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RESEARCH ARTICLE

Effect of salinity stress on phenotypic plasticity, yield stability, and signature of stable isotopes of carbon and nitrogen in safflower

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Abstract

Salinity is one of the major factors contributing in land degradation, disturbance of soil biology, a structure that leads to unproductive land with low crop yield potential especially in arid and semiarid regions of the world. Appropriate crops with sufficient stress tolerance capacity and non-conventional water resources should have to be managed in a sustainable way to bring these marginal lands under cultivation for future food security. The goal of the present study was to evaluate salinity tolerant potential (0, 7, and 14 dS m^{-1}) of six safflower genotypes that can be adapted to the hyper arid climate of UAE and its marginal soil. Several agro-morphological and physiological traits such as plant dry biomass (PDM), number of branches (BN), number of capitula (CN), seed yield (SY), stable isotope composition of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), intercellular CO_2 concentration from inside to ambient air (*Ci*/*Ca*), intrinsic water use efficiency (iWUE), carbon (C%) and nitrogen (N %), and harvest index (HI) were evaluated as indicative of the functional performance of safflower genotypes under salt stress. Results indicated that salinity significantly affected the seed yield at all levels and varied significantly among genotypes. The BN, PDM, CN, and $\delta^{13}C$ attributes showed clear differentiation between tolerant and susceptible genotypes. The $\delta^{13}C$ results indicate that the tolerant genotypes suffer less from stress, may be due to better rooting. Tolerant genotypes showed lower iWUE values but possess higher yield. Safflower genotypes (PI248836 and PI167390) proved to be salt tolerant, stable, and higher seed and biomass yielder. There was no G × E interaction but the genotypes that produce higher yield under control were still best even under salt stress conditions. Although salinity reduced crop yield, some tolerant genotypes demonstrate adaptation and good yield potential under saline marginal environment.

Keywords $\delta^{13}C$ · $\delta^{15}N$ · *Carthamus tinctorius* · Growth · Genotype evaluation · Salinity · Yield

Introduction

The Arabian Peninsula is the largest region with hyper arid climate and low unreliable rainfall. The temperature in the summer may exceed than 50 °C and soils are poor, sandy, and without any available plant nutrient elements. Moreover, the United Arab Emirates (UAE), in general, lack of any river and canal irrigation systems. Therefore, majority of agriculture depends upon the groundwater resources for crop irrigation. With increase in the population growth and agriculture activities, large-scale extraction of groundwater leads to water depletion and without aquifer recharge. This entire situation put enormous pressure on freshwater resources, leaving agriculture to use low-quality saline water or treated wastewater that might increase the risks of soil salinization and land degradation (Al-Dakheel et al. 2015; Qureshi et al. 2016).

Due to the narrow range of the crops that can be grown in this region, the UAE mostly imports food grains and oil seed crops. However, it is imperative to focus research on diversified crops that can tolerate high temperature, drought, salinity, low water consuming, and also play a significant role to achieve the regional food security. In this context, introduction of such diversified crops that will be beneficial for the sustainable development of degraded marginal lands should be given priority. Safflower (*Carthamus tinctorius* L.) is an oil seed crop, grown in arid and semiarid regions as winter and

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summer crop, renowned for its range of phenotypic plasticity, wide genetic diversity, and drought- (Movahhedy-Dehnavy et al. 2009) and salt-tolerant cash crop (Gengmao et al. 2015). Safflower seed constitutes 35–50% oil (Camas et al. 2007); minerals (Zn, Cu, Mn, and Fe); vitamins (thiamine and β-carotene); tocopherols α, β, and γ (Velasco et al. 2005); and bioactive compounds (Khalid et al. 2017).

Safflower oil has numerous applications in food, cosmetics, pharmaceutical, and feed industry. The functional properties of safflower oil can be used to treat skin infections, bone-related disorders, menopause, and atherosclerosis (Khalid et al. 2017). Safflower oil contains high proportion of polyunsaturated fatty acids such as linoleic acid and tocopherol that are used for medicinal as well as dietetic purposes (Han et al. 2009). Being deep rooted, safflower has shown good potential for adaptation to semiarid environments (Beyyavas et al. 2011). The deep root system of safflower may be able to take up moisture and nutrients, especially nitrogen that has been leached below the rooting zone of most other crops, especially in sandy soils which are already deprived of essential plant nutrients. The water use efficiency of safflower was compared with other oilseed species and has shown to be higher than that of flax and mustard (Kar et al. 2007) while lower than rapeseed (Wachsmann et al. 2008). However, water use efficiency among different safflower genotypes has not been extensively studied yet.

Salinity is the major abiotic stress factor that reduced the growth, yield, and quality attributes of major cereals, food, and oil seed crops globally (Shabala 2013; Guo et al. 2014; Hussain et al. 2016). It was estimated that more than 800 million hectares of land are affected by soil and water salinity (Munns and Tester 2008). Impacts of drought and salinity are more obvious in arid and semiarid regions where limited rainfall and high evapotranspiration and temperature are the major constraints for low crop yield (Azevedo Neto et al. 2006). The selection of appropriate crop genotypes and crop management practices that can play a pivotal role for adaptation and improvement under water scarcity and saline environment is a better option to mitigate effects of salinity (Jaradat and Shahid 2006; Munns and Tester 2008; Al-Dakheel et al. 2015; Al-Dakheel and Hussain 2016; Hussain et al. 2016). Moreover, selection of suitable agro-physiological and biochemical traits should be given priority in order to discover insight mechanism involved for abiotic stresses tolerance (Araus et al. 2008). Salinity induces osmotic stress (Roy et al. 2014) and inhibits the stomatal conductance and photosynthesis (Flowers et al. 2015), ultimately leaving a negative impact on plant growth and yield via decreased carbon assimilation, cell number, and plant tissue expansion (Hirel et al. 2007; Hussain et al. 2016). Water use efficiency can be used as a proxy for physiological feedback from the plant facing environmental challenges (Blum 2005, 2009; Hussain and Reigosa 2017). Studies of WUE at the whole plant and field levels are cumbersome due to the workload and costs involved in assessing whole plant or crop water use, especially when large plant populations in plant breeding are considered.

Carbon isotope composition ($\delta^{13}C$), ratio of CO_2 fluxes from inside the cell to outer atmosphere, and water use efficiency measurements in the plant dry matter have been recently proposed to evaluate the ecophysiological cascades of plants under stress (Farquhar and Cernusak 2012; Farquhar et al. 2007; Cernusak et al. 2009; Zobitz et al. 2008; Hussain and Reigosa 2014, 2015). Farquhar et al. (2007) explained that difference in $\delta^{13}C$ values might be the result of changes in intrinsic photosynthetic capacity (A) or stomatal conductance (g_s), and thus provides information on the long-term transpiration efficiency of plants. Meanwhile, a better understanding of the effects of genotype, environment, and their interaction on seed yield, agronomic, and isotopic traits is necessary to further improve the crop yield under the restricting environmental conditions (Araus et al. 2008; Yousfi et al. 2012). The salt tolerance of different genotypes needs to be evaluated to test their suitability for marginal environments to offer a more practical solution for effective utilization of salt-affected soils. Therefore, assessment of physiological, biochemical, and isotopic characterization of safflower genotypes and their response to salinity is of paramount importance before considering the adoption of these traits in crop management studies and release of selected genotypes for saline environment. Meanwhile, there is little information available on the relative importance of genotype (genetic variation) and genotype × environment interaction effects on the isotopic and agronomic traits of safflower grown under saline field conditions. Additional studies are therefore needed to investigate the effects of field salinity on seed yield, yield components, and stable isotopic attributes in safflower genotypes. Previous experiments in our group reported yield potential among a global collection of 265 safflower genotypes at different water salinity levels (Fraj et al. 2013). However, in the present study, six selected safflower genotypes were further evaluated for detailed physiological, agronomical, and isotopic responses under saline field condition that could provide a significant background regarding ecophysiological crosstalks to plant biologists, agronomists, and breeders with integrative traits to predict differences in plant growth, biomass, and yield traits of safflower genotypes subjected to a range of water salinities.

Materials and methods

Plant material and growth conditions

Six safflower (*Carthamus tinctorius* L.) genotypes were chosen for this study to represent the wide genetic variation and selection was based on previous seed yield trials from a global population (Table 1; Fraj et al. 2013). The experiment was conducted at the research station of the International Center for Biosaline Agriculture (ICBA), Dubai, UAE (25° 5′ N and 55° 23′ E). The field area has Hyperthermic Typic Torripsamment soil type. The compost was applied and mixed into the experimental plot as per recommendation of extension staff. Nitrogen, phosphorus, and potash (N-P-K) fertilizer (20-20-20 Growfert Solub™ fertilizer) was applied at a rate of 100 kg ha^{-1} split into applications of 50, 30, and 20 kg ha^{-1} at the early vigor, mid stem elongation, and heading stages, respectively, although fertilizer quantities applied were based on soil analysis and long-term management requirements to maintain soil fertility. The safflower seeds were sown manually during the first week of November 2013. The field plots measuring 2 × 4 m (plot area of 8 m^2) were established in a split-plot design with three replications. The main factor was the salinity level (S1, 0, or control; S2, 7 dS m^{-1} and S3, 14 dS m^{-1}) and the sub factor was the safflower genotypes. The salinity levels in the irrigation water were keep the same and checked biweekly. There were four rows (each row of 4 m in length) in each plot with a row space of 0.5 m between them. Drip irrigation system was installed as one main line in the middle of each main plot with several lateral irrigating lines arising towards both sides of the main line. Irrigation timing, quantity, and frequency were controlled with automatic control valves. Irrigation lines were laid out at 50 cm as R × R distance, whereas drippers were fixed at 25 cm as P × P distance. The irrigation cycle consisted of four applications of 15 min each, thus giving a discharge of 4 l/dripper/day or 32 l of water was applied to one square meter per day. Irrigation schedule was adjusted with the crop growth cycle and applied at rates equivalent to ET_0 plus 10% for leaching requirements. The climatic data (temperature, evapotranspiration, and relative humidity) are presented in Fig. 1. Figure 2 illustrates the safflower production system management (seedling establishment and vegetative and reproductive stages) during the course of the study.

Agronomical and physiological measurements

The data was collected for number of branches per square meter and number of flower heads (capitula) per square meter from the two central rows to avoid edge effects. The samples were obtained to measure the fresh biomass

Table 1 Genotype and treatment effects on biomass, agro-physiological traits, yield and yield components, and carbon isotope composition of six safflower genotypes grown under different water salinity levels

	BN	PDM	CN	SY	HI	*Ci/Ca*	δ^{13}C	Δ^{13}C	iWUE
Genotypes									
Tolerant—PI248836	42.2b	9.7a	598b	3.6a	36.7a	0.94a	− 32.73a	25.6a	1.3d
Tolerant—PI167390	50.0a	10a	727.1a	3.1a	30.4bc	0.92a	− 32.38a	25.2a	1.7d
M. tolerant—PI253387	29.9d	8.9b	448.4d	2.7b	30.4bc	0.82b	− 32.19c	22.8c	3.9b
M. tolerant—PI250714	37.3c	7.5b	546.2c	2.4bc	31.6b	0.88b	− 31.63b	24.4b	2.5c
Sensitive—PI253385	29.8d	6.8c	362.4f	2.3c	31.3b	0.78c	− 29.55d	22.2c	4.6b
Sensitive—PI239707	30.9d	8.6b	379.7e	2.2c	25.4d	0.67d	− 27.11e	19.6d	7.1a
Treatment									
S1—0 (control)	43.6a	9.8a	599.3a	3.5a	35.8a	0.800b	− 31.70a	24.5a	2.3c
S2—7 dS m^{-1}	34.7b	8.3b	499.5b	2.6b	31.9b	0.836a	− 30.50b	23.2b	3.6b
S3—14 m^{-1}	31.6c	7.7c	432.1c	1.9c	25.1c	0.786c	− 29.40c	22.1c	4.7a
Level of significance									
Genotype (G)	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00
Treatment (T)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G × T interaction	ns	**	ns	ns	ns	ns	ns	ns	ns

BN, number of branches m^{-2}; *PDM*, plant dry biomass (t ha^{-1}); *CN*, number of capitula per m^{-2}; *SY*, seed yield (t ha^{-1}); *HI*, harvest index (%); *Ci/Ca*, ratio of intercellular to ambient CO_2 concentration; $\delta^{13}C$, stable carbon isotope composition (‰); $\Delta^{13}C$, carbon isotope discrimination (‰). Genotype values are the means of 9 measurements (three treatments and three replications per treatment), while treatment values are the means of the 54 measurements (six genotypes and three replications per genotype). Means followed by different letters are significantly different ($p < 0.05$) according to Tukey's honestly significant difference (HSD) test. *M. Tolerant*, medium tolerant. Treatments S1—0 (control); medium salinity —S2, 7 dS m^{-1}; high salinity—S3, 14 dS m^{-1}; *ns*, not significant; *G*, genotypes; *T*, treatment

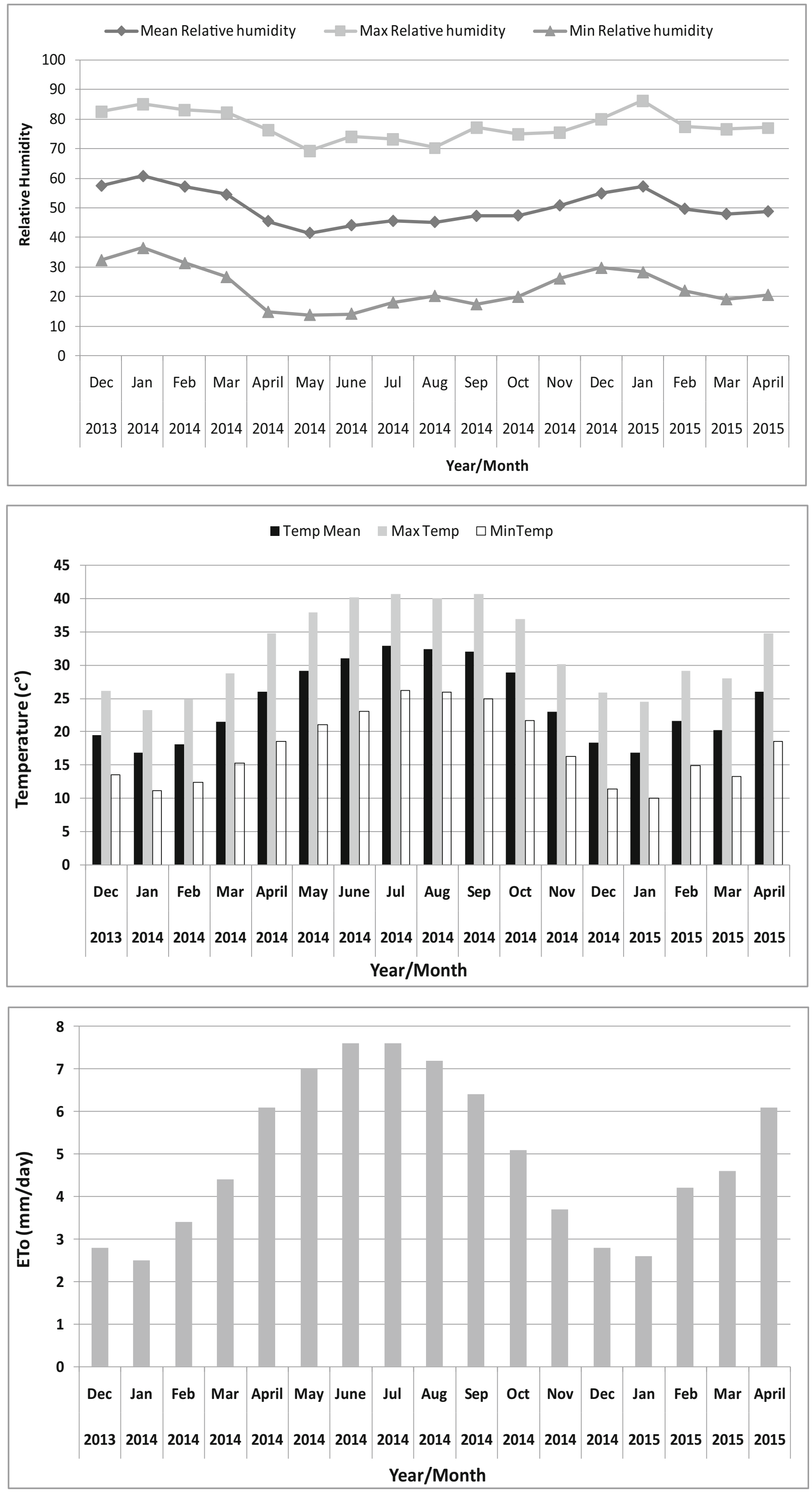

Fig. 1 Monthly average values of mean (T mean), maximum (T maxi), and minimum (T mim) air temperature and reference evapotranspiration (Eto) in the ICBA weather station, Dubai, UAE, from December 2013 to April 2015

(FW) at physiological maturity stage. The dry biomass (DW) yield was calculated following drying the plant samples at 70 °C until constant weight. For grain yield, a sample line of 1 m length from central rows was harvested and grains were removed from the capitula, threshed, and weighed (g/m^2), and values were converted into tons per hectare. The harvest index was calculated by using the following formula:

$$\text{Harvest index}(\%) = \text{Grain yield}/\text{dry biomass} \times 100$$

Fig. 2 Safflower production system management for sustainable development of marginal sandy desert soils at ICBA, Dubai, UAE. **a** Irrigation systems and seedling growth of safflower. **b** Safflower crop at vegetative stage. **c** Safflower crop at flowering stage. **d** Safflower capitulas and flowers

Stable isotope composition of nitrogen and carbon

The nitrogen isotope ($\delta^{15}N$), carbon isotope composition ($\delta^{13}C$), ratio of CO_2 from inside leaf to outside environment (Ci/Ca), carbon isotope discrimination ($\Delta^{13}C$), and intrinsic water use efficiency (iWUE) were determined according to the procedure of Farquhar et al. (1989), Farquhar and Richards (1984), and as documented previously (Hussain and Reigosa 2012, 2015, 2017). Briefly, the plant leaf samples were collected from each treatment and dried in an oven at 70 °C. The dry leaf samples were grinded using a ball mill and converted into fine powder. The powdered samples (weight 1700–2100 μg) were saved in the tin capsules (5 × 3.5 mm) and sealed. The capsules were entered automatically in combustion oven at 1600–1800 °C. The stable isotope ratios of carbon and nitrogen were determined through an isotopic ratio mass spectrometry (IRSM, Finnegan: Thermo Fisher Scientific, model MAT-253, Schwerte, Germany) (PINSTECH, Islamabad, Stable Isotope Facility). The precisions were better than 0.2‰ for ^{15}N and 0.05‰ for ^{13}C.

Statistical analysis

To evaluate the effect of different treatments (saline water (S), genotypes (G), and G × S interaction), the data was analyzed by using factorial ANOVA. Treatment means were compared by Tukey's honest significant test using SPSS 19.0. For each parameter, genotype, treatment, and their interaction (6 genotypes × 3 treatments), data was analyzed by recruiting the Pearson's correlation procedure. This analysis was done to evaluate the performance of salinity treatment on genotypes as a cause of changes in the observed plant responses. The relative contribution of carbon and nitrogen isotopes was carried out to check their impact on biological yield (plant dry biomass) and N concentrations.

Results

Growth, physiological traits, biomass and seed yield, and carbon isotope signatures

The salinity caused significant reduction in the number of branches (BN) that was 38% less than the control. Plant dry biomass (PDM) was significantly affected following saline water treatment that results in 15 and 21% reduction at 7 and 14 dS m^{-1}, respectively, compared to the control (Table 1). The yield components (number of capitula (CN), seed yield (SY), harvest index (HI), and the physiological characteristics (Ci/Ca ratios, and $\delta^{13}C$) were decreased significantly as compared to the control at all salinity levels (Table 1).

The number of capitula decreased from 24 to 44% at 7 and 14 dS m^{-1}, respectively. The seed yield was significantly higher in control plants and highest seed yield was recorded in genotype PI248836 and the lowest in PI239707. Harvest index (HI) was decreased with salt water treatments by an average of 10.24 and 32.6% at 7 and 14 dS m^{-1}, respectively, as compared with the control (Table 1). Harvest index greatly varied among the safflower genotypes and ranged between 36.7–25.4% with highest HI observed in genotype PI248836 and the lowest in PI239707. Among the genotypes, the $\Delta^{13}C$ values varied significantly ($p > 0.05$) and safflower genotype PI239707 had lowest Δ value (19.6‰), while PI248836 showed the highest Δ (25.6‰) (Table 1). Safflower genotypes were separated into three grades according to their Δ values.

The first grade included salt-sensitive genotypes, with the lowest Δ values, ranging from 19.6 to 22.2‰ but relatively high iWUE (Table 1). These genotypes had relatively lower SY that were in the range of 2.2–2.3 t ha^{-1}. The case of PI253387 and PI239707 was interesting, because these genotypes had a lower grain yield despite a relatively higher iWUE. The second grade included genotypes with Δ values slightly higher than those from the first grade, ranging from 22.8 to 24.4 ‰. Seed yield in this category was quite variable, ranging from 2.4 to 2.7 t ha^{-1}. In the third grade, genotypes had the highest Δ values, ranging from 25.2 to 25.6‰ and also had the highest seed yield with a range of 3.1 to 3.6 t ha^{-1} (Table 1).

Carbon and nitrogen concentrations and δ^{15}N isotope composition

The C% in tolerant genotypes, PI248836 and PI167390, was significantly higher than the sensitive genotype PI239707 (Table 2). Safflower genotype PI167390 showed higher foliar N% values than all the other genotypes (Table 2). Water salinity treatments decreased the C% in treated safflower plants at 7 and 14 dS m^{-1} as compared to the control. Following the salinity treatment, the N% values were increased significantly as compared to control. The G × T interaction was not significant. Interestingly, it was also noticed that nitrogen isotope composition (δ^{15}N) was significantly increased following treatment with saline water (Table 2).

Table 2 Genotype and treatment effects on carbon and nitrogen concentrations and nitrogen isotope composition (δ^{15}N) of six safflower genotypes grown under different water salinity levels

	C%	N%	C/N ratio	δ^{15}N
Genotypes				
Tolerant—PI248836	28.2a	2.5b	11.3b	6.5a
Tolerant—PI167390	28.9a	3.0a	9.6d	5.7b
M. tolerant—PI253387	27.5b	2.2c	13.2a	6.4a
M. tolerant—PI250714	27.9b	2.6b	11.5b	6.6a
Sensitive—PI253385	28.3a	2.5b	11.8b	6.5a
Sensitive—PI239707	27.8b	2.6b	10.0c	5.7b
Treatment				
S1—0 (control)	29.8a	2.2c	14.7b	5.7b
S2—7 dS m^{-1}	27.5b	2.8b	9.8a	6.4a
S3—14 dS m^{-1}	27.1b	2.9a	9.7a	6.6a
Level of significance				
Genotype (G)	0.250	0.021	0.079	0.006
Treatment (T)	0.00	0.00	0.00	0.00
G × T interaction	ns	ns	ns	ns

C%, foliage carbon concentration; *N%*, foliage nitrogen concentration; *C/N ratio*, ratio of carbon to nitrogen; *δ^{15} N*, stable nitrogen isotope composition (‰). Genotype values are the means of 9 measurements (three treatments and three replications per treatment), while treatment values are the means of the 54 measurements (six genotypes and three replications per genotype). Means followed by different letters are significantly different ($p < 0.05$) according to Tukey's honestly significant difference (HSD) test. *M. Tolerant*, medium tolerant. Treatments S1—0 (control); medium salinity—S2, 7 dS m^{-1} ; high salinity—S3, 14 dS m^{-1} ; *ns*, not significant. *G*, genotypes; *T*, treatment

Phenotypic association for growth, yield, and isotopic composition attributes

The phenotypic correlation (*r*) estimates among different traits ranged from − 0.068 to 0.999 and were significant at 0.05 level (Table 3). Several *r* estimates were negative and highly significant; however, the magnitude of associations varied. The highest *r* value was observed between the seed yield and harvest index ($r = 0.839$), and between the carbon isotope discrimination and number of capitula ($r = 0.703$). Plant dry biomass (PDM) highlights the significant and positive correlation ($r = 0.667$) with seed yield and also positive and significant correlation with CN ($r = 0.561$) (Table 3). The number of branches (BN) demonstrated significant and positive correlation ($r = 0.543$) with number of capitulas. But contrary, BN had significant and negative correlation ($r = - 0.522$) with carbon isotope composition. The number of capitula has significant but negative correlation ($r = - 0.702$) with carbon isotope composition. It was noticed that the N% has highly significant negative correlation ($r = - 0.919$) with C:N ratio but positively correlated ($r = 0.591$) with nitrogen isotope composition.

Discussion

Are branch numbers, capitula numbers, plant dry biomass, and *Ci*/*Ca* traits good indicators of genotypic tolerance to salinity?

High accumulation of salts in saline soils led to reduced soil water potential which causes impairment for plants to extract water and nutrients from soil and ultimately experience the "osmotic stress". Salinity typically impacts the different physiological processes (decreased respiration, low water uptake, decreased photosynthesis), and ultimately crop yield (Hussain et al. 2016; Mustafa et al. 2014). Generally, salt induced suppression in the rate of photosynthesis and thus reduced the plant biomass (Ashraf and Harris 2004). The C isotope results revealed that the tolerant genotypes suffer less from salt stress, which may be due to better rooting. These results in a lower WUE but still produced the higher yield. This is in the line of Blum (2009) who frequently commented on this. The sensitive genotypes were more stressed, which may be due to the closed stomata, and had lower C isotope discrimination. Since a positive relationship between biomass and *Ci*/*Ca* was observed in most of the genotypes, these results are analogous to earlier findings in which very strong relationship was found

Table 3 Pearson's correlation among physiological and seed yield traits of safflower genotypes evaluated at different water salinities

	SY	BN	PDM	CN	HI	N%	C%	C/N Ratio	δ^{15}N	δ^{13}C	Δ^{13}C
SY	1										
BN	0.511**	1									
PDM	*0.667***	0.489**	1								
CN	0.546**	*0.543***	*0.561***	1							
HI	*0.839***	0.327**	0.189^{ns}	0.325**	1						
N%	− .325**	0.0319^{ns}	− 0.219**	-0.010^{ns}	− 0.260**	1					
C%	0.459**	0.449**	0.336**	0.36**	0.351**	− 0.026**	1				
C:N Ratio	0.43**	0.131^{ns}	0.316^{ns}	0.119^{ns}	0.323^{ns}	− *0.919***	0.247**	1			
δ^{15}N	− .234**	-0.044^{ns}	-0.202^{ns}	0.01^{ns}	-0.132^{ns}	*0.591***	-0.198^{ns}	− *0.587***	1		
δ^{13}C	− .508**	− *0.522***	− 0.373**	− *0.702***	− 0.417**	0.074^{ns}	− 0.342**	-0.173^{ns}	-0.068^{ns}	1	
Δ^{13}C	0.508**	*0.522***	0.374**	*0.703***	0.417**	-0.074^{ns}	0.342**	0.172^{ns}	0.068^{ns}	− *0.999***	1

**Correlation significant at $p > 0.05$ according to Tukey's HSD test; *SY*, seed yield; *BN*, branch number; *PDM*, plant dry mass; *CN*, capitula number; *HI*, harvest index; *N%*, nitrogen concentration; *C%*, carbon concentration; $\delta^{15}N$, nitrogen isotope composition; $\delta^{13}C$, carbon isotope composition; $\Delta^{13}C$, carbon isotope discrimination

between these two variables in canola (Ulfat et al. 2007) and wheat (Yousfi et al. 2012). Some researchers found that safflower can be a potential alternate oil seed crop in saline and drought-prone environment due to the ability to grow under water stress conditions (Yau 2004; Kar et al. 2007).

Growing conditions and genotypic effects on δ^{13}C and intrinsic water use efficiency under salinity

Under different abiotic stress conditions, plants have developed many defense strategies that help the plant to maintain the normal plant functions by adaptation and adjustment in the physiological and biochemical processes (Hussain and Reigosa 2011; Hussain et al. 2015, 2016). Plant δ^{13}C has less negative values under saline conditions, as reported elsewhere (Yousfi et al. 2012). As long as the salt stress was more severe, safflower plants became enriched in the heavier isotope ^{13}C (δ^{13}C less negative values) compared with untreated control plants.

Several researchers demonstrated that difference in carbon isotope discrimination (Δ^{13}C) among the various genotypes indicates an appropriate criterion for enhancing the water use efficiency in different crops such as *Phaseolus vulgaris* (Ehleringer 1990), wheat (*Triticum spp.*) (Farquhar and Richards 1984), chickpea (*Cicer arietinum*) (Kashiwagi et al. 2006), and cowpea (*Vigna unguiculata*) (Ismail et al. 1994). Variation in Δ values has been reported by researchers in different oilseed crops such as rapeseed (Luckett and Cowley 2011), soybean (Kumar et al. 2012), and sunflower (Lambrides et al. 2004). As compared to safflower, the sunflower has been extensively studied for intraspecific variability in Δ (belonging to the same family) (Lambrides et al. 2004; Virgona and Farquhar 1996). Recently, Mihoub et al. (2016) demonstrated that Δ varied from 21.3 to 25.2‰ among safflower genotypes under drought stress. In the present study, safflower genotypes also showed significant variation in Δ^{13}C ranging from 19.6 to 25.6‰. However, heritability estimation of this trait is still needed to confirm if a selection is possible for Δ in safflower. Large amplitude of variation in Δ (1.0–6‰) was also noted within the safflower genotypes. Safflower genotype "PI248836" elaborated extraordinary performance showing a higher Δ (25.6‰) and seed yield (3.6 t ha^{-1}) but lower iWUE. The achievement of higher digits in these characteristics might be due to higher photosynthetic assimilates (*Ci*/*C*a) as compared to other genotypes. Therefore, this genotype combines both benefits sought by breeders and agronomists and elucidate a model plant for studies to better understand the physiological mechanisms that may link these two antagonistic characters. Fraj et al. (2013) demonstrated previously that genotype PI248836 showed excellent growth and higher yield under salt stress, and thus support the results of this study. Safflower genotype, PI239707, had the lowest Δ value, therefore the highest WUE (7.1). This genotype could be an appropriate model plant species that can be employed to improve the WUE for high-yielding varieties. Furthermore, the possessing of substantial large root system might facilitate safflower plant to adapt under water scarcity and marginal environments that is a particular characteristic of arid regions.

Growing conditions and genotypic effects on N concentration

The salinity caused significant reduction in leaf N concentration and tolerant genotypes exhibit higher biomass and N% as compared to susceptible ones. The present results were agreed with previous reports from Hirel et al. (2007).

The N isotopes have the potential to provide integrated information for nitrogen fluxes, assimilation pathways, and allocation (Evans 2001). The present results demonstrate that there was minor difference among safflower genotype for N isotope composition (Table 2). Different reports indicate that abiotic stresses such as salinity and drought can either decrease (Handley et al. 1997; Robinson et al. 2000) or increase $\delta^{15}N$ (Ellis et al. 2002; Lopes and Araus 2006) relative to controls. Saini and Westgate (2000) narrated that some growth and physiological stages of safflower are salt and drought sensitive, especially at reproductive stage. Irrigation with saline water during all growth stages in general and at flowering and capitula development stage in particular was mainly responsible for lower grain yield. Consequently, reduction of grain yield showed the accumulation and toxicity of sodium at this stage. This indicates that high rate of genetic diversity exists among safflower genotypes for salinity tolerance. The decrease in yield and yield components in different safflower genotypes due to salinity, osmotic stress, and water deficiency has also been reported by Kar et al. (2007).

Correlation between agro-morphological traits, yield, and Δ

Improving WUE of crops is a way to increase their production under a given water supply condition (Richards et al. 2002). In the past, there was a misunderstanding for interpreting the correlation between **Δ** and yield that sometimes could lead to oversight data and wrong decisions. This technical cascade does not allow a successful breeding program for drought-prone and water-scarce regions (Hussain et al. 2016). Previously, there was confusion regarding Δ (or WUE) and yield parameters that might be positive, negative, and sometimes no correlation (Ngugi et al. 1996; Blum 2009; Condon et al. 2002). The correlations between various agro-morphological attributes and the relative yield of safflower genotypes at overall salinity are shown in Table 3. Branch number, capitula number, plant dry biomass, and harvest index showed significant ($p < 0.05$) correlations with the yield. Total dry biomass has the highest predictive value for the overall salt tolerance among the tested traits and showed highest correlation with the relative yield ($R^2 = 0.667$). The safflower heavily relied on harvest index values followed by total dry weight, while stem number and capitula number showed relatively low significant ($p > 0.05$) correlation with the yields. Significant positive correlations were detected among capitula number, harvest index, and grain yield; therefore, the genotypes with considerable plant height and total dry biomass expressed in saline environment will have high grain yield.

Conclusion

The carbon isotope discrimination traits demonstrate unique insights into biochemical and ecophysiological cascade of safflower growth and development. The large amplitude of variations (6‰) in Δ demonstrates the consequence of employing Δ as an excellent suitable criterion and a useful indicator for salinity stress of safflower genotypes and screens out high and stable yield. These results also demonstrate the useful contribution of *Ci/Ca* was encouraging regarding the possibility of using them as an effective selection index in safflower breeding programs. The results did not demonstrate a significant G × T interaction, because the response of most of the genotypes was similar and the one which was superior under low/no salinity was also superior under salinity stress. The safflower genotypes, PI248836 and PI167390, revealed high seed yield and were stable at stressed and non-stressed conditions. This study encourages us to investigate the relationship between Δ, iWUE, and seed yield for different architectures in more details and with a large set of genotypes.

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