NSH. As a yield component of safflower NHP is affected by drought that can consecutively decrease the yield production (Shahrokhnia and Sepaskhah 2017).

Our results are consistent with Singh *et al.* (2016), who found a reduction in safflower NSH under drought stress. The reduction in NSH under drought stress conditions may be attributed to lower photosynthetic production (Rauf, 2008).

Yeilaghi *et al.* (2012) reported a significant reduction in seed yield in 64 safflower genotypes following salinity treatment. Similarly, Siddiqi *et al.* (2011) have pointed out that the seed yield, NSH and TSW of safflower were affected by salinity stress.

The results showed that the oil content of safflower seeds decreased with increasing salinity and drought stress. This is in agreement with Yeilaghi *et al.* (2012) who stated that salinity stress reduced the oil content of safflower by 7.7% on average as compared with the control conditions. Furthermore, a reduction in safflower oil content with an increase in drought severity was reported by Nabipour *et al.* (2007), Ashrafi and Razmjoo (2010), Bagheri and SamDaliri (2011), and Mohammadi *et al.* (2018).

CONCLUSIONS

1. Drought and salinity stress negatively affected the photosynthesis, plant growth and yield production of safflower. Exposure to mild salinity-drought stress revealed no harmful effects on safflower productivity during the stem elongation and flowering stages as shown by observations of seed yield.

2. The reduction in seed yield caused by salinitydrought stress was higher at the heading stage as compared with the stem elongation and flowering stages, this may be attributed to the sensitivity of this growth stage to combined salinity-drought stress.

3. As a general guide, it may be concluded that increasing number of heads per plant, leaf area index, plant height, relative water content, and number of seeds per head at the stem elongation stage and number of heads per plant, leaf area index, and 1000-seed weight at the heading stage and finally, number of seeds per head at the flowering stage may be effective methods in safflower production under salinitydrought stress conditions.

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REFERENCES

Ahmed I.M., Dai H.X., Zheng W., Cao F.B., Zhang G.P., Sun D.F., and Wu F.B., 2013. Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between Tibetan wild and cultivated barley. Plant Physiol. Biochem., 63: 49-60. doi: 10.1016/j.plaphy.2012.11.004

- Amooaghaie R., and Moghym S., 2011. Effect of polyamines on thermo tolerance and membrane stability of soybean seedling. African J. Biotechnol., 10: 9673-9679. doi: 10.5897/ AJB10.2446
- Ashrafi E., and Razmjoo K., 2010. Effect of irrigation regimes on oil content and composition of safflower (*Carthamus tinctorius* L.) cultivars. J. American Oil Chemists' Society, 87(5): 499-506. doi: org/10.1007/s11746-009-1527-8
- AOCS, 1993. Official Methods and Recommended Practices of the American Oil Chemists Society. American Oil Chemists Society, 1608, Broadmoor Drive, Champaign, Illionis 61826-3489.
- Babazadeh H., Sarai Tabrizi M., and Hassanpour Darvishi H., 2016. Adopting adequate leaching requirement for practical response models of basil to salinity. Int. Agrophys., 30(3): 275-283. doi: 10.1515/intag-2016-0002
- Babazadeh H., Tabrizi M.S., and Homaee M., 2017. Assessing and modifying macroscopic root water extraction basil (*Ocimum basilicum*) models under simultaneous water and salinity stresses. Soil Sci. Soc. Am. J., 81: 10-19. https:// doi.org/10.2136/sssaj2016.07.0217
- Bagheri H., and Sam-Daliri M., 2011. Effect of water stress on agronomic traits of spring safflower cultivars (*Carthamus tinctorius* L.). Australian J. Basic Applied Sci., 5: 2621-2624.
- Bates L.S., 1973. Rapid determination of free proline for waterstress studies. Plant Soil, 39: 205-207. doi: org/10.1007/ BF00018060
- Campos P.S., Quartin V., Ramalho J.C., and Nunes M.A., 2003. Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants. J. Plant Physiol., 160: 283-292.

doi: org/10.1078/0176-1617-00833

- Chaves M.M., Flexas J., and Pinheiro C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals Botany, 103: 551-560. doi: 10.1093/aob/ mcn125
- Chen S., Wang Z., Guo X., Rasool G., Zhang J., Xie Y., Hamoud Y.A., and Shao G., 2019. Effects of vertically heterogeneous soil salinity on tomato photosynthesis and related physiological parameters. Scientia Hort., 249: 120-130. doi: org/10.1016/j.scienta.2019.01.049
- Cho K., Toler H., Lee J., Ownley B., Stutz J.C., Moore J.L., and Auge R.M., 2006. Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. J. Plant Physiol., 163:517-528. doi: 10.1016/j. jplph.2005.05.003
- Farahbakhsh H., Pasandi Pour A., and Reiahi N., 2017. Physiological response of henna (*Lawsonia inermise* L.) to salicylic acid and salinity. Plant Production Sci., 20(2): 237-247. doi: org/10.1080/1343943X.2017.1299581.
- Flemmer A., Franchini M., and Lindström L., 2015. Description of safflower (*Carthamus tinctorius*) phenological growth stages according to the extended BBCH scale. Ann. Appl. Biol., 166: 331-339. https://doi.org/10.1111/aab.12186

- **Flexas J., Bota J., Loreto F., Cornic G., and Sharkey T., 2004.** Diffusive and metabolic limitations to photosynthesis under drought and salinity in C₃ plants. Plant Biol., 6: 269-279. doi: 10.1055/s-2004-820867
- Gardner F.P., Pearce R.B., and Mitchell R.L., 1985. Physiology of Crop Plants. Iowa State University Press, Ames, Iowa, USA.
- Gilbert J., 2008. International safflower production an overview. 7th Int. Safflower Conf., November 3-6, Wagga Wagga, Australia.
- Grassi G., and Magnani F., 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant, Cell Environ., 28: 834-849. doi: 10.1016/j. indcrop.2014.10.058
- Grzesiak T.M., Janowiak F., Szczyrek P., Kaczanowska K., Ostrowska A., Rut G., Hura T., Rzepka A., and Grzesiak S., 2016. Impact of soil compaction stress combined with drought or water logging on physiological and biochemical markers in two maize hybrids. Acta Physiologiae Plantarum, 38: 109. doi: org/10.1007/s11738-016-2128-4
- Guo J., Ling H., Wu Q., Xu L., and Que Y., 2014. The choice of reference genes for assessing gene expression in sugarcane under salinity and drought stresses. Scientific Report. 4: 7042. doi: 10.1038/srep07042. doi: 10.1038/srep07042
- Hayat S., Hayat Q., Alyemeni M.N., Wani A.S., Pichtel J., and Ahmad A., 2012. Role of proline under changing environments: a review. Plant Signal Behavior, 7: 1456-1466. doi: 10.4161/psb.21949
- Hernandez-Santana V., Fernández J.E., Cuevas M.V., Perez-Martin A., and Diaz-Espejo A., 2017. Photosynthetic limitations by water deficit: effect on fruit and olive oil yield, leaf area and trunk diameter and its potential use to control vegetative growth of super-high density olive orchards. Agric. Water Manag., 184: 9-18. doi: org/10.1016/j.agwat.2016.12.016.
- Hojati M., Modarres-Sanavy S.A.M., Karimi M., and Ghanati F., 2011. Responses of growth and antioxidant systems in *Carthamus tinctorius* L. under water deficit stress. Acta Physiologiae Plantarum, 33: 105-112. doi: org/10.1007/ s11738-010-0521-y
- Hsiao T.C., 1973. Physiological effects of plant in response to water stress. Plant Physiol., 24: 519-570. doi: org/10.3389/ fpls.2014.00086
- Iftikhar Hussain M., Lyra D.A., Farooq M., Nikoloudakis N., and Khalid N., 2016. Salt and drought stresses in safflower: a review. Agronomy Sustain. Developt., 36: 4-31. doi: org/10.1007/s13593-015-0344-8
- Ikkonen E.N., Shibaeva T.G., and Titov A.F., 2018. Influence of daily short-term temperature drops on respiration to photosynthesis ratio in chilling-sensitive plants. Russian J. Plant Physiol., 56: 78-83. doi: org/10.1134/S1021443718010041
- Jamil S., Riaz M., Ashraf M., and Foolad M.R., 2011. Gene expression profiling of plants under salt stress. Crit. Reviews Plant Sci., 30: 435-458. doi: org/10.1080/0735268 9.2011.605739
- Jin J., Niu J., Guo T., Zhou R., and Sun L.Z., 2020. The effect of drought on physiological responses of forage plants to salt stresses depends on occurring time. Acta Physiologiae Plantarum, 42:91. doi: org/10.1007/s11738-020-03083-3

- Johnson R., Petrie S., Franchini M.C., and Evans M., 2012. Yield and yield components of winter-type safflower. Crop Sci., 52: 2358-2364. doi: 10.2135/cropsci2011.12.0659.
- Kalaji H.M., Baba W., Gediga K., Goltsev V., Samborska I.A., Cetner M.D., Dimitrova S., Piszcz U., Bielecki K., Karmowska K., Dankov K., and Kompala-Baba A., 2018. Chlorophyll fluorescence as a tool for nutrient status identification in rapeseed plants. Photosynth Res., 136: 329-343. doi: org/10.1007/s11120-017-0467-7
- Kar G., Kumar A., and Martha M., 2007. Water use efficiency and crop coefficients of dry season oilseed crops. Agric. Water Manag., 87(1): 73-82. doi: org/10.1016/j. agwat.2006.06.002
- Kaya M.D., 2009. The role of hull in germination and salinity tolerance in some sunflower (*Helianthus annuus* L.) cultivars. African J. Biotechnol., 8(4): 597-600.
- Khan A., Anwar Y., Hasan M.M., Iqbal A., Ali M., Alharby H.F., Hakeem K.R., and Hasanuzzaman M., 2017. Attenuation of drought stress in brassica seedlings with exogenous application of Ca²⁺ and H₂O₂. Plants, 6: 1-13. doi: 10.3390/plants6020020
- Kong X., Luo Z., Dong H., Eneji A.E., and Li W., 2012. Effects of non-uniform root zone salinity on water use, Na⁺ recirculation, and Na⁺ and H⁺ flux in cotton. J. Experim. Botany, 63(5):2105–2116, doi:10.1093/jxb/err420
- Kong X.Q., Luo Z., Dong H.H., Li W.J., and Chen Y.Z., 2017. Non-uniform salinity in the root zone alleviates salt damage by increasing sodium, water and nutrient transport genes expression in cotton. Scientific Reports, 7: (1). doi: 10.1038/ s41598-017-03302-x.
- Krasensky J., and Jonak C., 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Experim. Botany, 63: 1593-1608. doi: 10.1093/jxb/err460
- Mahlooji M., Seyed Sharifi R., Razmjoo J., Sabzalian M.R., and Sedghi M., 2018. Effect of salt stress on photosynthesis and physiological parameters of three contrasting barley genotypes. Photosynthetica, 56: 549-556. doi:10.1007/ s11099-017-0699-y
- Manuchehri R., and Salehi H., 2014. Physiological and biochemical changes of common bermudagrass (*Cynodon dactylon* [L.] Pers.) under combined salinity and deficit irrigation stresses. South African J. Botany, 92:82-88. doi: org/10.1016/j.sajb.2014.02.006
- Masoumi A., Kafi M., Khazaei H., and Davari K., 2010. Effect of drought stress on water status, elecrolyte leakage and enzymatic antioxidants of kochia (*Kochia scoparia*) under saline condition. Pakistan J. Botany, 42: 3517-3524.
- Mohammadi M., Ghassemi-Golezani K., Chaichi M.R., and Safikhani S., 2018. Seed oil accumulation and yield of safflower affected by water supply and harvest time. Agronomy J., 110: 1-8. doi: org/10.2134/agronj2017.06.0365
- Munns R., 2011. Plant adaptation to salt and water stress: Differences and commonalities. Advances Botanical Res., 557: 1-32. doi: org/10.1016/B978-0-12-387692-8.00001-1
- Munns R., and Tester M., 2008. Mechanisms of salinity tolerance. Ann. Review Plant Biol., 59: 651-681. doi: 10.1146/ annurev.arplant.59.032607.092911

- Nabipour M., Meskarbashee M., and Yousefpour H., 2007. The effect of water deficit on yield and yield components of safflower (*Carthamus tinctorius* L.). Pakistan J. Biol. Sci., 10: 421-426. doi: 10.3923/pjbs.2007.421.426
- Nonami H., 1998. Plant water relations and control of cell elongation at low water potentials. J. Plant Res., 111(3): 373-382. doi: org/10.1007/BF02507801
- Parida A.K., and Das A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology Environ. Safety, 60: 324-349. doi: org/10.1016/j.ecoenv.2004.06.010
- Patil N.M., 2012. Adaptations in response to salinity in safflower cv. Bhima. Asian J. Crop Sci., 4: 50-62. doi: 10.3923/ ajcs.2012.50.62
- Pinheiro C., and Chaves M., 2011. Photosynthesis and drought: can we make metabolic connections from available data? J. Experim. Botany, 62: 869-882. doi: org/10.1093/jxb/ erq340
- Pourdad S., 2008. Study on drought resistance indices in spring safflower. Acta Agronomica Hungarica, 56: 203-212. doi: 10.1556/AAgr.56.2008.2.9
- Rauf S., 2008. Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. Communications Biometry Crop Sci., 3: 29-44.
- Sahin U., Ekinci M., Ors S., Turan M., Yildiz S., and Yildirim E., 2018. Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea* var. capitata). Scientia Horticulturae, 240: 196-204. doi: org/10.1016/j.scienta.2018.06.016
- Sajedi N.A., Ferasat M., Mirzakhani M., and Mashhadi Akbar Boojar M., 2012. Impact of water deficit stress on biochemical characteristics of safflower cultivars. Physiol. Molecular Biol. Plants, 18(4): 323-329. doi: 10.1007/ s12298-012-0129-3
- Shahrokhnia M.H., and Sepaskhah A.R., 2017. Physiologic and agronomic traits in safflower under various irrigation strategies, planting methods and nitrogen fertilization. Industrial Crops Products, 95: 126-139. doi:10.1016/j. indcrop.2016.10.021
- Shrivastava P., and Kumar R., 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J. Biological Sci., 22: 123-131. doi:org/10.1016/j.sjbs.2014.12.001
- Siddiqi E.H., Ashraf M., Al-Qurainy F., and Akram N.A., 2011. Salt-induced modulation in inorganic nutrients, anti-

oxidant enzymes, proline content and seed oil composition in safflower (*Carthamus tinctorius* L.). J. Science Food Agric., 91(15): 2785-2793. doi: 10.1002/jsfa.4522

- Siddiqi E.H., Ashraf M., Hussain M., and Jamil A., 2009. Assessment of inter cultivar variation for salt tolerance in safflower (*Carthamus tinctorius* L.) using gas exchange characteristics as selection criteria. Pakistan J. Botany, 41(5): 2251-2259.
- Siddiqui M.S., Thodey K., Trenchard I., and Smolke Ch.D., 2012. Advancing secondary metabolite biosynthesis in yeast with synthetic biology tools. FEMS Yeast Res., 12(2):144-170, doi:10.1111/j.1567-1364.2011.00774.x
- Singh S., Angadi S.V., Grover K., Begna S., and Auld D., 2016. Drought response and yield formation of spring safflower under different water regimes in the semiarid Southern High Plains. Agric. Water Manag., 163: 354-362. doi: org/10.1016/j.agwat.2015.10.010
- Sreenivasulu N., Grimm B., Wobus U., and Weschke W., 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). Physiologia Plantarum, 109: 435-442. doi:10.1034/j.1399-3054.2000.100410.x
- Umar M., and Siddiqui Z.S., 2018. Physiological performance of sunflower genotypes under combined salt and drought stress environment. Acta Botanica Croatica, 77 (1): 36-44. doi:org/10.2478/botcro-2018-0002
- Valentovič P., Luxová M., Kolarovič L., and Gašparíková O., 2006. Effect of osmotic stress on compatible solutes content, membrane stability and water relationsin two maize cultivars. Plant Soil Environ., 52, 186-191.
- Weiss E., 1971. Castor, sesame and safflower. Cambridge University Press, London, UK.
- Yan M., 2015. Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. South Africa J. Botany, 99: 88-92. doi: org/10.1016/j.sajb. 2015.03.195
- Yeilaghi H., Arzani A., Ghaderian M., Fotovat R., Feizi M., and Pourdad S.S., 2012. Effect of salinity on seed oil content and fatty acid composition of safflower (*Carthamus tinctorius* L.) genotypes. Food Chem., 130(3): 618-625. doi: org/10.1016/j.foodchem.2011.07.085
- Zhang X., Lu G., Long W., Zou X., Li F., and Nishio T., 2014. Recent progress in drought and salt tolerance studies in Brassica crops. Breeding Sci., 64: 60-73. doi: 10.1270/ jsbbs.64.60