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RESEARCH ARTICLE

Effects of water, salt and nitrogen stress on sunflower (*Helianthus annuus* L.) at different growth stages

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Abstract

Experiments in soil columns were conducted to evaluate the single and interactive effects of water, salt and nitrogen stress at different sunflower (*Helianthus annuus* L.) growth stages in Hetao Irrigation District, China. The study factors included soil salinity (S_0: EC_e=2.5-3.6 dS m^{-1}; S_1: EC_e=9.6-10.7 dS m^{-1}), soil moisture (W_0: 35％-55％ of field water capacity; W_1: 75％-100％ of field water capacity), and nitrogen application rates (N_0: 0 kg N ha^{-1}; N_1: 135 kg N ha^{-1}). The results indicated that the S_1 treatments increased the duration of the seedling stages by 23.91% but decreased the duration of maturity by 33.09% on average compared with the S_0 treatments. Similarly, water deficit significantly retarded anthesis and prolonged the total growth period. The comprehensive stress assessment index (CSAI) was obtained using principal component analysis (PCA) and membership function analysis (MFA). The CSAIs in different treatments showed that soil salinity was the main limiting factor for sunflower vegetative growth from seeding to bud (SS_1), whereas water stress dominated the development from bud to flowering (SS_2) and flowering to maturity (SS_3). Although statistically non-significant, nitrogen stress was intensified after bud initiation and the CSAI in $W_1S_0N_0$ treatment was 40.68% lower than $W_1S_1N_1$ treatment in SS_3. Moreover, the interactive effects of the three factors were complicated. Our experiments suggested that adequate water supply after bud initiation and the reasonable nitrogen application rate (135 kg N ha^{-1}) can alleviate adverse effects on sunflower reproductive growth under different saline conditions.

Keywords: Sunflower, soil moisture, salinity, nitrogen application rate, interactive effects, growth stages

1. Introduction

Continuing population and consumption growth indicate that food security will still be a great challenge for the world for at least another 40 years (Godfray *et al*., 2010). Soil salinization is one of the most important issues that can seriously decrease food production. Take China as an example as more than 8 million hectares of farmland are suffering from salinity. Therefore, alleviating the adverse effects of soil salinity on crops has become a hot global research area that is of great importance in ensuring sustainable food security.

Irrigation and fertilization have been two major management tools for enhancing crop production. In non–saline soils, numerous studies have shown the effects of irrigation and fertilization on crop growth and illustrated their coupling mechanism (Mahajan *et al*., 2012; Nájera *et al*., 2015). Generally, irrigation and fertilization can cause synergistic, superimposed or antagonistic effects on crop growth. More specifically, a synergistic and superimposed effect happens only under a condition where both irrigation and fertilization are within appropriate ranges; otherwise, an antagonistic effect may occur and decrease crop production (Sadras and Richards, 2014). Meanwhile, irrigation and fertilization also interact. For example, irrigation changes soil moisture, which influences the transformation of fertilizer. Another factor is nitrogen, which has been proven to affect root water uptake and crop water use efficiency (Morgan, 1984).

In salt–affected regions, crops are affected by soil water, nitrogen, salinity and their interactions simultaneously, which leads to three major differences compared with non-saline soils. First, salinity affects the crop-water relationship. Munns (2002) proved that salt stress is harmful to crops by indicating the damage caused to the water uptake ability of crops. To describe the crop-water relationship under saline soils, researchers have developed models based on statistical analyses of experiments. Wang *et al*., (2007) established a dynamic crop water and salinity response model and determined the optimal brackish water irrigation strategy for winter wheat and corn based on long time observations. Haghverdi *et al*., (2014) also developed two production functions based on the Decision Tree and Neural Network methods to estimate spring wheat grain yield under simultaneous salinity and water stress.

Second, soil salinity also affects nitrogen transformation processes, such as hydrolysis, ammonia volatilization, nitrification, and denitrification. Most research has indicated that salinity restrains nitrogen mineralization and nitrification, which decreases crop nitrogen uptake and crop production (Zhang *et al*., 2015). Meanwhile, this inhibiting effect increases with soil salt content. However, Zeng *et al*., (2013) also found that the effect of soil salinity on the nitrification rate had a threshold. In his incubation experiment, the rates of mineralization or nitrification decreased with increasing soil salt content when the soil salinity level exceeded the threshold value.

Third, salinity also affects the interaction of water and nitrogen. Due to the complex interactions among water, nitrogen, and salinity, few studies have focused on this issue.Tian, (2011) found that the optimal irrigation amount and nitrogen application rate for sunflower differed in slight (<0.1%) and moderate saline (0.1%-0.32%) soils. However, Yan, (2014) conducted experiments in the same area as Tian but found no significant difference in the optimal irrigation amount and nitrogen application rate for maize between the two salinity levels. Furthermore, Azizian *et al*., (2015) modified the integrated water and nitrogen Maize Simulation Model (MSM) for salinity conditions and achieved a good estimation of soil water content, evapotranspiration, leaf area index and grain yield for maize in the southwest of Iran.

Overall, the interactive effects among soil water, nitrogen, and salinity are still ambiguous, and some inconsistent results about this issue have been reported in the literature. Therefore, the objectives of this study were to quantificationally evaluate single and interactive effects of soil water, salt, and nitrogen on crop growth. More specifically, we chose the sunflower (*Helianthus annuus* L.) – a widely planted industrial crop in the Hetao irrigation District (the largest irrigation district of China that is troubled with soil salinization) – as the study crop and conducted a column experiment (Section 2). Based on the experiment, we analyzed the CSAIs obtained from some agronomy and physiological characteristics at different sunflower growth stages (Section 3) and provided some practical suggestions for irrigation and fertilizer management in saline soils (Section 4).

2. Materials and Methods

2.1. Experimental site

The experiment was conducted at the Yichang Experimental station, located in the Hetao Irrigation District (40°19′–41°18′ N, 106°20′–109°19′ E) of Inner Mongolia, China, from June 2014 to September 2014 (Figure 1(a)). The climate of the experimental area is temperate continental and monsoonal. The average annual precipitation is 139–222 mm, with approximately 60% falling in July and August. The annual potential evaporation is approximately 2200–2400 mm. Strong evaporation forces the groundwater and soil water to migrate upward constantly, eventually resulting in salt accumulation on the soil surface after the evaporation of water from soil.

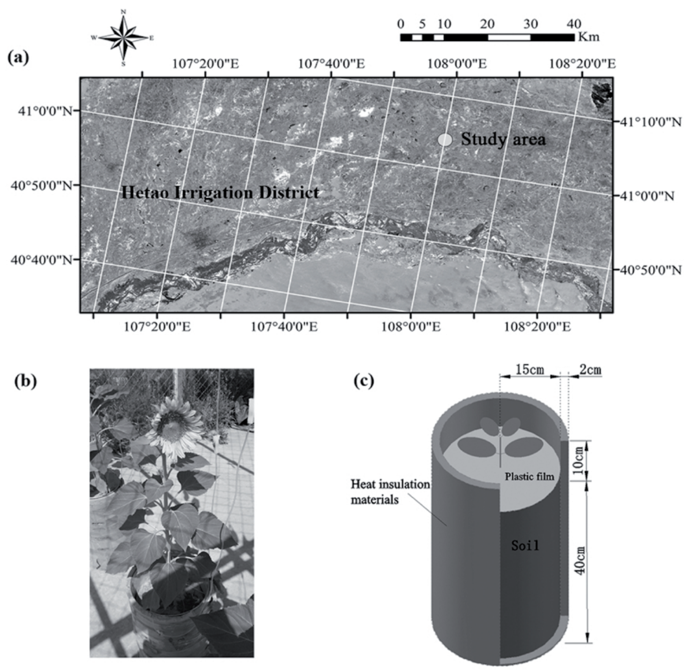

Figure 1. Study site. (a) Location of the study area. (b) Photo of column experiment. (c) Schematic diagram of the column.

2.2. Experimental equipment and treatments

Treatments were arranged in 24 cylindrical columns of 30 cm inner diameter and 50 cm height (Figure 1(b)-1(c)). Each column contained backfilled soil of 40 cm depth. Due to the high temperature in the daytime and all columns being placed on the ground, heat insulation materials of Polystyrene foam with 2 cm thickness were wrapped outside the columns. Soil samples were taken from the Yichang Experimental station. All soil samples were pretreated by crushing, smoothing and air-drying. Then, the pretreated samples were put through a 2 mm sieve and set aside. The basic physical and chemical properties of the soil samples are shown in Table 1. The study factors included soil salinity, soil moisture, and nitrogen application rate. More specifically, the soil salinity level was indicated as the saturated electrical conductivity of soil (EC_e). Salts were mixed using $MgCl_2$, $CaCl_2$, Na_2CO_3, $NaHCO_3$, and Na_2SO_4 with molar concentration ratios of 11.74: 8.54: 1.00: 15.39: 20.83, which represents the averaged salt constitution in the local area. Then, mixed salts were added into the pretreated soils, followed by division of the soils into two salinity levels (FAO, 1985): slight salinity level (S_0, EC_e=2.5-3.6 dSm^{-1}) and high salinity level (S_1, EC_e=9.6-10.7 dSm^{-1}). All of the treated soils were backfilled into columns at the specified bulk density (1.3 g cm^{-3}). Soil moisture was also divided into two levels: deficit irrigation (W_0), which had a soil moisture ranging from 35% to 55% of field capacity, and sufficient irrigation (W_1), which had a field capacity of 75%-100% throughout the experimental period. Two nitrogen application rates, 0 kg N ha^{-1} (N_0) and 135 kg N ha^{-1} (N_1) (Zeng *et al*., 2013), were applied in our study. There were eight treatments, and each treatment had three replicates. Among them, $W_1S_0N_1$was regarded as the control treatment; $W_0S_0N_1$ represented the water stress treatment; $W_1S_1N_1$ represented the salt stress treatment; $W_1S_0N_0$ represented the nitrogen stress treatment; $W_0S_0N_0$ represented the water-nitrogen stress treatment; $W_0S_1N_1$ represented the water-salt stress treatment; $W_1S_1N_0$ represented the salt-nitrogen stress treatment; and $W_0S_1N_0$ represented the water-salt-nitrogen stress treatment.

Table 1. Basic physical and chemical properties of the soil samples

Bulk density	Particle size distribution			Texture	Organic	Total Nitrogen	pH
	Clay	Silt	Sand				
g cm^{-3}		%			g kg^{-1}	mg kg^{-1}	
1.3	3.95	73.19	22.86	Silty loam	5.51	572.51	8.31

2.3. Field management

The nitrogen application rates mentioned above were achieved using urea (46% N). In addition, all columns received 78.59 kgha^{-1} P as superphosphate (7.86% P). All fertilizers were applied to each column on June 2nd, 2014 as base manure. Each column was then mulched with a plastic film of 30 cm diameter. Sunflowers (LD5009) were sown on June 5th 2014 and harvested around September 15th-27th. Each column was planted with one sunflower. Other management practices, including insect and weed control, were conducted according to local agronomic practices unless otherwise indicated.

2.4. Data collection

Data were collected for plant height (H, cm), leaf area index (LAI), bud or flower disk diameter (BFD, cm), SPAD value, growth stage period (GSP, day), up–ground dry matter (UDM, g), N content of up–ground part (NCU, mg), dry root mass (RDM, g), seed yield (SY, g), precipitation (mm), air temperature (℃) and evapotranspiration (ET, mm).

H and BFD were measured using a measuring tape. The length (L) and width (W) of each leaf were determined with a measuring tape to calculate leaf area as follows: leaf area = 0.6564 × L × W (Chen, 1984). LAI was defined as the leaf area per unit ground surface area. SPAD value was measured by a SPAD-502 meter (Minolta corporation, Ltd., Osaka, Japan). H, BFD, LAI, and SPAD measurements were performed once every four days from 28 days after sowing. The growth situations for the sunflower in each column were visually checked every day, and the GSP for each growth stage was recorded.

Furthermore, we divided the total growth period for the sunflowers into three different sub–stages: from seedling to bud (SS_1), from bud to flowering (SS_2), and from flowering to maturity (SS_3). At the end of each sub–stage, UDM and RDM were measured by drying plants at 70 °C to a constant weight. NCU was determined by the micro–Kjeldahl method (Schuman *et al.*, 1973). Meanwhile, SY was air dried to obtain constant moisture (approximately 8% g g^{-1}) and measured at the end of SS_3. In addition, column weight was measured by electronic balance (precision: 0.001 kg) 39 times during the crop cycle. Daily precipitation, maximum and minimum air temperature were recorded by an automated weather station beside the columns (Figure 2(a)). Irrigation amount was recorded by measuring cylinder and this amount was different between columns to guarantee the soil moisture treatment. ET was determined by the difference in the column weight measurements, precipitation, and irrigation amounts using the water balance method (Equation 1). The total ET of every entire growth stage for each column is shown in Figure 2(b).

$$ET_i = 10 \times \frac{W_{i-1} - W_i}{\pi \times 15^2} + I_i + P_i \qquad (1 \le \mathrm{i} \le 39) \quad (1)$$

Where ET_i is the evapotranspiration between the i-1th and ith measurement (mm); W_i is the column weight on the ith measurement (g); and I_i and P_i are the irrigation and precipitation amount between the i-1th and ith measurements (mm), respectively.

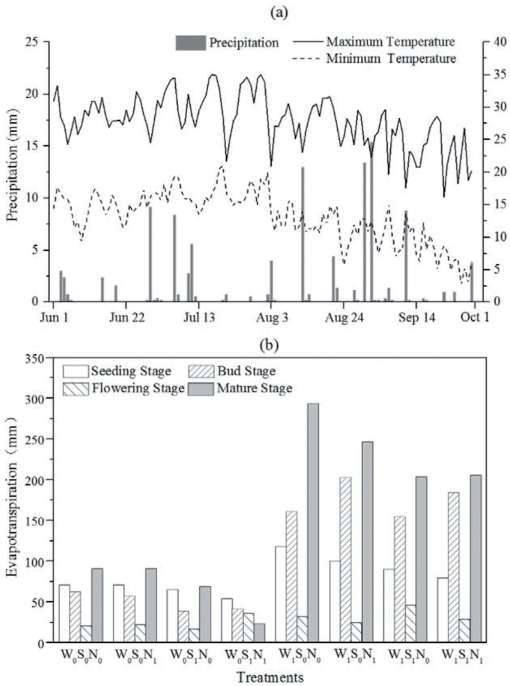

Figure 2. (a) Daily weather data including maximum temperature (℃), minimum temperature (℃), and precipitation (mm) during the experimental period, (b) Evapotranspiration (ET, mm) during different growth stages for each column

2.5. Data analysis and the comprehensive tress assessment index (CSAI)

All data obtained were subjected to analysis of variance (ANOVA), principal component analysis (PCA) andmembership function analysis (MFA) (Dombi, 1990). Computations and statistical analyses were carried out using SPSS software, version 20.0 (SPSS, Inc., Chicago, Ill).

The membership function value (U_j) and the comprehensive stress assessment index (CSAI) is calculated as:

$$U_j = (PC_j - PC_{j\min}) / (PC_{j\max} - PC_{j\min}) \qquad \text{j=1,2} \cdots n \qquad (2)$$

$$W_j = P_j / \sum_{j=1}^{n} \left| P_j \right| \qquad \text{j=1,2} \cdots n \qquad (3)$$

$$CSAI = \sum_{j=1}^{n} \left| U_j * W_j \right| \qquad \text{j=1,2} \cdots n \qquad (4)$$

Where U_j is the membership function value of extracted principle component j (PC_j); PC_{jmin} and PC_{jmax} are the maximum value and minimum value of PC_j for all the treatments, respectively; W_j is the weight of U_j among all the membership function values, P_j is the percentage of variance of PC_j.

3. Results

3.1. Growth stages of sunflower

The durations of the different sunflower growth stages for each treatment are shown in Figure 3. Our study showed that soil salinity significantly increased the duration of the sunflower seedling stage (P=0.001). Meanwhile, the durations of the mature stages were reduced under saline conditions (P<0.001). For example, the durations of the seedling stages for four S_1 treatments had an average increase of 23.91% on average compared with the S_0 treatments, as shown in Figure 3(c). However, for the mature stages, the durations for four S_1 treatments decreased by 33.09% on average compared with the S_0 treatments. Figure 3(b) showed that water stress significantly prolonged the duration of the sunflower bud stages (P=0.045) and the total growth period (P=0.002). Consequently, the average duration of bud stages and total growth period for four W_0 treatments increased by 7.78% and 5.51%, respectively, compared with four W_1 treatments. Moreover, no significant influence of nitrogen application rate was shown in Figure 3(d).

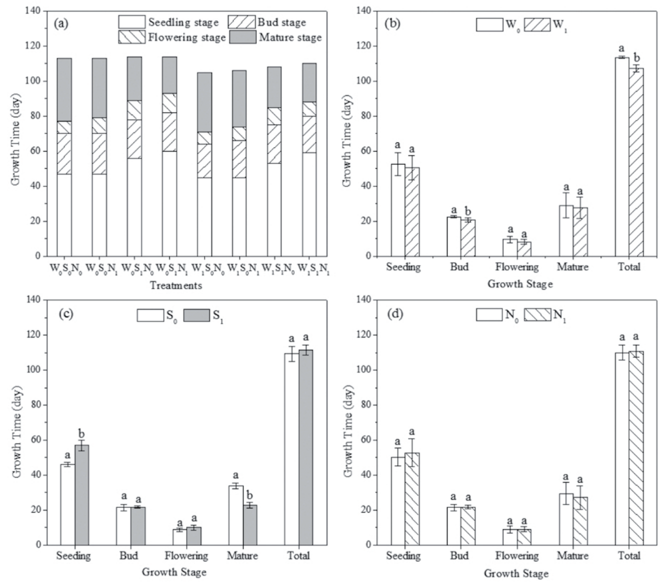

Figure 3. Durations of different sunflower growth stages: (a) indicates all of the eight treatments, whereas (b)-(d) indicate the durations (mean ± standard deviation) of different sunflower growth stages and the total growth periods under water (W_0) and no water (W_1) stress (b), salt (S_1) and no salt (S_0) stress (c) and nitrogen (N_0) and no nitrogen (N_1) stress (d). Different letters above the bar indicate significant difference at $P \leq 0.05$.

3.2. Principal component analysis

PCA was performed on sunflower growth characteristic variables, such as UDM, NC, RDM, ET, and the mean values of H, LAI, SPAD, and BFD in each sub–stage (7 variables in SS_1, 8 variables in SS_2 and SS_3). Two principal components (PC_1 and PC_2) with eigenvalues higher than one were obtained, which accounted for 91.507%, 96.414% and 93.819% of the total variance in the three sub-stages, respectively. The value of loading in Table 2 is an indicator of the participation of the variables in the PCs.For PC_1, all characteristics except BFD in SS_2,SPAD in $SS_1$and SS_3 were the dominant features (>0.75). Meanwhile, PC_2 was dominated by BFD and NCU in SS_2, and the dominant features that affected PC_2 in both SS_1 and SS_3 are SPAD.

Table 2. Loadings of eight sunflower growth characteristic variables on two significant principal components for the three sub-stages

Variable	Seedling-Bud(SS_1)		Bud-Flowering(SS_2)		Flowering-Maturity(SS_3)	
	PC1	PC2	PC1	PC2	PC1	PC2
H	0.920	0.248	0.953	0.044	0.970	-0.038
LAI	0.974	0.011	0.978	0.187	0.965	0.006
SPAD	-0.180	0.975	0.895	-0.414	0.172	0.966
BFD	——	——	-0.736	0.661	0.963	-0.025
UDM	0.979	0.010	0.951	0.279	0.997	0.026
RDM	0.990	-0.053	0.983	0.077	0.971	-0.148
NCU	0.835	-0.107	0.759	0.634	0.887	0.299
ET	0.954	0.072	0.943	-0.200	0.907	-0.285

3.3. Analysis of the comprehensive stress assessment index (CSAI)

The PC_1 and PC_2 scores, the membership function values (U_1 and U_2), and the comprehensive stress assessment index (CSAI) of different treatments for the three sub-stages are shown in Table 3. Main effects of water, salt, and nitrogen on the CSAIs for different sub-stages by ANOVA are shown in Table 4. In SS_1, soil salinity significantly affected the CSAIs (P=0.012), whereas the effects of soil moisture and nitrogen were not significant. In SS_2 and SS_3, soil moisture significantly affected the CSAIs (P=0.002 and P=0.003), whereas the effects of soil salinity and nitrogen were not significant. Moreover, our study also showed that the CSAIs had a significant linear relationship with seed yield in all the three sub-stages (Figure 4(a)). The determination coefficients between CSAI and seed yield in SS_2 (R^2=0.925) was 78.57% and 34.64% higher than SS_1 and SS_3 (R^2=0.518 and 0.687), respectively.

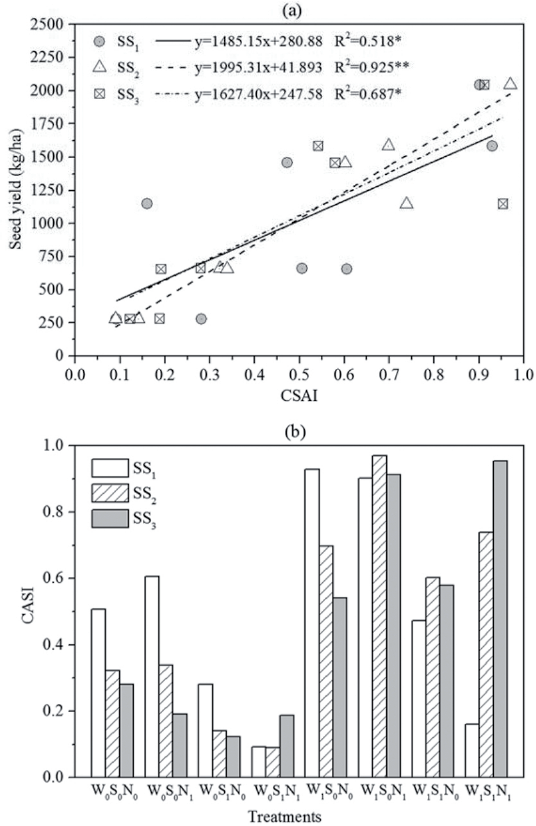

Figure 4. The comprehensive stress assessment index (CSAI): (a) the linear relationship between the CSAIs and seed yield. (b) The CSAIs in different treatments for the three sub-stages.

Table 3. The PC_1 and PC_2 scores, the membership function values (U_1 and U_2), and the comprehensive stress assessment index (CSAI) of different treatments for the three sub-stages

Sub-stages	Treatments	PC1	PC2	U1	U2	CSAI
Seedling-Bud(SS1)	$W_0S_0N_0$	-0.029	0.457	0.467	0.712	0.506
	$W_0S_0N_1$	0.315	0.314	0.594	0.671	0.606
	$W_0S_1N_0$	-0.903	1.450	0.143	1.000	0.281
	$W_0S_1N_1$	-1.291	-0.048	0.000	0.567	0.091
	$W_1S_0N_0$	1.347	0.389	0.975	0.693	0.929
	$W_1S_0N_1$	1.414	-0.641	1.000	0.395	0.902
	$W_1S_1N_0$	-0.080	0.084	0.448	0.605	0.473
	$W_1S_1N_1$	-0.774	-2.005	0.191	0.000	0.160
Bud-Flowering (SS2)	$W_0S_0N_0$	-0.529	-0.048	0.269	0.614	0.322
	$W_0S_0N_1$	-0.539	0.386	0.266	0.742	0.338
	$W_0S_1N_0$	-1.074	-0.327	0.071	0.533	0.141
	$W_0S_1N_1$	-1.270	-0.129	0.000	0.591	0.090
	$W_1S_0N_0$	0.992	-2.146	0.823	0.000	0.698
	$W_1S_0N_1$	1.478	0.588	1.000	0.801	0.970
	$W_1S_1N_0$	0.311	0.407	0.575	0.748	0.601
	$W_1S_1N_1$	0.631	1.269	0.692	1.000	0.739
Flowering-Maturity(SS3)	$W_0S_0N_0$	-0.518	-0.427	0.274	0.316	0.280
	$W_0S_0N_1$	-0.706	-0.962	0.202	0.131	0.191
	$W_0S_1N_0$	-1.233	1.018	0.000	0.813	0.122
	$W_0S_1N_1$	-0.976	0.671	0.099	0.694	0.188
	$W_1S_0N_0$	0.429	-1.344	0.637	0.000	0.541
	$W_1S_0N_1$	1.234	1.561	0.945	1.000	0.954
	$W_1S_1N_0$	0.394	-0.381	0.623	0.332	0.579
	$W_1S_1N_1$	1.377	-0.137	1.000	0.416	0.912

Table 4. Main effects of water, salinity, and nitrogen on PC1 for the three sub-stages by ANOVA

Factors	Seedling-Bud(SS_1)	Bud-Flowering(SS_2)	Flowering-Maturity(SS_3)
	$(P>F)^a$	$(P>F)^a$	$(P>F)^a$
Water	0.303	0.002^{**}	0.003^{**}
Salinity	0.012^{*}	0.433	0.938
Nitrogen	0.664	0.705	0.482

[a] Probability that a significant F value would occur by chance; *, significance level=0.05; **, significance level=0.01

More specifically, all of the CSAIs obtained under salt conditions (S_1) in SS_1 were below 0.5, whereas all of the CSAIs in the S_0 treatments were above 0.5 (Figure 4(b)). However, in SS_2 andSS_3, all of the W_0 treatments had relatively high CSAIs ranging from 0.541 to 0.970, whereas the W_1 treatments always had relatively low CSAIs ranging from 0.09 to 0.338. For the eight different treatments, the control treatment ($W_1S_0N_1$) had the highest CSAIs in both SS_2 and SS_3 compared with the other treatments. The CSAIs in $W_1S_1N_1$ increased constantly and rapidly from 0.160 in SS_1 to 0.912 in SS_3, which made it become the second highest CSAI in SS_3. However, unlike the $W_1S_1N_1$ treatment, the CSAIs in $W_1S_0N_0$ showed a constant decrease from 0.929 in SS_1 to 0.541 in SS_3.$W_1S_1N_0$had an increase of 27.16% from SS_1 to SS_2, but went through a slight decrease of 3.64% from SS_2 to SS_3. Among four W_0treatments, $W_0S_1N_0$ and $W_0S_1N_1$ always had the lowest CSAIs in SS_2 and SS_3 among all treatments. The former decreased constantly, whereas the latter had a slight increase (13.71%) in SS_3. Moreover, $W_0S_0N_0$ and $W_0S_0N_1$also decreased constantly by 44.64% and 68.41% from SS_1 to SS_3.

4. Discussion

Understanding water-salt-nitrogen relationships is of great economic importance to crop production in arid and semi-arid areas (Pang and Letey, 1998). Single effects of water, salinity or nitrogen fertilizer on phenology period, plant growth, biomass accumulation and yield have long been recognized in many literatures(Phogat *et al*., 2014). However, fewstudies have been conducted to evaluate their interaction effect.

In particular, crop phenologyis known to be mainly affected by climate factors, such as temperature, photoperiod, and precipitation (Cleland *et al*., 2007), but little definite evidence is available about the effects of soil factors on phenology. In our study, the results indicated that salt stress extended the seedling stage but compress the mature stage. Similar results were found in Kong *et al*., (2004), who showed that the duration of sunflower seedling stage increased with soil salinity and that the bud stage was consequently delayed. Furthermore, Maas *et al*., (1986) also reported that salt stress retarded the seedling stage for sorghum but hastened its maturity. This phenomenon was attributed to high soil salt content lowering soil solute potential, which made it difficult for the seed to absorb water and also decreased the water absorption rate during early seedling growth (Jaleel *et al*., 2007). Meanwhile, for the effect of water deficit, retarded anthesis and prolonged total growth period were reported in Figure 3(b), which was consistent with the findings of Zeng *et al*., (2014) in the same study area. Boonjung and Fukai, (1996) also found that the anthesis date of maize was postponed when drought occurred during panicle development. Moreover, plant maturity would be delayed if the nitrogen content in the soil could not meet the demand of reproductive growth (Agüera *et al*., 2010). For example, Gungula *et al*., (2003) found that the dates of silking and maturity were delayed with a decrease in the nitrogen application rate, which indicated that nitrogen influenced phenology.

In the present study, the CSAIs, which integrated several agronomy and physiology indexes, were used to evaluate the effects of water, salinity, nitrogen application rate and their interactions on sunflower growth at three different sub–stages. SS_1represented the vegetative stage of plant development before bud initiation. In $SS_{2,}$ the vegetative growth continued and reached its peak at anthesis, while the reproductive growth began with the first appearance of the inflorescence. SS_3 was the reproductive stage with leaf senescence and grain filling. The linear relationship between the CSAIs and seed yield at all the three sub–stages demonstrated that the CSAI was a valuable evaluation index with practical significance in sunflower yield prediction, especially when the data was collected in SS_2.

The results reported here indicated that salt stress could strongly inhibit sunflower growth in SS_1. In the studies of other crop species, the seedling growth of corn, sorghum, cowpea and wheat (Maas and Poss, 1989), was all proven to be much more sensitive to salinity than other growth stages. Possible reasons may be that the crop root in SS_1 was fragile, which resulted in a weak ability for soil water and nitrogen uptake (Munns *et al*., 1988). Higher CSAIs were obtained in W_1treatments compared with W_0 treatments under the same salinity and nitrogen conditions (Figure 4(b)), which indicated that soil moisture could also exert slight effects on sunflower growth in SS_1. However, the fact that the CSAI in $W_0S_0N_1$ (0.606) were higher than in $W_1S_1N_1$ (0.160) also illustrated that salt stress was the main factor affecting sunflower growth in SS_1. This was because the small plant in SS_1 had low ET rate (Figure 2), which rendered the effect of water deficit less significant (Kong *et al*., 2004). Nitrogen stress in SS_1 was not significant compared with water and salt stress (Table 4). This was because the basal nitrogen in soils sampled from the fields may be sufficient for the nitrogen demand of sunflower in SS_1. Fang and Ke, (1990) proved that crop nitrogen in SS_1 was mainly from soils with ^{15}N labeling and suggested that nitrogen application was unnecessary during the early growth stages in areas with high soil fertility. In addition, evidence in our experiments suggested that nitrogen application in SS_1 could achieve counterproductive results under saline conditions, as lower CSAIs was found in $W_0S_1N_1$ and $W_1S_1N_1$ compared with $W_0S_1N_0$ and $W_1S_1N_0$, respectively. Among them, $W_0S_1N_1$ had the lowest CSAI in SS_1. This was because the interactive effects of salt and fertilizer nitrogen dissolved in soil solution could severely reduce soil solute potential, especially when water deficit took place simultaneously.

It is generally accepted that salt tolerance increased with time after the seedling stage (Läuchli and Grattan, 2007). In SS_2 and SS_3, our results indicated that sunflower salt tolerance was enhanced. $W_1S_1N_1$ had the second highest CSAI in SS_3, which showed that salt stress had less effect than water and nitrogen stress in reproductive growth. A more detailed study (Winicov, 2000) suggested that sunflower salt tolerance was enhanced as root growth reached a high level after seedling growth. Moreover, $W_1S_1N_0$ had much lower CSAI than $W_1S_1N_1$ in SS_3, which indicated that reasonable nitrogen application rate (135 kg N ha^{-1}) alleviated the effects of salt stress on reproductive growth of sunflower when the water supply was sufficient.

The effect of water stress was aggravated and became the predominant factor in SS_2 and SS_3. Higher water demand for faster vegetative and reproductive growth could account for the aggravation of water stress. The transpiration rate of sunflower increased significantly after bud initiation, and great difference in ET were found between the W_0 and W_1 treatments (Figure 2). A few studies in non-saline areas have shown that the critical water demand period of sunflower lasted from the bud stage to the early maturity stage (Unger, 1982). Meanwhile, Qiao *et a*l., (2006) also found that the root water uptake rate of sunflower increased sharply during the bud stage and reached its maximum in the flowering stage in saline areas.

Although the effect of nitrogen stress was still not significant in SS_2 and SS_3, it was intensified after bud initiation and became stronger than salt stress in SS_3. Similar findings was reported by Deng *et al*., (2002), who studied the effect of nitrogen application rate on biological responses of sunflower in different salinity level soils and showed that nitrogen fertilizer resulted in great differences in reproductive stage. Moreover, except for $W_0S_0N_0$ and $W_0S_0N_1$, all of the N_1 treatments had higher CSAIs in SS_3 than the N_0 treatments under the same water and salinity conditions. Thus, it could be further confirmed that nitrogen stress significantly affected the reproductive growth of sunflower. Using the CSAI, our study made a quantitative evaluation onthe interactive effects of water, salinity and nitrogen stress in SS_2 and SS_3. The results reported here indicated that the intensity sequence ofthe interactive stress effects in SS_2 was $W_0S_1N_1$> $W_0S_1N_0$>$W_0S_0N_0$>$W_1S_1N_0$. Because water was the predominant factor after bud initiation, all the interactions with water stress had severe stress effects on sunflower growth, especially when water deficit coupled with high salinity level. With regard to the evaluationafter anthesis, the CSAI in $W_0S_1N_1$ was slightly increased and $W_0S_1N_0$ became the most serious interaction. This result further demonstrated that the reasonable nitrogen application rate (135 kg N ha^{-1}) could alleviate the interactive effects on post-anthesis reproductive growth under both water deficit and saline soil conditions. Similar phenomenon was reported by Xue *et al.*, (2007) and Kütük *et al*., (2004), who suggested that increasing the nitrogen fertilizer supply to a certain extent could attenuate the interactive effects of water-salt stress on sunflower and tomato development, respectively.

5. Conclusion

Sunflower growth is affected by soil moisture, salinity and the nitrogen application rate. Our study proved that water and salt have a significant influence on sunflower phenology. Soil salinity delays bud initiation but hastens maturity, whereas water deficit retards anthesis and prolongs the total growth period. Moreover, the soil salinity level was the main limiting factor for vegetative sunflower growth in SS_1. Sunflower salt tolerance increased with growth, and water stress rather than salt became the main limiting factor in SS_2 and SS_3. In addition, the nitrogen effect was not significant in SS_1 but increased after bud initiation and became stronger than salt stress in SS_3. Furthermore, the interactive effects of water, salt and nitrogen stress showed considerable variation at different growth stages. For example, salt-nitrogen stress was weaker than salt stress but was stronger than nitrogen stress in SS_1, and it had the most negative effect in SS_2 and SS_3 when the water supply was sufficient. The effect of water-salt-nitrogen stress was intensified constantly during the crop cycle and had the most severe effect on sunflower growth in SS_3, whereas water-salt stress significantly inhibited sunflower growth in SS_1 and SS_2 but was mitigated slightly in SS_3. Therefore, we suggest increasing the irrigation amount after bud initiation to avoid the severe adverse effect of salt and nitrogen stress coupling with water deficit. Meanwhile, applying a reasonable nitrogen application rate (135 kg N ha^{-1}) can alleviate the interactive effects of salt and nitrogen stress on sunflower reproductive growth under saline conditions, regardless of whether the water supply is sufficient or not.

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