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Physiological Response of Diverse Origin Spring Safflower Genotypes to Salinity

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Abstract: Increased salinity of irrigation water is a major constraint affecting crop productivity in the Southern High Plains of United States of America. Adapting salt tolerant crops could be a viable option for the region. Yield formation and physiology of diverse spring safflower (*Carthamus tinctorius* L.) genotypes was studied under a range of salinity levels in a greenhouse experiment. Five irrigation salinity levels of 0.5 (control), 2.5, 5.0, 7.5 and 10 dS m^{-1} along with five genotypes of diverse origin were arranged in a randomized complete block design with four replications. Seeds per head and harvest index were the most sensitive parameters to salinity, declining 98% and 99% at 10 dS m^{-1} compared to control, respectively. In contrast, biomass per plant and 1000-seed weight were reduced only 43% and 56% with the same 10 dS m^{-1}. Plant height and head numbers were only affected at the highest salinity level (10 dS m^{-1}). Increasing salinity levels decreased relative water content (RWC), leaf water potential (Ψ_1), osmotic potential at full turgor ($\Psi_{\pi 100}$), photosynthesis and transpiration. However, chlorophyll fluorescence was unaffected by salinity treatments. Salt tolerance of PI199898, among all the genotypes, was manifested by higher 1000-seed weight and Harvest Index (HI); this tolerance was associated with its ability to maintain higher Ψ_1, osmotic concentration, RWC, photosynthesis and transpiration. Safflower can be planted under moderate salinity levels of < 5 dS m^{-1}.

Key Words: Photosynthesis, Safflower, Salinity, Water potential, Yield formation

1. Introduction

Salinity is one of the major abiotic stresses affecting agricultural productivity, particularly in arid and semi-arid regions of the world including the Southern High Plains of United States of America. Globally, about half of the irrigated land or 20% of the cultivated land or 7% of the total land area is affected by salinity (Sudhir and Murthy, 2004). Saline irrigation water, high evaporation, and low rainfall are the contributing factors to salinity problems in New Mexico and West Texas. Adverse effects of salinity on plant growth and development are mainly due to osmotic stress, salt stress, nutritional imbalance, and sometimes a combination of these factors (Ashraf, 1994). Salinity also restricts the supply of photosynthetic assimilates to growing tissues (Munns, 1993). These adverse effects of salinity have made agriculture less economical for farmers as low yields or sometimes complete crop failures are quite common under saline conditions. Hence, in the Southern High Plains where salinity is higher for sensitive crops, inclusion of salt tolerant crops in crop rotation could be a viable alternative.

Safflower is generally considered as a moderately salt tolerant crop and is grown in arid and semi-arid regions of the world where salinity can restrict the growth of many other crops (Bassil and Kaffka, 2002). However genotypic variation for salt tolerance has been reported in safflower in the Middle East (Yeilaghi *et al*., 2012). Hence, a better understanding of physiological aspects of salinity stress tolerance mechanisms will not only help in identifying a well-adapted, salinity tolerant germplasm for the Southern High Plains but also help breeders to use genes involved in salt stress tolerance for crop improvement in saline conditions. The objective of the current study was to assess physiological and yield formation responses of diverse spring safflower genotypes collected from different geographic locations under range of salinity levels.

2. Materials and Methods

2.1. Experimental site description

A greenhouse experiment was conducted at the Agricultural Science Center (ASC) of New Mexico State University, located 23 km north of Clovis, New Mexico (34°35'N, 103°12' W) at an altitude of 1348 m above sea level. The study location is characterized as semi-arid climate with annual average precipitation of 445 mm, and the mean maximum and minimum temperatures of 22°C and 7°C,

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respectively (Contreras-Govea *et al*., 2011). The average air temperature in the greenhouse during the whole experiment period was maintained at 30°C during day and 24°C at night. The sunlit greenhouse used for the study transmitted most of the sunlight, and mid-day light intensities were 1500 μmol m^{-2} sec^{-1}. Sandy loam soil was used in the plastic pots of 15 kg capacity. The bulk density and electrical conductivity (EC) of the soil were 1.34 g cm^{-3} and 1.2 dS m^{-1}, respectively.

2.2. Planting and experimental design

Five safflower genotypes PI199898, PI248866, PI304453, PI304507, and S333 were collected from National Plant Germplasm System (NPGS), Pullman, WA, USA. These genotypes originated in different countries (India, Iran, Turkey and United States). Before planting, pots were watered to field capacity with domestic well water (≈ 0.5 dS m^{-1}). Four seeds were planted on 21st March, 2012 in the middle of each pot. After establishment, thinning was done to maintain two plants per pot. Salinity treatments were imposed from 8th May, 2012 to 30th June, 2012. Salt solutions were prepared by adding a calculated amount of NaCl per liter of domestic well water. Five salinity levels of 0.5 (control), 2.5, 5.0, 7.5 and 10 dS m^{-1} were prepared. Salt solutions were applied manually and EC was determined by an EC meter (Model Orion Star A215, Thermo Scientific, Singapore). All pots were maintained above 60% available water by frequent weighing to avoid water stress to the plants. The experiment was laid out in a Randomized Complete Block Design with four replications.

2.3. Physiological measurements

All the physiological observations were recorded during midday and pots were divided into two sets to complete observations in four days. First set comprised of all five genotypes irrigated with two extreme salinity levels of 0.5 dS m^{-1} (control) and 10 dS m^{-1}. The second set included two genotypes (PI304507 and PI248866), irrigated with all the salinity levels (0.5, 2.5, 5.0, 7.5 and 10 dS m^{-1}). Observations were taken at 62 days after planting when salinity stress started affecting plants.

Photosynthesis measurements were recorded using a portable photosynthesis system (Model LI-COR 6400, Lincoln, NE, USA) selecting the two youngest, fully expanded and illuminated leaves, one from each plant in the pot. Chlorophyll fluorescence was measured simultaneously with photosynthesis measurements. The ratio of variable to maximal fluorescence (F_v/F_m) was measured after dark adapting two leaves per pot for 30 minutes using a continuous source fluorometer (Model OS 30p, Opti-Science, Hudson, NH, USA).

Relative water content of two leaves per pot was measured using the following equation Relative Water Content = (Fresh weight - Dry weight / Turgid weight - Dry weight) × 100 (Eq. 1).

Pressure bomb (Model 615, PMS instrument company, Albany, OR, USA) was used to measure leaf water potential (Ψ_1) (Turner, 1981) on two young branches per pot. At the same time, two leaf samples were collected, frozen and later thawed to measure leaf osmotic potential (Ψ_π) of the expressed sap using a pre-calibrated Wescor Vapor Pressure Osmometer (Model Wescor 5520, Logan, UT, USA). Relative water content was used to convert osmotic potential (Ψ_π) to osmotic potential at full turgor ($\Psi_\pi 100$) using the following formula $\Psi_{\pi 100} = (\Psi_\pi \times RWC)/100$ (Eq. 2).

2.4. Agronomic measurements

Plant height from the soil surface to tip of two plants was measured at the end of the growing period. Both plants were hand harvested and oven dried at a temperature of 55°C for 3 days to measure the shoot dry weight. Data on number of heads, number of seeds and seed weight was recorded and 1000-seed weight was calculated. Harvest index was computed as the ratio of seed yield to the total dry matter yield (Eq. 3).

The analysis of variance was performed on all the data using proc GLM procedure of SAS software (Version 9.2, SAS Institute, Inc., NC, USA). The means were separated using LSD at 5% significance level ($P < 0.05$).

3. Results

3.1. Effect of salt stress on safflower physiology

A significant salt stress effect was observed on safflower leaf water relation characteristics (Ψ_1, $\Psi_{\pi 100}$ and RWC), photosynthesis, transpiration and fluorescence (**Tables 1** and **2**) as the salinity level increased from control (0.5 dS m^{-1}) to 10 dS m^{-1}.

Genotypes did not show any significant differences for RWC when averaged across the salinity treatments (Tables 1 and 2). Average RWC for all safflower genotypes under low salinity was 80.3%, which decreased to 69.9% at 10 dS m^{-1} (Table 1). Mean RWC of two genotypes decreased with an increase in salinity level (Table 2). The highest RWC was observed in the control which was not significantly different than 2.5 and 5 dS m^{-1} salinity levels. The lowest RWC was observed when salinity increased above 7 dS m^{-1}.

Genotypes differed significantly for Ψ_1 across salinity levels (Tables 1 and 2). PI304507 was the most stressed in both observations, while PI199898 was the least stressed genotype across two extreme salinity levels. The mean Ψ_1 of

Table 1. Effect of salinity levels on water potential (Ψ_1), osmotic potential at full turgor ($\Psi_{\pi 100}$), relative water content (RWC), photosynthesis (Pn), transpiration (Tr) and fluorescence (Fv/Fm) of five safflower genotypes in 2012 at Clovis, NM.

Genotype	Ψ_1 (MPa)	$\Psi_{\pi 100}$ (MPa)	RWC (%)	Pn µmol m^{-2} sec^{-1}	Tr (mmol m^{-2} sec^{-1})	Fv/Fm
PI304453	-1.50ab†	-0.95a	71.6a	23.8a	13.7ab	0.775c
S333	-1.50ab	-1.07ab	74.4a	18.7a	11.2b	0.798b
PI304507	-1.54b	-1.08ab	76.0a	20.4a	13.1ab	0.813ab
PI248866	-1.41ab	-1.21b	75.3a	20.7a	14.1ab	0.824a
PI199898	-1.36a	-1.23b	78.2a	23.5a	15.4a	0.813ab
Salinity (dS m^{-1})						
0.5	-1.15a	-0.97a	80.3a	23.1a	15.7a	0.800a
10	-1.77b	-1.23b	69.9b	19.7a	11.3b	0.809a
G x S	NS	NS	NS	NS	NS	NS

† Values within each column followed by same letter are not significantly different at P≤0.05.
NS= Non-significant at P≤0.05.

Table 2. Effect of salinity levels on water potential (Ψ_1), osmotic potential at full turgor ($\Psi_{\pi 100}$), relative water content (RWC), photosynthesis (Pn), transpiration (Tr) and fluorescence (F_v/F_m) of two safflower genotypes in 2012 at Clovis, NM.

Salinity level (dS m^{-1})	Ψ_1 (MPa)	$\Psi_{\pi 100}$ (MPa)	RWC (%)	Pn (µmol m^{-2} sec^{-1})	Tr (mmol m^{-2} sec^{-1})	Fv/Fm
0.5	-1.13a†	-1.07a	86.6a	27.7a	17.8a	0.819a
2.5	-1.38b	-1.24ab	80.3ab	26.1ab	16.3ab	0.815a
5	-1.70c	-1.26ab	75.8ab	23.6abc	17.5a	0.813a
7.5	-1.96d	-1.41b	72.5b	22.9bc	14.9b	0.812a
10	-2.03d	-1.44b	72.2b	20.6c	15.3b	0.821a
Genotype						
PI304507	-1.72b	-1.22a	76.2a	24.3a	16.7a	0.805b
PI248866	-1.53a	-1.32a	79.5a	24.0a	16.0a	0.827a
G x S	NS	NS	NS	NS	NS	NS

† Values within each column followed by same letter are not significantly different at P≤0.05.
NS= Non-significant at P≤0.05.

two genotypes decreased with increasing salinity levels and the lowest Ψ_1 or the highest stress level was observed above 7.5 dS m^{-1} salinity level (Table 2). Mean Ψ_1 values of five genotypes also increased with higher salinity level (Table 1). An increase in the solute accumulation was observed with the decrease in Ψ_1 as the salinity level increased from control to 10 dS m^{-1} (Table 2). The lowest $\Psi_{\pi 100}$ was observed by PI199898 across two extreme two salinity levels. Salt stress at the highest salinity level resulted in the lowest $\Psi_{\pi 100}$, which was not significantly different from 7.5 dS m^{-1}. The highest $\Psi_{\pi 100}$ was observed in the control.

No significant differences were noticed for photosynthesis among genotypes (Tables 1 and 2). The average photosynthesis for safflower ranged from 19.7 to 23.1 µmol m^{-2} sec^{-1} for 10 dS m^{-1} and control, respectively (Table 1). Although photosynthesis rate decreased with successive salinity levels it was not statistically different at control, 2.5 and 5 dS m^{-1} levels (Table 2). Transpiration variation among the genotypes was also small (Tables 1 and 2). Decrease in transpiration with increasing salinity was significant only

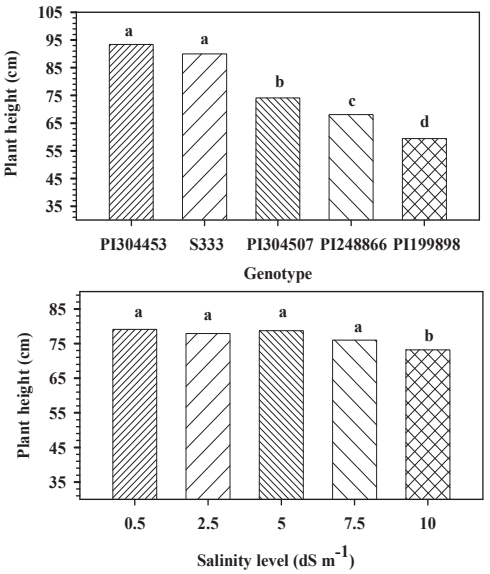

Fig. 1. Plant height of safflower genotypes as affected by different salinity levels in 2012 at Clovis, NM. Bars with the same letter are not significantly different at P≤0.05.

above 5 dS m^{-1} (Table 2). Decrease in photosynthesis and transpiration with increase in salinity is also observed in other crops (Burman *et al*., 2003). Significant fluorescence differences were observed among the genotypes with PI248866 showing the highest F_v/F_m (Tables 1 and 2). However, the F_v/F_m was not affected by an increase in salinity (Table 2).

No significant genotype by salinity interactions were observed for RWC, Ψ_1, $\Psi_{\pi 100}$, photosynthesis, transpiration and fluorescence. This indicates that in spite of diverse origin, genotypes included in this study responded to salinity similarly.

3.2. Effect of salt stress on growth and yield

Plant height varied significantly among the genotypes. Genotype PI304453 had the tallest plants with an average height of 93.4 cm, while genotype PI199898 had the shortest plants with a height of 59.4 cm (**Fig. 1**). Only two safflower genotypes PI304453 and S333 did not differ significantly in plant heights. Thus, genetic diversity for plant height among the tested genotypes was large. The decrease in plant height with increase in salinity level was only significant at the highest salinity level (10 dS m^{-1}) and the interaction effect was not significant.

Genotype S333 produced the highest number of heads per plant while the lowest was observed in PI304507 (**Table 3**). Genotypes PI248866, PI304453 and PI199898 did not differ for number of heads per plant, but differed significantly from the highest and the lowest head producing genotypes. A significant decrease in number of heads per plant was observed only above 7.5 dS m^{-1} salinity level with the lowest number recorded at 10 dS m^{-1}.

Number of seeds per head was affected by genotype,

Table 3. Yield attributing characters of five safflower genotypes under different salinity levels in 2012 at Clovis, NM.

Genotype	Heads plant^{-1}	Seeds head^{-1}	1000 seed Wt (g)	Biomass Plant^{-1} (g)	HI (%)
PI304453	9.9b†	13.7a	26.7c	30.1a	12.3b
S333	11.0a	8.9b	28.8bc	27.5a	10.0b
PI304507	7.7c	9.4b	33.9ab	20.3b	12.3b
PI248866	9.8b	7.0b	34.8ab	18.9bc	13.9ab
PI199898	9.1b	9.1b	35.1a	16.9c	16.8a
Salinity (dS m^{-1})					
0.5	9.6a	19.3a	46.9a	27.3a	29.6a
2.5	10.1a	19.3a	35.7b	28.4a	23.6b
5	10.3a	7.0b	25.9c	22.9b	9.3c
7.5	9.6a	2.2c	23.8c	19.5c	2.5d
10	7.9b	0.3c	20.5c	15.6d	0.3d
G x S	NS	*	NS	*	NS

† Values within each column followed by same letter are not significantly different at P≤0.05.
*, NS Significant and non-significant at P≤0.05, respectively.

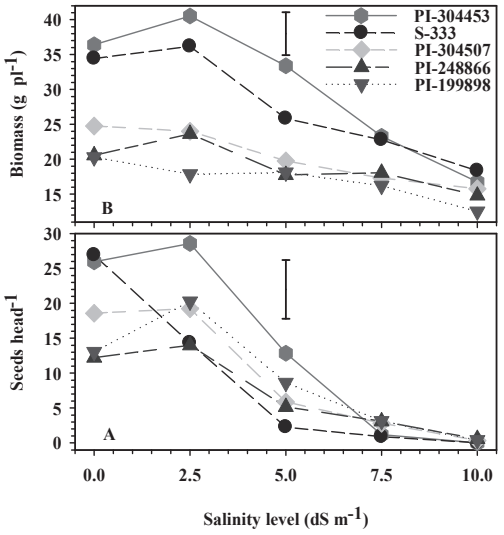

Fig. 2. Interactions of safflower genotypes and salinity on (A) seeds per head and (B) biomass production in 2012 at Clovis, NM. Vertical bar is LSD at P≤0.05.

salinity level and genotype x salinity interactions (Table 3). In general, genotypes did not differ in the number of seeds per head except for PI304453. Mean seeds per head did not change up to 2.5 dS m^{-1} and started declining with further increase in salinity. Number of seeds per head was a sensitive parameter to salinity in this study. Among all the genotypes, S333 recorded the highest number of seeds per head, however, decrease in seeds per head at 2.5 dS m^{-1} showed its sensitivity to salinity (**Fig. 2A**). In the rest of safflower genotypes, decline in seeds per head started after 2.5 dS m^{-1}, which indicated moderate salinity tolerance. Negligible seed production was noticed under higher salinity levels (7.5 and 10 dS m^{-1}) in all genotypes.

Effect of genotype and elevated soil salinity was observed on 1000-seed weight (Table 3). PI199898 and PI304453, averaged across salinity levels, showed the maximum and the minimum 1000-seed weights, respectively. A rapid decrease in 1000-seed weight across all genotypes was noticed with each stress level up to 5 dS m^{-1} and after that, decrease was non-significant. The highest salinity level exhibited 56% decrease in mean 1000-seed weight of all genotypes over the control.

Genotype, salinity level and their interactions significantly affected total aboveground biomass production (Table 3). Averaged across salinity levels, genotype PI304453 produced the highest biomass while PI199898 produced the lowest biomass which was almost half of the former. Averaged over genotypes, a significant reduction in biomass production was observed as the salinity level increased above 2.5 dS m^{-1} and the lowest biomass production was recorded at 10 dS m^{-1} salinity level. Two higher biomass producing genotypes, PI304453 and S333, showed rapid declines in biomass as salinity increased above 2.5 dS m^{-1}, while other three genotypes did not differ in their biomass across salinity levels (Fig. 2**B**). Biomass of PI304453 was significantly higher compared to the three lowest biomass producing genotypes up to 5 dS m^{-1}, but biomass production did not differ among genotypes at salinity levels above 5 dS m^{-1}.

Significant differences in biomass partitioning into seed or HI were noticed with genotype and salinity (Table 3). Across salinity levels, PI199898 recorded the highest HI, which was significantly higher compared to all other genotypes except PI248866. A rapid decrease was noticed in average HI of genotypes with increase in salinity, particularly above 5 dS m^{-1}, which indicates the role of biomass partitioning on seed yield at higher salinity levels.

4. Discussion

Negative effects of increasing salinity on Ψ_1 and RWC were observed in this study. Reduction in RWC of safflower plants with increase in salt content in soil was also reported by Siddiqi and Ashraf (2008). Higher salt concentration in the soil might be resisting the extraction of enough water to maintain its water status. Decrease in Ψ_1 with increase in salinity was also observed, which is also supported by results of Bassil and Kaffka (2002). Decline in $\Psi_{\pi 100}$ under salt stress conditions was observed in the present study. Solute accumulation or osmotic adjustments due to salinity in plant cells is an important mechanism for salt tolerance of plants (Ashraf, 2004). The highest salt accumulation along with maintenance of higher Ψ_1 in the PI199898 genotype suggests its superior salt tolerance under salinity conditions among all the genotypes.

Photosynthesis and transpiration of safflower genotypes were only affected by salinity levels above 5 dS m^{-1}. Even at

the highest salinity level used in this trial, photosynthesis and transpiration decreased by 26% and 14%, respectively. Reduction in stomatal and mesophyll conductance to CO_2 diffusion of salt stressed leaves results in low CO_2 concentration in the chloroplast which inhibits photosynthesis under stress conditions (Delfine *et al*., 1999). Genotype PI199898 maintained higher photosynthesis and transpiration among all the genotypes. Maintenance of net photosynthesis and stomatal conductance is related to salt tolerance (Lakshmi *et al*., 1996). Salt stress could not affect the dark adapted fluorescence in this experiment, which suggests that the photochemistry of photosynthesis was not directly affected by salt stress (Brugnoli and Lauteri, 1991).

Biomass production by safflower genotypes was stable up to 2.5 dS m^{-1} and started declining gradually at higher salinity levels. Reduced photosynthesis at higher salinity levels was driving the decline in biomass production (Siddiqi *et al*., 2009). At the highest salinity level used in this trial, biomass declined by 43% compared to control which was similar to observations of Bassil and Kaffka (2002). Interestingly, plant height was stable across salinity levels except at 10 dS m^{-1}, where it was significantly reduced by 7%. However, genetic variation was observed among genotypes for photosynthetic rate and biomass production relationship. Pooled over salinity levels, PI304453 recorded the highest photosynthesis rate and produced the highest biomass under lower salinity levels. But, it was also one of the more sensitive genotypes under higher salinity levels. On the other hand, PI199898 had stable biomass under a wide range of salinity levels.

Results showed that HI, an indicator of biomass partitioning into seed, was sensitive to salinity in this trial. PI199898 recorded the highest HI among genotypes. Lack of genotype by salinity interaction for the parameter suggested that HI of all genotypes responded similarly. The HI at the highest salinity level of 10 dS m^{-1} was reduced by 99% compared to control, while biomass production reduced by only 43%. Among the yield formation traits, averaged over genotypes, heads per plant and 1000-seed weight were relatively stable and recorded 18% and 56% reductions with 10 dS m^{-1} salinity over control. Feizi *et al*. (2010) reported 52.3% reduction in 1000-seed weight under high salt conditions (11.2 dS m^{-1}) which is very similar to our findings. Across salinity levels, PI199898 showed higher 1000-seed weight than other genotypes. Averaged across genotypes, seeds per head reduced rapidly at higher salinity levels and recorded 98% reduction with the highest salinity level. Similar results were reported by Francois and Bernstein (1964). Genotypes varied in their response and seeds per head declined most rapidly in S333 at higher salinity levels, while it was almost stable in PI199898. Thus, in the current trial, source of photosynthate production was less affected by increased salinity levels, while sink for photosynthate assimilation was more affected. Among sink parameters, number of seeds and not number of heads or inflorescence was more sensitive.

Considering agronomic data discussed above, PI199898 showed more salt tolerance due to higher HI, 1000-seed weight and less fluctuation in biomass production under different salinity levels than other genotypes. Head and seed production of PI199898 was also comparable with other genotypes. This salt tolerance of PI199898 was associated with its ability to maintain higher Ψ_1, osmotic concentration, RWC, photosynthesis and transpiration. It is thus apparent that a combination of characters like higher osmotic adjustment, higher water status leading to higher photosynthesis and transpiration contribute to salinity tolerance of safflower genotypes. Further assessment of PI199898 is needed to assess the salinity response in depth and potential to use it in developing commercial cultivars.

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