

*Full Length Research Paper*

## **Selection of high yielding and stable safflower (*Carthamus tinctorius* L.) genotypes under salinity stress**

**Makram Belhaj Fraj<sup>1\*</sup>, Abdullah J. Al-Dakheel<sup>1</sup>, Ian R. McCann<sup>1</sup>, Ghulam M. Shabbir<sup>1,2</sup>, Ghazi Abu Rumman<sup>1,3</sup> and Abdul Qader Abdul M. Al Gailani<sup>1</sup>**

<sup>1</sup> ICBA, International Center for Biosaline Agriculture, Dubai, PO Box 14660, United Arab Emirates ;

<sup>1,2</sup> PAAFR, Public Authority of Agriculture and Fish Resources - Plant Resources, PO Box 21422 Safat, Code 13075, Kuwait

<sup>1,3</sup> ICT International-Australia, MENA Region Office

\* Corresponding author: [m.belhaj@biosaline.org.ae](mailto:m.belhaj@biosaline.org.ae)

### **Abstract**

Safflower is among the most tolerant cash crops and many genotypes could display high yield under high water irrigation salinity. The objective of this study was to investigate salinity tolerance on a large panel of safflower composed of 265 entries selected among the international collection. During 2002/2003, all entries were evaluated in pots under 10 dS m<sup>-1</sup> and only 52 entries were selected for field plot trials. Field plot trials were conducted over two consecutive cropping seasons (2003/2005) using three replications of irrigation water salinities corresponding to electrical conductivities of 10 and 15 dS m<sup>-1</sup>. During 2003/2004, 52 entries were evaluated and in 2003/2004, eight additional entries were added to the trials (total of 60 entries). All salinity levels, biological and grain yields and flower number averaged 6, 1.9 t ha<sup>-1</sup> and 300 flowers per meter square, respectively. Salinity reduced the latter variables by 50, 75 and 25%, respectively. Some genotypes maintained their biological and grain yields at 7 and 2 t ha<sup>-1</sup>, respectively. Relationships between yield components showed a threshold of 300 flowers under 15 dS m<sup>-1</sup>, which enabled many genotypes to display higher yields due to higher salinity tolerance during branching, flowering and grain filling stages compared to that of susceptible genotypes. Stability analysis using genotypic ecovalence parameter showed that 10 genotypes were adapted to high salinity and 20 genotypes were adapted to intermediate salinity. Based on yield building patterns and yield stability there were 13 genotypes that were selected out of the collection to constitute a nursery that is representative of the original collection over the whole range of variation for salinity response.

**Key words:** salt-tolerant genotypes, International collection, pot and field plot trials, nursery selection.

**Abbreviations:** G x E, genotype x environment interaction; GY, grain yield; BY, biological yield; FN, flower number

### **INTRODUCTION**

Cropping systems in which conventional crops are included become less profitable due to a significant reduction in yield. This occurs more often in irrigated agricultural fields where salinity increases constantly (Ayres and Westcott, 1976). The enhancement of crop diversity through the introduction of new salt-tolerant

crops is one of the management tools that can increase income for the farmer (Maas, 1986; Zohary and Hopf, 1993; Shannon, 1997; Shannon and Grieve, 1999; Steppuhn et al. 2005). Safflower (*Carthamus tinctorius* L.) is one of the most salt-tolerant cash crops (Li et al., 1993).

Safflower is underutilized economically important crop despite its usefulness purposes and adaptation to various marginal dryland growing conditions. Safflower can be grazed or stored as hay or silage (Bar-Tal *et al.*, 2008) and its feed value is similar to oats and alfalfa (Smith, 1996; Witchman, 1996). Most of the research achieved on safflower focused evaluation of safflower entries for disease resistance in context of intensive agricultural practices in temperate climate regions. Safflower is cultivated under the semi-arid conditions as an important oil yielding crop in many countries including India and Iran. The crop was ranked fourth among the salt-tolerant crops by Bernstein (1964).

A high genetic variation exists among the international safflower collection in terms of morpho-physiological traits (USDA Regional Plant Introduction, Station, Pullman, WA, USA; Zhang and Johnson, 1999). This crop has a variety of uses including food colouring and textile dye from the colourful flower petals, vegetable oil with low amounts of saturated fats from the grain, spice powder and animal feed from its vegetative parts (Witchman, 1996). Several genotypes are used in landscaping because of its high flower number and size (Knowles, 1969; McGuire *et al.*, 2012). Safflower can be grown under rain fed and irrigated conditions, which makes it suitable for inclusion in various cropping systems (Li *et al.*, 1993).

A safflower germplasm subset of 265 entries was assessed for salinity tolerance in the harsh environmental conditions of Dubai (UAE). This collection was firstly screened in pots, during 2002/2003, against flower weight, biomass and grain yield and flower number under water salinity of 10 dS m<sup>-1</sup>. About 20% of the initial collection was then selected for evaluation in field plot trials planted over two consecutive growing seasons (2003/2004 and 2004/2005). Field plot trials were irrigated using water with salinity levels of 10 and 15 dS m<sup>-1</sup>.

Previous studies that focused on correlation analysis between yield components showed that yield depended mainly on flower number and flower weight (Arslan, 2007). During this study a model of yield building was developed which was based on flower number since safflower is a crop with strong branching characteristics. Yield potential and the threshold for flower numbers of the tested genotypes were determined at each salinity level. Stability of yield parameters important for grain, forage and landscape purposes were determined to select entries that are representative of the initial collection. These entries will be used for more detailed ecophysiological studies and field trials under Dubai farming conditions. The current study will provide a base to promote safflower cultivation on a large scale in salt affected agro systems. In addition, genotypes that showed stable salt-tolerance during this study will be included in the safflower breeding programme for the development and release of salt-tolerant cultivars with acceptable characteristics for release onto the market.

## MATERIALS AND METHODS

### Safflower collection

Table 1 provides the 265 genotypes representative of the whole international collection (USDA Regional Plant Introduction, Station, Pullman, WA, USA) that were tested for yield production, quality (oil extraction) and landscape characteristics. These genotypes were chosen based on results of Jaradat and Shahid (2006) in terms of their phenotypic diversity. Experiments were conducted at the Experiment Station of the International Center for Biosaline Agriculture (ICBA), Dubai, United Arab Emirates (25° 13' and 55° 17'E).

### Pot trial

A pot trial was conducted in 2002/2003 and irrigated with water containing a water salinity of 10 dS m<sup>-1</sup>. All the 265 entries were sown in pots of 20 kg capacity. The substratum was composed of a mixture of 18 kg dune sand (Carbonatic, Hyperthermic Typic Torripsamment having a negligible level of inherent soil salinity 0.2 dS m<sup>-1</sup>) and 2 kg organic compost from cow manure (41% organic matter, 1.64% moisture, pH=7.7, C/N=16.5, 1.5% N, 1.65% K and 1.22% Na, Al Bayadir®, Jabel Ali, Dubai, UAE). One seed per pot was sown around November and irrigation was applied at rates equivalent to ET<sub>0</sub> plus 10% for leaching requirements.

The trial layout consisted of randomised complete block design which included three replications for each entry. The trial was harvested at maturity and the flower number (denoted by FN<sub>p</sub>) and biomass yield (denoted by BY<sub>p</sub>) were measured. About 20% of the initial collection was selected for evaluation in field trials.

### Field trials

Field trials were conducted during 2003/2004 and 2004/2005 cropping seasons at the Experiment Station of the International Center for Biosaline Agriculture (ICBA), Dubai, United Arab Emirates (25°13'N and 55°17'E). In the first cropping season, 52 entries were evaluated while in the second cropping season 60 entries (52 entries of the first season plus additional eight entries) were evaluated. The experimental station is located in an arid desert climate where temperatures are high and rainfall is negligible from April to November (Karim and Al-Dakheel, 2006). The soil is a Carbonatic, Hyperthermic Typic Torripsamment. Two salinity treatments were established, corresponding to irrigation water salinities of 10 and 15 dS m<sup>-1</sup>, denoted as S1 and S2 respectively. The S1 level correspond to the prevailing level in the farmer's fields of the Arabian Peninsula, while S2 is the maximum level recommended by the extension services for growing safflower. The 10 and 15 dS m<sup>-1</sup> irrigation

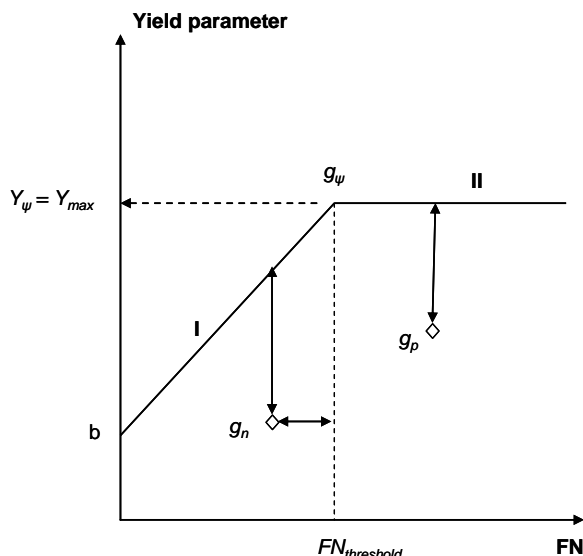
**Table 1.** Safflower collection tested for yield and its components. Genotype PI corresponding to codes used

Code	PI	Code	PI	Code	PI	Code	PI	Code	PI	Code	PI	Code	PI
1	167390	42	199894	82	226546	122	248798	162	250081	202	250717	244	543987
2	170274	43	199895	83	226993	123	248807	163	250083	203	250718	245	543990
3	173885	44	199896	84	235659	124	248808	164	250182	204	250719	246	543992
4	174080	45	199898	85	237538	125	248809	165	250183	205	250721	247	543994
5	175624	46	199902	86	237539	126	248810	166	250187	206	250822	248	543995
6	177302	47	199904	87	237541	127	248813	167	250188	207	250826	249	543998
7	181866	48	199907	88	237542	128	248814	168	250189	208	250827	250	543999
8	182165	49	199908	89	237544	129	248815	169	250190	209	250829	251	544009
9	183669	50	199910	90	237545	130	248833	170	250191	210	250830	252	544011
10	193473	51	199911	91	237546	131	248834	171	250193	211	250831	253	544012
11	193474	52	199912	92	237547	132	248836	172	250195	212	250833	254	544013
12	193475	53	199913	93	237548	133	248837	173	250196	213	250836	255	544014
13	193764	54	199914	94	237549	134	248838	174	250198	214	250837	256	544016
14	193765	55	199916	95	237550	135	248839	175	250199	215	250838	257	544017
15	195925	56	199918	96	237551	136	248840	176	250201	216	250839	258	544021
16	198293	57	199919	97	239042	137	248841	177	250202	217	250840	259	544024
18	198843	58	199920	98	239043	138	248844	178	250204	218	250924	260	544038
19	198844	59	199922	99	239226	139	248846	179	250205	219	250925	261	544047
20	198845	60	199923	100	239227	140	248848	180	250337	220	250926	262	544051
21	198990	61	199924	101	239706	141	248849	181	250338	221	251262	263	568782
22	199873	62	199926	102	239707	142	248851	182	250477	222	251264	264	568784
23	199874	63	199929	103	239708	143	248864	183	250479	223	251266	265	568785
24	199875	64	199932	104	240709	144	248868	184	250526	224	251267	266	568787
25	199876	65	199933	105	242418	145	248869	185	250528	226	251288	267	568788
26	199877	66	199934	106	243070	146	248872	186	250530	227	251289	268	568792
27	199878	67	199937	107	248356	147	248874	187	250531	228	251290		
28	199879	68	199938	108	248359	148	248875	188	250534	229	251291		
29	199881	69	199939	109	248360	149	248877	189	250537	230	251981		
30	199882	70	205179	110	248362	150	248880	190	250539	231	251984		
31	199883	71	208678	111	248363	151	249081	191	250595	232	251986		
32	199884	72	209280	112	248364	152	250000	192	250597	234	251988		
33	199885	73	209281	113	248377	153	250006	193	250600	235	251989		
34	199886	74	209294	114	248385	154	250007	194	250607	236	252040		
35	199887	75	209296	115	248386	155	250009	195	250608	237	253384		
36	199888	76	209297	116	248388	156	250010	196	250610	238	253385		
37	199889	77	209301	117	248620	157	250075	197	250709	239	253386		
38	199890	78	210834	118	248625	158	250076	198	250710	240	253387		
39	199891	79	212346	119	248626	159	250077	199	250714	241	278879		
40	199892	80	212886	120	248794	160	250078	200	250715	242	543976		
41	199893	81	214150	121	248795	161	250080	201	250716	243	543984		

salinities were accomplished by mixing highly saline groundwater (with EC<sub>w</sub> up to 25 dS m<sup>-1</sup>, SAR>26 mmol/l with Na and Cl concentrations higher than 190 meq/l and pH=7.6) with the 2 dS m<sup>-1</sup> water, which was the lowest saline water available (SAR=4 mmol/l with Na and Cl concentrations lower than 11 meq/l and pH=8.5). The two salinity levels were maintained constant throughout each season. Each salinity level was monitored twice a week using a portable EC meter (TetraCon® 325 Cond 197i,

WTW, USA). Irrigation was applied at rates equivalent to ET<sub>0</sub> plus 10% for leaching requirements. After harvest, all plots were irrigated at ET<sub>0</sub> plus 25% for additional leaching. All plot data were collected from the middle 1 m of the two central rows so as to avoid edge effects.

The trial layout consisted of a split-plot design with three replicates. The main factor was the salinity level and the sub factor was the entry tested. Prior to planting, the site was harrowed to ensure an even seedbed.



**Figure 1.** Safflower equation of yield parameter (biomass and grain yields or flower weight) building as a function of flower number (FN) for whole collection assessed.

Organic compost from cow manure was spread and incorporated at the rate of 10 tons  $\text{ha}^{-1}$ . Plot measuring 2 m x 4 m, (for a plot area of 8  $\text{m}^2$ ) were established and seeded manually with a row spacing of 0.5 m to enable manual weeding. An equal number of 128 seeds per entry were used since the germination rate from prior tests did not differ between entries. The plots were sown around mid November to avoid high temperatures and desiccating winds. N-P-K fertilizer (20-20-20%) was applied at a rate of 100  $\text{kg ha}^{-1}$  (Growfert Solub<sup>TM</sup>), corresponding to the recommended rate for the region. A drip irrigation system was used with a dripline for each row and an emitter spacing of 0.25 m.

Physiological maturity extended from late April to May. The plots were harvested at maturity to measure yields of biomass (BY) and straw (SY) at 0% moisture. Grain yield (GY) was measured at 15% moisture. Harvest index (HI) was calculated as  $\text{GY}/\text{BY}$ . All yields are expressed in units of tons per hectare. Flower number (FN) and weight (FW) were recorded for each sample. Reference value of a given yield parameter (biomass yield, flower weight or flower number) is determined by the maximal value recorded for the whole safflower collection.

The relationship of flower number (FN) to a given yield parameter showed a boundary line beyond which the data do not extend. This boundary line is characterized by three parameters (Figure 1): (1) potential yield denoted by  $Y_\psi$ , which is the maximum value of yield parameter, only reached when FN is low; (2) FN threshold, beyond which the boundary Y decreases; and (3) Y, which reaches its maximum value at FN x boundary Y.

$Y = a \times x + b$ , when  $\text{FN} < \text{FN}_{\text{threshold}}$ ,  
with  $Y = Y_{\text{max}}$  when  $\text{FN} \geq \text{FN}_{\text{threshold}}$ ;

Where  $a$  is the slope of the ascendant phase and  $b$  is the constant at FN equal to 0.

This equation allowed identification of high potential entries out of the 265 entries and to select representative subset of entries.

Reduction of yield parameter to potential denoted by RY was defined as following:

$$\text{RY} = \frac{Y_\psi - Y}{Y_\psi} \times 100,$$

with  $Y_\psi = Y_{\text{max}}$  when  $\text{FN} \geq \text{FN}_{\text{threshold}}$ ;

This parameter quantifies the effect of salinity factor on percent yield decrease.

Similarly, reduction of flower number to threshold was calculated as following:

$$\text{RFN} = \frac{\text{FN}_{\text{threshold}} - \text{FN}}{\text{FN}_{\text{threshold}}} \times 100,$$

with  $\text{RFN} = 0$  when  $\text{FN} \geq \text{FN}_{\text{threshold}}$ ;

This parameter was used as environmental covariate for distinction between levels of the salinity factor and to estimate salinity tolerance index.

Vertical dashed line represents the flower number threshold and the horizontal dashed line represents the yield potential value of the maximal yield displayed by the top-performing entry. Values beyond this plateau represents yields likely limited by thousand kernel weight due to limiting factor during grain filling period.

I, growing phase where yield was limited by FN that was lower than threshold value;

II, plateau phase where yield did not increase when FN exceed threshold value. This phase showed effect of inter-competition for assimilates;

$Y_\psi$ , correspond to potential values of Y following potential boundary curve

$Y_{\text{max}}$ , maximal value of Y reached by a potential genotype

$g_\psi$  representing the top performing of the collection.

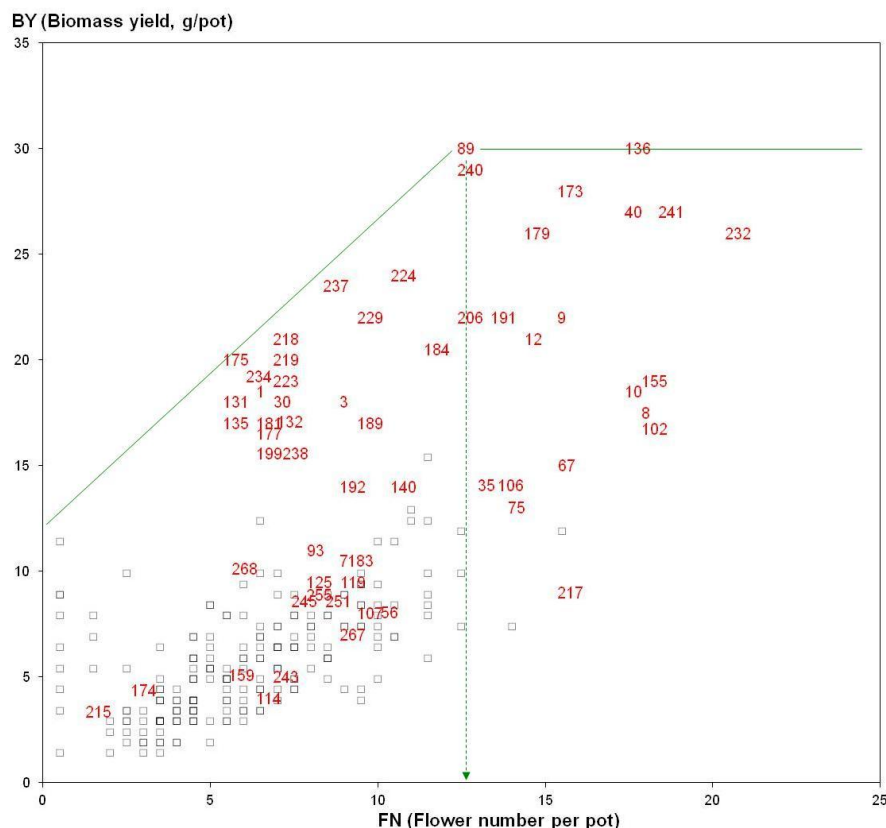
$g_n$ , genotype displaying yield lower than potential curve due to reduction of FN to threshold

$g_p$ , genotype displaying yield lower than potential curve due to limitation of grain weight during grain filling period

## Statistical Analyses

Note that 'block' stand for any replication. Statistical analyses were performed in three stages:

(1) Analysis of variance (ANOVA) was done according to split-plot design. The 265 total entries for the pot trial grown in 2002/2003, and 52 and 60 total entries for the field trials grown in 2003/2005 seasons, were compared using Fisher's protected LSD test at the  $P < 0.05$  level.



**Figure 2.** Relationships between biomass yield (BY, g/pot) and flower number per pot (FN) of the 265 assessed genotypes at discriminating salinity level  $S1=10 \text{ dS m}^{-1}$  applied during first screening season. Boundary curve and thresholds for BY and FN determined for the entire safflower collection.

Y, genotype not selected for field evaluation.

Vertical dashed arrow symbolized flower number threshold

(2) Principal component analysis (PCA) was performed on trait means recorded at each salinity level in order to cluster the tested entries according to their end-uses purposes, either grain, forage, or both (dual use), and landscape for each salinity level. Note that end-use ability of an entry was determined using its loadings on the two components of PCA at each salinity level.

(3) Stability assessment aims at characterization of the observed yield variation for each entry under different salinity levels. The more stable an entry is, the lower will be its yield variation with salinity. Stability was estimated using genotype ecovalences (von Wricke, 1962) and computed for each entry of the selected entries. Ecovalence describes stability type 2 (Lin et al., 1986) in which stable genotypes respond as a parallel line to the mean of all tested genotypes. This parameter quantifies genotype  $\times$  environment interaction. Higher values of ecovalence mean lower stability. Quantitative estimation of stability was achieved on 51 entries common to the two year field experiment (2003/2004 and 2004/2005). All analyses were performed with SAS Software System

Version 6.1 (SAS Institute, 1990, Cary, NC, USA) using GLM procedure and FACTOR procedure, respectively.

## RESULTS AND DISCUSSIONS

### Pot trial of the entire collection of 265 entries

Averages of FN and BY were estimated to be equal to 6 flowers per pot and 7 g/pot, respectively. Reference value of FN equal to 12.5 was obtained by a single boundary curve (Figure 2). Data collected for all entries showed potential value of BY equal to 30 g/pot. Yield potential was obtained by entries 89 and 36. Criteria measured in the current trial were the reduction of biomass yield and threshold of flower number. Reduction of FN from the reference value (threshold value) estimated intensity of the effect of salinity as a main yield limiting factor acting in the present experiment. Reduction of FN and BY varied from 0 to 90%, thus showing the efficiency of selection pressure applied which was the discriminating

irrigation water salinity equal to  $10 \text{ dS m}^{-1}$ . Reduction of FN was due to salinity limiting factor that was the most intense before flowering stage. Accordingly, tolerant entries were those less affected by salinity stress during branching compared to susceptible entries experiencing high reduction in ramification. As consequence, tolerant entries produced higher FN and eventually higher grain number compared to the susceptible entries. This is consistent with the literature as salinity has been reported as having diverse effects on safflower. Salinity reduces germination and lower transpiration rate resulting in reduction in plant density up to 64% (Feizi et al., 2010). Plants grown under salinity have hastened flowering and maturation because of Na toxicity and nutrient deficiencies. In addition, flower number and grain number per head are reduced for genotypes characterized by low earliness to flowering (Kafka and Kearney, 1998). Salinity is often accompanied by other soil properties, such as sodicity, alkalinity, or boron toxicity influencing plant growth (Munns, 2002). Soil salinity of  $7 \text{ dS m}^{-1}$  in arable layers reduced safflower yield by 12.5% (Francois and Bernstein, 1964). This reduction was twice higher when salinity reaches  $11 \text{ dS m}^{-1}$ . However, Ayers and Westcott (1976) showed yield reduction of 50% when soil salinity was equal to  $10 \text{ dS m}^{-1}$ . In Experiments in Isfahan province (central Iran) safflower shoots yield was decreased 44.2 and 71.1 % in 8.8 and  $11.2 \text{ dS m}^{-1}$  treatments, respectively, as compared to  $3.4 \text{ dS m}^{-1}$  (Feizi et al., 2010).

Soil salinity depressed the seed contribution to total yield of the heads (Yermanos et al., 1964). Effects of seven salinity levels (0 to 2% NaCl) on germination of three safflower varieties were determined under controlled temperature ( $30 \pm 2 \text{ C}$ ) and salt tolerant genotypes were identified (Ghorashy et al., 1972).

The present study showed that identified top performing entries were those close to the boundary curve and that displaying flower number higher than the threshold value. Selected entries were among those having FN higher than the threshold. In addition, among the lowest yielding genotypes there was only 8% that were selected as checks.

### Field trials of the selected entries in 2003-2005

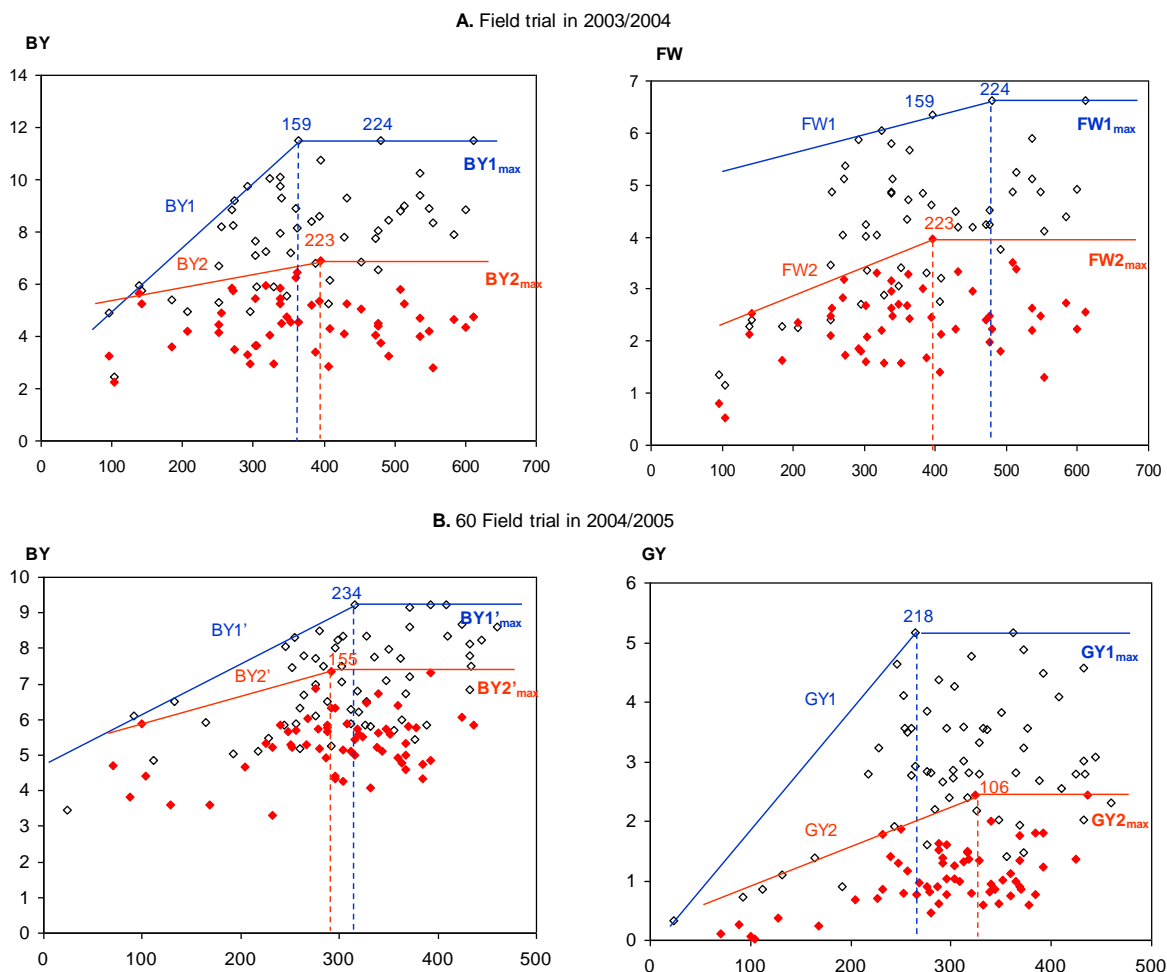
Average FN was equal to 300 flowers per meter square. Flower number varied from 20 to 620 which highly influenced variation of FW, BY and GY (Figure 3). The shape of all relationships were similar with ascendant phase showing increase of yield when flower number increase until a threshold value beyond which yield did not increase showing a plateau. Accordingly, maximal BY recorded can be considered as potential value obtained. Relationships between FN and yields established at 10 and  $15 \text{ dS m}^{-1}$  showed different potentials and thresholds.

During 2003/2004 season where 52 safflower entries were grown, potential values of BY and FW were equal to

11.5 and  $6.6 \text{ t ha}^{-1}$ , respectively. These values decreased on average by 40% when salinity reached  $15 \text{ dS m}^{-1}$ . Threshold of FN varied from 364 to 480 showing that beyond these values several top yielding entries were identified. The best entry at intermediate salinity level was 159 and at high salinity level was 223. Salt-tolerant top performing entries at  $15 \text{ dS m}^{-1}$  displayed FN threshold value 8% higher than that obtained at  $10 \text{ dS m}^{-1}$ . Consequently, genotypic differences for salinity tolerance were established before flowering stage and 35% of the collection was considered tolerant to salinity. Tolerant entries displayed FN higher than thresholds can achieve BY higher than  $4 \text{ t ha}^{-1}$ . Entries adapted to intermediate salinity were identified as those having FN higher than threshold and BY and FW higher than plateau obtained at high salinity level. Values on the plateau were equal to 6.8 and  $4 \text{ t ha}$  of BY and FW, respectively. Besides, threshold of FN under high salinity level obtained in relation to FW was lower compared to that recorded under intermediate salinity level. Consequently, salt-tolerant entries reached potential FW at low FN showing high grain filling activity. Even FN was curtailed by salinity, therefore BY would be compensated by higher grain weight. Indeed, best performing entries for BY were the same for FW.

During 2004/2005 season where 60 entries were grown, potential values of BY and GY were equal to 9.2 and  $5.1 \text{ t ha}^{-1}$ , respectively. When salinity reached  $15 \text{ dS m}^{-1}$ , these values decreased on average by 15% and 70%, respectively. Consequently, salinity limiting factor influenced the highest grain formation than ramification. Action of salinity factor continued after flowering and caused most probably flower sterility. Indeed, FN threshold value obtained for GY building was higher than that for BY building at high salinity level. Consequently, top performing entries at high intensity of salinity stress might build 18% more flowers in order to sustain its grain yields. This fact means that selection of salt-tolerant entries for BY was easier than that for grain yield. Threshold of FN varied from 250 to 450 showing that beyond these values top yielding entries were identified. The best entries at intermediate salinity level were 155 and 106 and at high salinity level were 234 and 218 for BY and GY, respectively. Genotypic differences for salinity tolerance were established after flowering stage and 33% of the collection was considered tolerant to salinity. Tolerant genotypes displayed FN higher than thresholds can achieve BY higher than  $4.5 \text{ t ha}^{-1}$ . Entries adapted to intermediate salinity were identified as those having FN higher than threshold and BY and GY higher than plateau obtained at high salinity level.

Salinity tolerance was mainly estimated at plant germination and emergency stages; however safflower yield can be highly reduced after these stages due to irrigation water salinity increase during branching. Safflower is a branching plant so model of yield building can be considered thistle-like herbaceous annual crops in term of compensation of low plant density by increased



**Figure 3.** Determination of flower number (FN) thresholds for biomass yield (BY, t ha<sup>-1</sup>), flower weight (FW, t ha<sup>-1</sup>) and grain yield (GY, t ha<sup>-1</sup>) of safflower genotypes under intermediate (in blue) and high salinity levels (in red).

branching after stem elongation stage (Emongor, 2010). As consequence, final yield could be highly related to flower number and grain number per flower (Mündel et al., 1992). In marginal conditions where drought and irrigation water salinity reached their maxima in post-flowering period, grain yield is related the highest to grain number which is in turn closely related to flower number. So be it, the 9-point scale based on ability for germination and emergency as recommended by IBPGR is not sufficient to estimate salinity tolerance for field extension of economically feasible cultivation. Improvement of safflower yield stability in these harsh environments would be achieved through the selection of genotypes displaying high number of heads (capitula) per plant strongly linked to field yield in safflower.

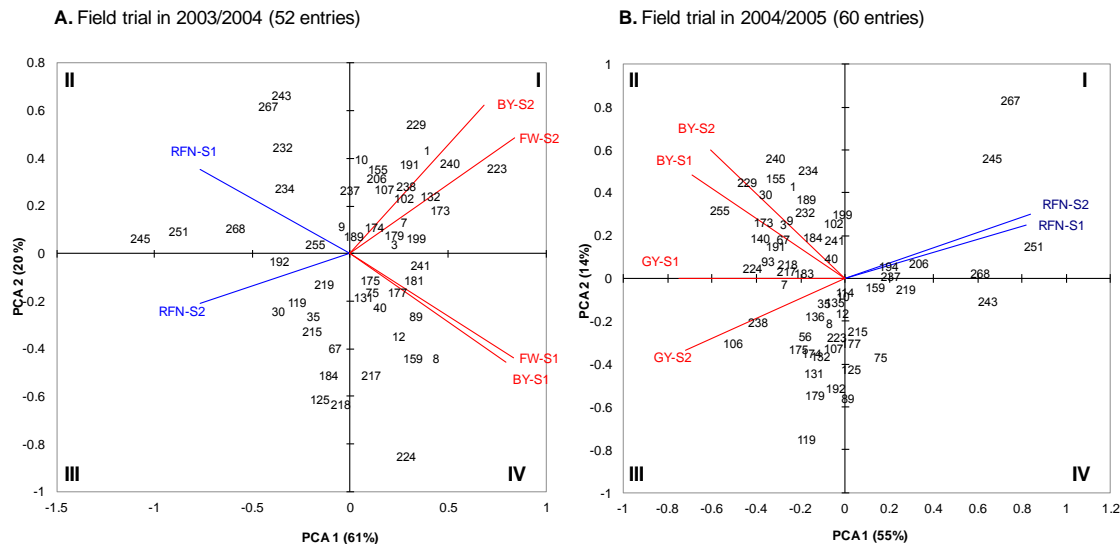
Potential relationships assumed were asymptotically equal to the following equations where, x was a given value of flower number per meter square.

*During 2003/2004 season:*

$BY1 = 0.2509 \cdot x + 2$  when  $FN < FN_{threshold}$ , and  $BY1 = BY1_{max} = 11.518$  when  $FN \geq FN_{threshold} = 364$ ;  
 $BY2 = 4.9 \cdot 10^{-3} \cdot x + 5$  when  $FN < FN_{threshold}$ , and  $BY2 = BY2_{max} = 6.916$  when  $FN \geq FN_{threshold} = 396$ ;  
 $FW1 = 6.5 \cdot 10^{-3} \cdot x + 4.2$  when  $FN < FN_{threshold}$ , and  $FW1 = FW1_{max} = 6.628$  when  $FN \geq FN_{threshold} = 480$ ;  
 $FW2 = 5.7 \cdot 10^{-3} \cdot x + 1.75$  when  $FN < FN_{threshold}$ , and  $FW2 = FW2_{max} = 3.962$  when  $FN \geq FN_{threshold} = 396$ ;

*During 2004/2005 season:*

$BY1' = 10^{-2} \cdot x + 6$  when  $FN < FN_{threshold}$ , and  $BY1' = BY1'_{max} = 9.224$  when  $FN \geq FN_{threshold} = 316$ ;  
 $BY2' = 8.7 \cdot 10^{-3} \cdot x + 5.2$  when  $FN < FN_{threshold}$ , and  $BY2' = BY2'_{max} = 7.556$  when  $FN \geq FN_{threshold} = 292$ ;  
 $GY1 = 2 \cdot 10^{-2} \cdot x - 0.2$  when  $FN < FN_{threshold}$ , and  $GY1 = GY1_{max} = 5.155$  when  $FN \geq FN_{threshold} = 264$ ;  
 $GY2 = 7 \cdot 10^{-3} \cdot x + 0.4$  when  $FN < FN_{threshold}$ , and  $GY2 = GY2_{max} = 2.448$  when  $FN \geq FN_{threshold} = 324$ ;



**Figure 4.** Bi plots of principal component analyses of the variates: biomass yield (BY,  $\text{t ha}^{-1}$ ), flower weight (FW,  $\text{t ha}^{-1}$ ), grain yield (GY,  $\text{t ha}^{-1}$ ) and reduction in flower number (RFN, per meter square) with levels indexed by S1 and S2 corresponding to salinity levels of  $10 \text{ dS m}^{-1}$ ,  $15 \text{ dS m}^{-1}$ , respectively; and the entries evaluated during two growing seasons 2003/2004 and 2004/2005. Projection of 52 and 60 entries on two axes: first component (PCA 1) and second component (PCA 2) during 2003/2004 and 2004/2005 seasons, respectively.

## Multivariate analyses

The correlation between the traits related to forage and grain yields in field trials (2003/2004 and 2004/2005 seasons) were analysed using two PCA, resuming more than 69% of the variation enabling the entries to be clustered into significantly different groups according to their end-uses (Figure 4). Genotypes close to variates GY and FW are more suitable for grain production purpose end-use and those close to RFN are the lesser suitable for landscape purpose end-use. Biomass yield was the highest correlated with FW compared to GY.

For 2003/2004 season, axis 1 (first component), accounting for 61% of the variation was influenced by reduction of FN to threshold (RFN-S1 and RFN-S2, see Figure 4-A). Biplot orders the best grain yielding entries with positive coordinates (quadrants I and IV) inversely to the low yielding entries having negative coordinates for both principal components (quadrant III). On the right side of the biplot, entries on the top quadrant (I) have high adaptation to  $15 \text{ dS m}^{-1}$ . In the bottom of the right side of the biplot (quadrant IV) were distinguished the genotypes having highest performance at  $10 \text{ dS m}^{-1}$ . Note that two entries 251 and 245 displayed weakness for all end-use purposes.

During 2004/2005 season, average yield was 25% curtailed due to higher heat and drought stresses (data not shown). In this condition, grain and biological yielding genotypes were easily distinguished. axis 1 accounting for 55% of the variation was influenced by GY and RFN,

whereas axis 2 was influenced by BY (Figure 4-B). PCA indicates that BY was the highest correlated with GY than with RFN and entries loadings showed a significant dimorphism between grain and forage end-use purposes. Biplot orders the best yielding entries with negative coordinates (quadrants II and III) inversely to the low yielding entries having positive coordinates for both principal components (quadrants I and IV).

Entries having loadings comprised between -0.1 and +0.1 on axis 1 and localized in quadrants III and IV were destined for landscape purpose end-use because reduction of FN to threshold was low and that in BY and GY were intermediate. Note that genotypes 251, 245, 243 and 268 displayed weakness for all end-use purposes. Otherwise, 23% of the collection tested showed contrasted loadings for the two PCA. Indeed, coordinates of these genotypes (8, 10, 30, 67, 107, 132, 159, 192, 206, 218, 223 and 255) were contrasted for the two PCA suggesting their low inter-year response variability.

## Stability analyses

Yield potential obtained in 2004/2005 season was 20% lower to that of 2003/2004 season due to higher drought and heat limiting factors. During 2003/2004 season, the 52 entries showed FW varying from 0.5 to  $6.6 \text{ t ha}^{-1}$  and BY varied from 2 to  $11.5 \text{ t ha}^{-1}$ , however during 2004/2005 season, BY of the 60 entries ranged from 3 to



9.2 t ha<sup>-1</sup> and GY varied between 0 and 5.1 t ha<sup>-1</sup> (see parameters of boundary curves in Figure 3). This fact means that when yield was highly reduced by additional limiting factors to salinity, tolerant entries build their yields based on higher development speed allowing lower number of flowers but better grain filling contrary to more favourable year where salt-tolerant entries build their yield based mainly on branching and flowering. Biomass yield, FW and GY were dependent to values obtained for FN.

Biomass yield and FN exhibit very different interaction ratios as shown by percentage of the genotypic contribution to G x E interaction reported in Figure 5. The interaction for BY and FW was as high as that for FN. However, the interaction was the lowest for GY. Entries responsible for the interaction for BY differed from those responsible for the interaction for FN. This suggested that there were different genotypic factors related to stability of yield and its components. The two genotypic factors were most likely segregated and observing an entry characterized by stability for both characters is scarce. The forage-type accessions, with profuse branching and long vegetative growth stage, were the most tolerant to salinity (Jaradat and Shahid, 2006). Li and Mündel (1986, 1996) reported that the average among 2039 accessions evaluated at Beijing was 20 capitula per plant. More than 50 capitula per plant were produced by 33 accessions from 14 countries and the maximum recorded was equal to 90 heads per plant. Our results showed that potential biological yield can be obtained with 17 heads per plant. Selected genotypes displayed flower number higher than 270 per m<sup>2</sup> and were potentially useful for crop production targeting 7 and 2.5 t ha<sup>-1</sup> of biological and grain yields under high salinity, respectively. Bassil and Kaffka (2002) research finding is similar to our results. Francois and Bernstein (1964); Yermanos *et al.* (1964) and Irving *et al.* (1988) concluded that 60% reduction in number of capitula (flower heads) per plant and seed number per capitula caused yield losses affecting dramatically cost-effectiveness of the crop.

Entries responsible for FN interaction with environment (salinity x year) were 8, 89, 179, 177 and 255, and those responsible for BY interaction were 224, 159, 30, 245, 223 and 234. Entry 224 accounted the highest for interaction sum of squares, whereas entries 240 and 238 accounted the less to this interaction term for BY. In contrast, 41% of the entries slightly interact for both criteria and displayed percent ecovalence lower than 2%.

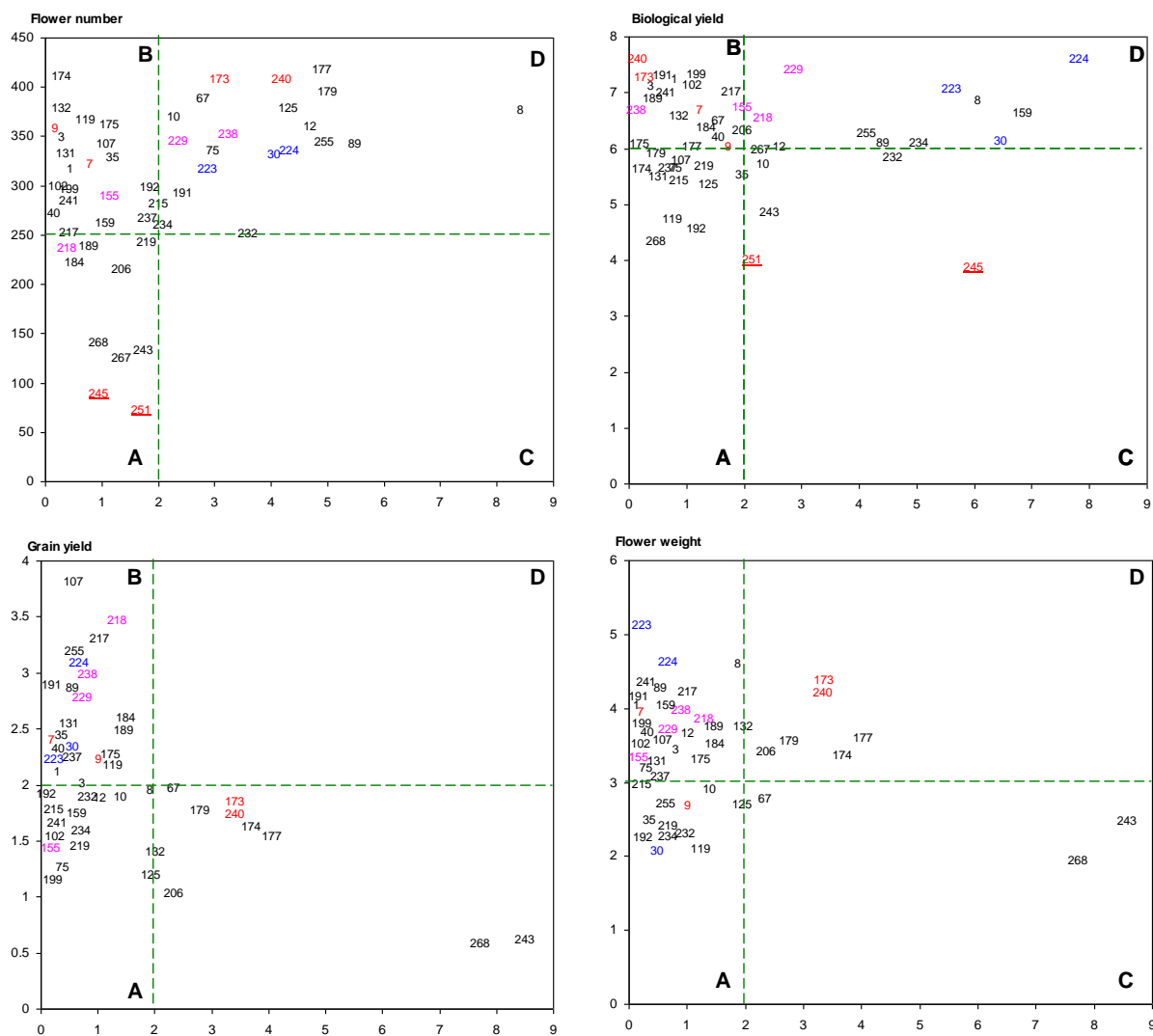
**Solid lines correspond to the variates.**

**Individuals of the PCA are the genotypes indicated by their corresponding codes (see Table 1).**

High yielding entries were those displaying FN higher than 250 (minimum threshold obtained for yield building)

and BY higher than 6 t ha<sup>-1</sup> (minimum potential value obtained at high salinity level). Simultaneous use of yield potential at high salinity level and minimum FN threshold, and entry ecovalence threshold allowed clustering the tested collection to four genotypic groups (denoted by A–D, Figure 5). These clusters were determined for flowering capacity and yielding, separately. Concerning landscape purpose end-uses, low flowering entries having low ecovalence were specifically adapted to high salinity (20% of the collection, cluster A) and those displaying high ecovalence were not adapted whatever the environment of production (no entry, cluster C). In contrast, high flowering entries displaying low ecovalence were characterized by wide adaptation (41% of the collection, cluster B) and those having high ecovalence were specifically adapted to intermediate salinity (39% of the collection, cluster D). The most interesting entries were those belonging to cluster A and B. Concerning grain purpose end-use, low yielding entries having low ecovalence were specifically adapted to high salinity (20–30% of the collection, cluster A) and those displaying high ecovalence were not adapted whatever the environment of production (10–20% of the collection, cluster C). In contrast, high flowering entries displaying low ecovalence were characterized by wide adaptation (32% of the collection, cluster B) and those having high ecovalence were specifically adapted to intermediate salinity (27% of the collection, cluster D).

Combining flowering and yielding abilities, entries displaying dual purpose end-use belonging to cluster A and B were among the most interesting. These genotypes represented 15% of the collection. A single landscape entry 268 was specifically adapted to unfavorable environments. Whereas, entries displaying dual purpose end-use and widely adapted to all environments were 1, 3, 7, 40, 102, 132, 199, 217 and 237. The selection of a nursery among the tested collection was achieved with the respect of representativeness of each group of end-use. Selection was preferentially achieved on entries having firstly high end-use potential for dual-purpose end-use; secondly we selected entries having high GY; and finally those having high landscape end-use. In addition, selection of the nursery takes also in consideration clusters of genotype stability. The identified entry clusters could serve as parents in breeding programs. Narkhede and Patil (1987) also reported that this character contributed most to a heterotic effect in 17 crosses of safflower. Number of primary and secondary branches was the next most important contributor to the heterotic effect. Most of the characters studied appeared to be controlled by non-additive gene action with a degree of over dominance. Correlated responses in various crosses showed that selection for capitula per plant was effective for the improvement of yield (Patil *et al.*, 1994). Capitula per plant seemed to be controlled by four groups of genes in a 10-parent incomplete diallel cross (Gupta and Singh



**Figure 5.** Entry repartition according to ecovalence and mean flower number, biological yield, grain yield and flower weight. A, genotypes specifically adapted to unfavorable environments (specific adaptation). B, genotypes generally adapted to all environments (wide adaptation). C, genotypes not adapted to all environments. D, genotypes specifically adapted to favorable environments (specific adaptation).

1988), with mainly non additive gene action. However, additive gene action controlled the number of primary branches. In studying yield-related traits over six generations, Narkhede *et al.* (1987) showed that dominance effects were predominant for capitula per plant (and for branches per plant) and duplicate epistasis was evident for all characters studied. These authors recommend reciprocal recurrent selection for the improvement of safflower yields.

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### References

- Arslan B (2007). The path analysis of yield and its components in safflower (*Carthamus tinctorius* L.), Journal of Biological Sciences 7(4): 668–672.
- Ayres RS, Westcott DW (1976). Water quality for agriculture. Irrigation and Drainage Paper 29, FAO Rome.
- Bar-Tal A, Lndau S, Lin-Xin Z, Markovitz, T, Keinan M, Dvash L, Brener S, Weinberg, ZG, 2008. Fodder quality of safflower across an irrigation gradient and with varied nitrogen rates.

- Agron. J., 100: 1499–1505.
- Bassil ES, Kaffka SR (2002). Response of safflower (*Carthamus tinctorius* L.) to saline soils and irrigation II. Crop response to salinity. Agr. Water Manage. 54: 81–92.
- Bernstein L (1964). Salt tolerance of plants; Agric. Inf. Bull No. 283 US Dept. Agric., Washington DC
- Emongor V (2010). Safflower (*Carthamus tinctorius* L.) the underutilized and Neglected Crop: A review. Asian Journal of Plant Science 9(6): 299–306.
- Feizi M, Hajabbasi MA, Mostafazadeh-Fard B (2010). Saline irrigation water management strategies for better yield of safflower (*Carthamus tinctorius* L.) in an arid region. Australian Journal of Crop Science 4(6): 408–414.
- Francois LE, Bernstein L (1964). Salt tolerance of safflower. Agron. J. 56: 38–40.
- Ghorashy SR, Sionit N, Kheradnam M (1972). Salt tolerance of safflower varieties (*Carthamus Tinctorius* L.) during germination. Crop science 4: 310–315.
- Gupta RK, SB Singh (1988). Diallel analysis for seed yield, oil content and other economic traits in safflower (*Carthamus tinctorius* L.). Genetika-Yugoslavia 20:161–173.
- Irving DW, Shanon MC, Breda VA, Mackey BE (1988). Salinity effects on yield and oil quality of high-linoleate and high-oleate cultivars of safflower (*Carthamus tinctorius* L.). J. Agric. Food Chem. 36: 37–42.
- Jaradat AA, M Shahid (2006). Patterns of phenotypic variation in a germoplasm collection of *Carthamus tinctorius* L. from the Middle East. Genetic Resources and Crop Evolution 53: 225–244.
- Kaffka SR, Kearney TE (1998). Safflower production in California. University of California, Agriculture and Natural Resources Publication 21565, Oakland, p. 29.
- Knowles PF (1969). Centers of plant diversity and conservation of crop germoplasm: Safflower. Econ. Bot. 23: 324–329.
- Lin CS, Bains MR, Lefkovitch LP (1986). Stability Analysis: Where Do We Stand? Crop Science 26: 894–900.
- Li D, Mündel HH (1996). Safflower *Carthamus tinctorius* L. Promoting the conservation and use of underutilized and neglected crops. 7. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.
- Li D, Mingde Z, Rao VR (1993). Characterization and Evaluation of Safflower Germplasm. Geological Publishing House, Beijing, 260 pp.
- Maas EV, 1986: Salt tolerance of plants. Appl. Agric. Res. 1: 12–26.
- McGuire PE, Damania AB, Qualset CO (2012). Safflower in California. The Paulden F. Knowles personal history of plant exploration and research on evolution, genetics, and breeding. Agronomy Progress Report No. 313, Dept. of Plant Sciences. University of California. Davis CA USA. 44p.
- Mündel HH, Blackshaw RE, Byers JR, Huang HC, Johnson DL, Keon R, Kubik J, McKenzie R, Otto B, Roth B, Stanford K (1992). Safflower Production on the Canadian Prairies: revisited in 2004. Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, Lethbridge, Alberta T1J 4B1
- Munns R (2002). Comparative physiology of salt and water stress. Plant, Cell and Environment 25: 239–250.
- Narkhede BN., Patil, AM (1987) Heterosis and inbreeding depression in safflower. J. Maharashtra Agric. Univ. 12(3): 337–340.
- Narkhede BN, Patil AM, Deokar AB (1987). Gene action of some characters in safflower. J. Maharashtra Agric. Univ. 17(1): 4–6.
- Patil VD, Reddy MVS, Nerkar YS (1994). Efficiency of early generation selections for yield and related characters in safflower (*Carthamus tinctorius* L.). Theor. Appl. Genet. 89: 293–296.
- SAS Institute (1990). SAS/STAT User's guide. Vol 1 and 2, Version 6, 4th edn. SAS Institute Cary, NC, USA.
- Shannon MC (1997). Adaptation of plants to salinity. Advances in Agronomy 60: 87–120.
- Shannon MC, CM Grieve (1999). Tolerance of vegetable crops to salinity. Scientia Horticulturae 78: 5–38.
- Smith JR (1996). Safflower, 1st Edn, AOCS Press, USA, pp 624.
- Steppuhn H, Van Genuchten MT, Grieve CM (2005). Root-zone salinity. I. Selecting a product-yield index and response functions for crop tolerance. Crop Science 45: 209–220.
- USDA-ARS (2005). George E. Brown Jr Salinity Laboratory, Riverside, CA, USA (<http://www.ars.usda.gov/Services/docs.htm?docid=8908>).
- Von Wricke G (1962). Field trials on a method for the detection of environmental stray into wide. Z Pflanzenzucht 47: 92–96.
- Witchman D (1996). Safflower for forage. Proceedings of North American Safflower Conference. Jan 17-18, Great Falls, Montana, Lethbridge, Canada, pp 56–60.
- Yermanos DM, Francois LE, Bernstein L (1964). Soil Salinity Effects on the Chemical Composition of the Oil and the Oil Content of Safflower Seed, Agron. J. 56: 35–37.
- Zhang Z, Johnson RC (1999). Safflower germplasm collection directory. IPGRI Office for East Asia, Beijing, China.
- Zohary D, Hopf M (1993). Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Oxford: Clarendon Press.