



### RESEARCH ARTICLE

#### EVALUATION AND POOLING OF SAFFLOWER (*Carthamus tinctorius* L.) ACCESSIONS FROM DIFFERENT WORLD ORIGINS USING AGRO-MORPHOLOGICAL TRAITS.

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#### Abstract

Safflower (*Carthamus tinctorius* L.) is a minor hardy crop well adapted to semi-arid conditions, which could be considered as alternative oilseed crop in climate change context. Evaluation of broad genetic base germplasm under such particular conditions is a crucial phase in breeding program. During 2013-2014, a total of 62 accessions from different origins of the world were evaluated in the INRA experimental station, at Douyet, for morphological, agronomic, pathological and technological traits. Results showed large variability among these accessions for spinelessness level, earliness, leaf rust resistance, thousand seed weight, seed yield per plant and seed oil content. Seed oil content ranged from 22.10 to 38.77%, with an average of 28.26%. Average seed yield is about 2 t/ha, and the highest seed yield ever observed was 4.490 t/ha. Presence of high genetic variability for agro-morphological, pathological and technological traits would ensure the effectiveness of breeding and selecting performant safflower varieties. The accessions studied were grouped into six groups based on desired traits: seed yield per plant, seed oil content, spineless, flowering earliness, resistance to leaf rust and resistance to broomrape. These groups will be considered as genetic pools and selected genotypes from each pool will be used as elite parents in our safflower hybridization program. The findings of this research would be useful for safflower breeding programs in Morocco as well as in other countries of the world.

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#### Introduction:-

Due to their importance in Moroccan cropping system and food security challenge in vegetable oils, oilseed crops are considered among the priority agricultural sectors in the country (Houmanat et al., 2016). Safflower is one of the oilseed crops that have some agronomic advantages, such as drought resistance and adaptation to arid and semiarid climatic conditions (Weiss, 2000). Safflower is among the rustic oilseed crops well adapted to Moroccan environmental conditions, especially in a climate change context, exhibiting a good potential in semiarid areas (Nabloussi and Boujghagh, 2006). Furthermore, this crop possesses considerable diversity across different regions

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of the world (Amini et al., 2007). In comparison with other oilseed crops, safflower is characterized by high adaptation ability in regions where winter months are cool and summer months are arid (Erbas et al., 2016).

About 734.000 tons of safflower are produced in an area of 937.000 ha worldwide. Mexico, USA, Kazakhstan, India and Argentina account for approximately 95% of the world's safflower production (FAOSTAT, 2014). Safflower is a multi-purpose plant cultivated for edible oil, birdseed, spices, dye of its flowers, medicinal and ornamental use (Mündel et al., 1992; Johnston et al., 2002).

In Morocco, safflower cultivation, for oil production, was initiated in 1965 but has been abandoned since 1992, mainly for commercial reason. In fact, the production sale prices were not fixed and guaranteed as was the case for sunflower and rapeseed. In addition, during safflower cultivation period, seed yield and oil content remained very low due to the lack of adapted cultivars and poor management techniques used by farmers (Nabloussi and Boujghagh, 2006). Recently, the Moroccan Ministry of Agriculture has called for the restart and development of safflower through a global action plan. In this context, a new safflower breeding program was launched. The objective is to release new spineless and productive safflower varieties with high technological value characterized by shorter vegetative cycle and higher seed yield and oil content than the existing varieties. Only three varieties have been registered, two of them are spineless, and could produce a quite high seed yield between 1.60 and 2.00 t/ha (Nabloussi and Boujghagh, 2006). However, all these varieties have a low oil content < 30%. The expected varieties should have oil content close to 40%. Furthermore, they must be early to avoid a late season drought and spineless to be suitable for traditional management practices, such as hand-harvesting.

A world safflower collection, comprising more than 200 accessions, was introduced to constitute the initial germplasm for our breeding program. A preliminary study was carried out to evaluate the adaptation and performance of those accessions under Moroccan conditions (Nabloussi et al., 2008). Among this material, 61 accessions were selected for further evaluation in other environments. The present study was conducted to assess the genetic diversity of 61 safflower genotypes from different geographic origins in the world, using agromorphological and technological traits during two seasons.

## Materials and Methods:-

### Plant material:-

The plant material used in this research consisted of 61 accessions previously selected among a world safflower collection provided by the Department of Agriculture and Research, Agricultural Service in the United States (Nabloussi et al., 2008), and a cultivar from Spain, Namely 'Rancho', provided by CSIC, IAS-Cordoba, and used as a check. All these accessions were planted during two seasons, 2013 and 2014, at the INRA Experimental Station of Douyet. Table 1 summarizes the INRA-codes, PI-codes and countries of origin of these accessions.

**Table 1:-** Codes of 62 safflower accessions from different geographic origins. The Spanish variety 'Rancho' was used as a check.

Code INRA	Code	Origin	Code INRA	Code	Origin
1	PI286199	KUWAIT	114	PI 250527	EGYPT
2	PI 262430	SYRIA	115	PI 250533	EGYPT
3	PI 237549	SUDAN	116	PI 250077	EGYPT
4	PI 209295	KENYA	117	PI 250528	EGYPT
8	PI 251979	TURKEY	118	PI 250537	EGYPT
10	PI 253391	SPAIN	119	PI 250538	EGYPT
29	PI 237550	UNKNOWN	120	PI 250539	EGYPT
31	PI 306684	UNKNOWN	122	PI 306612	EGYPT
32	PI 262431	UNKNOWN	123	PI 306611	EGYPT
35	PI 248631	PAKISTAN	124	PI 306609	EGYPT
52	PI 304592	AFGHANISTAN	126	PI 306607	EGYPT
68	PI 613498	USA	132	PI 306605	EGYPT
69	PI 537599	USA	133	PI 306602	EGYPT
75	PI 560172	USA	135	PI 306600	EGYPT
79	PI 560161	USA	136	PI 306598	EGYPT
84	PI 307060	INDIA	134	PI 306601	EGYPT

86	PI 306928	INDIA	137	PI 306596	EGYPT
90	PI 307085	INDIA	138	PI 250920	IRAN
91	PI 248381	INDIA	142	PI 250828	IRAN
94	PI 306847	INDIA	150	PI 250823	IRAN
98	PI 305212	INDIA	151	PI 250821	IRAN
99	PI 248382	INDIA	152	PI 250820	IRAN
102	PI 248362	INDIA	155	PI 222240	IRAN
104	PI 250611	EGYPT	157	PI 304467	IRAN
106	PI 250609	EGYPT	158	PI 250827	IRAN
107	PI 250610	EGYPT	160	PI 250825	IRAN
109	PI 250605	EGYPT	162	PI 304471	IRAN
110	PI 250540	EGYPT	163	PI 304472	IRAN
111	PI 250526	EGYPT	164	PI 304476	IRAN
112	PI 250079	EGYPT	165	PI 304462	IRAN
113	PI 250523	EGYPT	<b>Rancho</b>	<b>Cultivar</b>	SPAIN

#### Description of the experimental station:-

The INRA experimental station of Douyet is located at 10 km from Fez city (34°04' N, 5°07' W), at an elevation of 416 m and receives an average rainfall of 425 mm per year. The soil is a cracking clay type with vertic properties. Climate is of Mediterranean type, with cold and wet winter and hot and dry summer. This station is also characterized by a frequent presence of the sirocco wind which could be, to some extent, harmful for crop growing by aggravating hot and dry conditions. The 2013-2014 growing season was rainy, with a good rainfall distribution throughout the crop cycle. The cumulative rainfall recorded, from 1 September 2013 to the end of August 2014, reached 429 mm, versus 300 mm in the same period of the 2012-2013 growing season.

#### Planting design:-

The genotypes tested, long with the check, were planted late in February during 2013 and early in December during 2014. The field experiment was conducted during these two years using a completely randomized blocks design, with two replications. Each genotype was grown in a single 3 m-length row, with inter and intra row spacing of 80 and 30 cm, respectively.

#### Observations and Measurements:-

Data were gathered on morphological parameters (initial vigor, degree of spines presence, plant height, number of branches per plant, number of heads per plant, color of petals, homogeneity), phenological parameter (flowering time), agronomic parameters (number of heads per plant, NHP, number of seeds per head, HSN, thousand seeds weight (g), TSW, seed yield per plant (g), total seed yield per hectare (t/ha), TSY, technological parameter (seed oil content, SOC) and pathological parameters (degree of leaf rust presence, degree of broomrape presence). A random sample of five plants per genotype was taken for the different measurements.

The initial vigor (IV) was evaluated on a scale ranging from 1 (least vigorous accession) to 5 (most vigorous accession). The presence or absence of spines (SL) was assessed on a scale from 1 (spineless accession) to 4 (highly spiny accession). Similarly, homogeneity (HOM) was rated on a scale ranging from 1 (very homogeneous accession) to 4 (very heterogeneous accession). Number of days at flowering (DFS) was the number of days from planting to opening of the first flower on each sample plant. The reaction of accessions to rust (RR), was assessed according to a scale of attack severity ranging from 1 (no, or very light, attack) to 4 (very severe attack). Broomrape virulence (BV) was evaluated, only in the second year (2014), by accounting the number of broomrape stems infecting each sample plant. The oil content (SOC) was determined by Nuclear Magnetic Resonance (NMR, Oxford 4000) and was expressed in% of dry matter.

#### Statistical Analysis:-

Descriptive analysis of gathered data and analysis of variance were performed using anova procedure of SAS program. Duncan's new multiple range test was applied to compare genotypes means. This enabled to distinguish which accessions were significantly different from others and, thus, to select those interesting ones according to an established selection threshold. This threshold was fixed on basis of the parameters studied and their relation with

agronomic and technological performances, the overall average of each parameter and the check performance (Houmanat et al., 2016).

## Results and Discussion:-

### Genetic Diversity Among The Accessions:-

Results of analysis of variance of the accessions studied in two successive years (2013 and 2104) are shown in Table 2. There were significant differences ( $p < 0.001$ ) between the 62 accessions for all the traits studied, indicating the existence of a very large genetic diversity expressed by wide ranges of variation of these traits. These results confirmed those obtained by Nabloussi et al. (2008) who studied a larger safflower collection from which the 62 accessions of the present study had been selected. Other authors found similar results, in their previous researches, namely Mahasi et al. (2005) on a collection of 36 exotic safflower accessions in Kenya and Erbas et al. (2016) on a set of 39 safflower genotypes in Turkey. The 'year' factor had a significant effect on all parameters, except SL, TSW and SOC, which is in accordance with findings of Erbas et al. (2016). Figure 1 showed the inter-annual variation recorded for the parameters studied during two consecutive and contrasted cropping seasons. Analyzing the 'location' effect on the evaluated parameters, Nabloussi et al. (2008) had reported significant differences between locations studies, except for TSW. The variability observed between both years, for most of the parameters studied, could be explained by the contrasting environmental conditions, mainly rainfall and temperature, aggravated by planting date which was too late in 2013. On the other hand, the fact that both years were comparable for SL, TSW and SOC, might indicate that these characters were not significantly affected by those contrasting conditions. Also, the interaction Year  $\times$  Accession had significant effect on all parameters, except TSY and NHP. Interestingly, this indicated that the better accessions, regarding seed yield and number of heads per plant, were confirmed from one environment to the other. In other words, they maintained their higher performance in terms of seed yield and number of heads per plant under contrasted environmental conditions. Previous study on the original safflower collection had shown significant effect of the interaction Location  $\times$  Accession for all parameters, except TSW (Nabloussi et al., 2008).

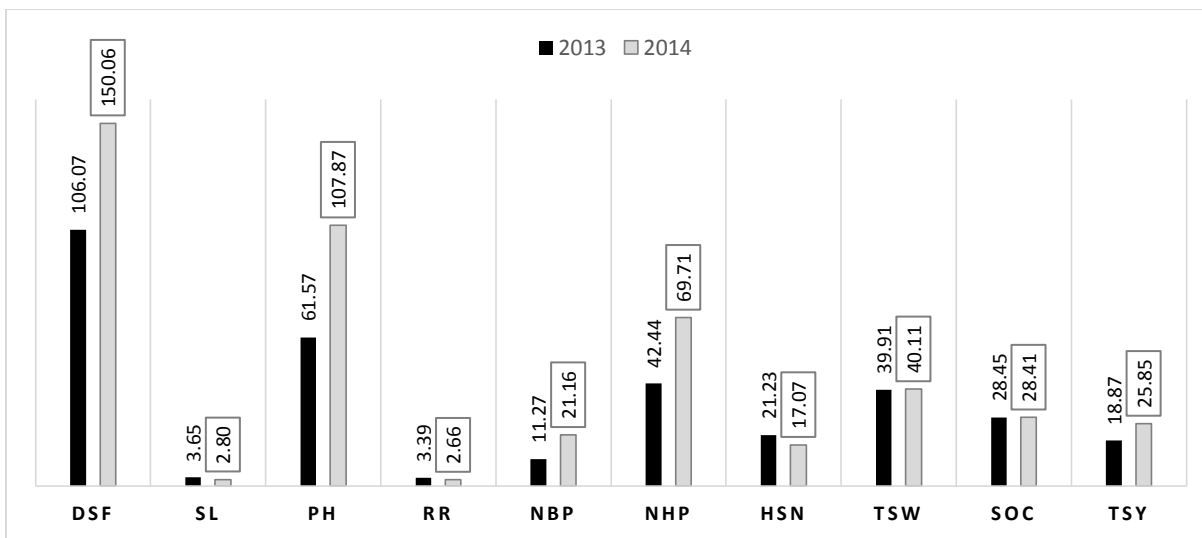
As mentioned above, there was a large variability among the accessions for all parameters studied. Hereafter, description and discussion of averages and variations recorded for morphological, pathological, agronomic and technological traits are given (Table 3).

**Table 2:-** Analysis of variance (Mean squares) for different parameters of 62 safflower genotypes evaluated at 'Douyet' during two years (2013 and 2014).

Source of variation	DSF <sup>1</sup>	SL	PH	RR	NBP	NHP	HSN	TSW	SOC	TSY
Year	20865 *** <sup>2</sup>	125,2 ns	23970 ***	39,5 **	8846,5 ***	41040 ***	1495,9 ***	5,06 ns	0,001 ns	1812,8 **
Accession (Acc)	253,74 ***	17,35 ***	689,35 ***	4,95 ***	126,55 ***	1231,3 ***	168,17 ***	443,4 ***	50,87 ***	330 ***
Year*Acc	263,77 ***	9,81 ***	287,35 ***	4,44 ***	130,14 ***	726,31 ns	75,42 ***	91,98 ***	17,57 ***	164,58 ns

<sup>1</sup> **DSF:** Days from sowing to flowering. **SL:** Spinelessness level. **PH:** Plant height. **RR:** Leaf rust resistance. **NBP:** Number of branches per plant. **NHP:** Number of heads per plant. **HSN:** Head seeds number. **TSW:** 1000 Seeds weight. **SOC:** Seed oil content. **TSY:** Total seed yield (per hectare).

<sup>2</sup> \*\*\* and \*\*: Significant at the probability level of 1% and 1%, respectively; ns: non-significant.



**Figure 1:-** Inter-annual variation of average values of the parameters studied in two cropping seasons (2013 and 2014).

**DSF:** Days from sowing to flowering. **SL:** Spinelessness level. **PH:** Plant height. **RR:** Leaf rust resistance. **NBP:** Number of branches per plant. **NHP:** Number of heads per plant. **HSN:** Head seeds number. **TSW:** 1000 Seeds weight. **SOC:** Seed oil content. **TSY:** Total seed yield (per hectare).

#### Morphological Parameters:-

The accessions showed a variation in the color of their petals (COP): red, white, yellow, orange and intermediate color (Fig.2). Some studies have highlighted the close correlation between color of safflower petals and their biochemical composition, mainly the total polyphenol content (carthamin) (Hiramatsu et al., 2008). This component, which is characterized by its high antioxidant properties, can be further explored and exploited for industrial and pharmaceutical purposes. Also, the accessions studied showed a large variation in the degree of spinelessness (SL) at leaves and bracts, varying from strongly spiny to completely spineless. On average, the accessions were spiny (2.96); therefore, a large number of genotypes had fewer spines than the check variety (Rancho), which is too spiny (4). Comparable findings had been reported by Nabloussi et al. (2008). Narkhede and Deokar, (1990) reported that the spiny type is generally dominant over the spineless type in safflower genotypes. It was reported that cultivation of safflower was not widely practiced because of the presence of spines, which is a major constraint for some operations, such as harvesting and threshing (Nabloussi and Boujghagh, 2006; Sujatha, 2008). Thus, one of the main objectives of safflower breeding programs is to develop spineless varieties with high yield and high oil content (Nabloussi and Boujghagh, 2006; Golkaret al., 2010).



**Figure 2:-** Diversity in colors of safflower petals.

In addition, the accessions were characterized by an average plant height (PH) of almost 100 cm, with a very large variation ranging from 33 to 150 cm. More recently, in a study of 39 safflower genotypes, the observed variation in plant height of safflower was 38.2 to 84.1 (Erbaset et al., 2016). Indeed, the height can be affected by environmental conditions (Camas et al., 2007; Beyyavas et al., 2011).

The crop vegetative cycle was too long and the average number of days between sowing and flowering (DFS) was 140 days, with a variation from 102 to 180 days. Shinwari et al. (2014) showed wider variation in the DSF from 76 to 170 days, when they evaluated a USDA collection. Contrarily, in a study of 45 pure and exotic lines, there was smaller variation in the DSF from 116 to 134 days (Alizadeh and Carapetian, 2006). Our results have also showed a large variation in number of branches per plant (NBP), ranging from 4 to 50. The average NBP was about 18. The check variety had 17 branches per plant. Similar results had been reported in previous studies (Weiss, 2000; Reddy et al., 2004; Omidi et al., 2009).

#### **Pathological parameters:-**

Broomrape virulence (BV) varied significantly among the studied accessions which showed, in general, a high level of resistance. In fact, the BV average did not exceed 2.18 broomrape stems per safflower plant. The accession '164' was the most susceptible, being infected by 16 broomrape stems, whilst, other accessions, like the check variety 'Rancho', were very resistant, with no broomrape attack. Previous studies have shown that broomrape (*Orobanche crenata*) is a holoparasite attacking certain legumes (beans, peas, lentils, etc.), being entirely devoid of chlorophyll and totally host dependent on organic carbon, water and nitrogen (Joel et al., 2007). It is also parasitic and dangerous for safflower (*Carthamus tinctorious* L.) and for many other cultivated or wild species (Restuccia et al., 2009). Broomrape on safflower was reported for the first time in Morocco during 2006 (Nabloussi, 2006).

Regarding rust resistance (RR), the accessions studied were in general moderately resistant, with an average score of 2.9. 'Rancho' had 3 as resistance score, and the accession '216' was the only one with no rust symptoms, which might indicate that it was immune. For the rest of accessions, the resistance score ranged from 1 (most resistant) to 4 (most susceptible). Nabloussi et al. (2008) found similar results on related safflower material. In a previous study on ten safflower genotypes of different origins in field conditions, Karakaya et al. (2004) showed that all of them were attacked by *Puccinia carthami*, the causative agent of safflower rust, but with different degrees of severity.

#### **Agronomic traits:-**

Safflower accessions were characterized by an average number of heads per plant (NHP) of about 55, and a range of variation from 10 to 98 heads. A large number of genotypes have been shown to be promising by producing a NHP much higher than that of the check variety (54). Among these accessions, the '135' had the highest NHP (98). Similarly, the number of seeds per head (HSN) varied significantly according to the accessions studied, from 5 to 48, and the recorded average was almost 16.5. The check variety had a mean HSN of 12. Our findings were more interesting than those of Safavi et al (2010), having reported a NHP variation from 3 to 16.4, Amini et al (2007), having showed NHP varied from 10.5 to 32.2 and HSN ranged from 12.3 to 27.8, and Tunçtürk and Çiftçi (2004) who reported HSN ranged from 10.5 to 32.2. In addition to high plant branching, a high number of heads per plant is a seed yield component that could be used as selection criterion for improving this latter (Lahane et al., 1999).

The mean TSW was 39.58 g, higher than the check (35 g), indicating that most of the accessions studied were characterized by bigger seeds, compared to the check variety. The variation of this parameter was very important ranging from 14 to 67g. These results were in agreement with those of Nabloussi et al. (2008), and Safavi et al. (2010).

The average seed yield (TSY) was almost 2 t/ha. However, there was a very large variation among the accessions, from 0.231 to 4.490 t/ha. The accession '164' was the least productive due to its high susceptibility to broomrape infection, while the accession '123' was the most performant. However, several genotypes were more productive than the check variety which produced 1.3 t/ha. Narrower variation ranges were reported in previous studies, from 1.5 to 2.7 t/ha (Panahiet al., 2013) and from 1.1 to 2.2 t/ha (Amini et al., 2007).

#### **Technological parameter:-**

The oil content (SOC) varied from 22.10 to 38.77%, for the accessions '164' and '78', respectively. The overall average SOC was about 28%, whilst SOC of the check variety 'Rancho' was 24%. This indicated that most of the accessions studied were characterized by higher SOC than the check. This will enable to select safflower germplasm with quite high seed oil content, which is the most important selection objective, in addition to seed yield. In previous studies, variation ranges observed for the oil content were 14-44% (Johnson et al., 1999), 23-34% (Alizadeh, 2005), 21.4-31.7% (Alizadeh and Carapetian, 2006), 19-47% (Nabloussi et al., 2008) and 23-37.4% (Panahiet al., 2013).

**Accessions pooling:-**

Overall, high genetic diversity was observed among the evaluated accessions, which belong to different geographical origins, for all the parameters studied. In particular, large variations were shown for some economically important traits, such as seed yield, seed oil content, flowering earliness, spinelessness, rust resistance and broomrape resistance. This would be potentially useful for our breeding program. As was suggested by Nabloussi et al. (2008), we could think of developing some genetic pools in the light of our findings. Six pools would be feasible, and each of them may comprise the most homogeneous, performant and stable accessions regarding a specific trait from the six ones mentioned before. Table 4 shows the proposed genetic pools with accessions selected according to a suggested threshold for each trait. The threshold established for the development of each pool was made taking into account the degree of variability observed among the safflower accessions and the performance of the check variety used in this study.

In the next future, crossings and interbreeding should be achieved between genitors belonging to different pools in order to combine various desirable traits in some particular safflower germplasms. These bred germplasms will be suitable and useful for developing highly productive and adapted safflower varieties to be cultivated in Morocco as well as other regions throughout the world.

**Table 3:-** Average, minimum and maximum values of studied parameters of safflower accessions evaluated at 'Douyet' during 2013 and 2014 and identification of accessions having extreme values.

Trait	Average	Minimum		Maximum		Rancho
		values	Genotypes	values	Genotype	
DSF <sup>1</sup> (d)	140.37	102	124	180	102	145
SL	2.96	1	35,68,94,102,116,120,151,152,160,157, 162, 164, 165.	4	1,2,4,10,29,31,32,52,60,68,69,75,78,84,104.	4
PH (cm)	98.09	33	69	150	104	99
RR	2.90	1	52,60,68,84,110,133,104,116,119,122,132,133,134,142,152,157.	4	1,2,94,151.	3
NBP	17.77	4	32,94,136,155,157.	50	111	17
NHP	55.20	10	162	98	135	54
HSN	16.48	5	35,114,2.	48	152	12
TSW (g)	39.88	14	94	67	118	35
SOC (%)	28.26	22.10	164	38.77	78	24
TSY (t/ha)	2.00	0.23	164	4.49	123	1.30
IV	3.43	1	102, 162	5	52, 69, 105, 115, 116, 122, 126	5
BP <sup>2</sup>	2.18	0	35, 58, 60, 68, 75, 100, 102, 111, 113, 114	16	164	0

1 IV: Initial vigor. HOM: Homogeneity. SL: Spinelessness level. CP: Color of the petals. BP: Broomrape. RR: Leaf rust resistance. PH: Plant height. DSF: Days from sowing to flowering. NBP: Number of branches per plant. NHP: Number of heads per plant. HSN: Head seed number. TSW: 1000 Seeds weight. SOC: Seed oil content. TSY: Total seed yield (per hectare).

2 Broomrape virulence was assessed only in the 2014 crop season.

**Table 4:-** Accessions pooling for each studied trait on basis of suggested threshold for selection and check's values.

Trait	Value of the check 'Rancho'	Threshold for selection	Pools
Degree of spinelessness (scale 1 to 4)	4	1	Pool-1 : 35,68,94,102,116,120,151,152,160,157,162,164,165.
Flowering earliness	145	<120	Pool- 2 : 1, 26, 142, 152, 165, 2,

(number of days between sowing and flowering)			118, 122, 124, 127, 132,135, 137.
Resistance to rust (scale 1 to 4)	3	1	Pool- 3 : 52, 60, 68, 84, 110, 133, 104, 116, 119, 122, 132, 133, 134, 142, 152, 157.
Broomrape virulence (Number of broomrape stems infecting safflower plant)	0	0	Pool-4 : 100, 102, 111, 113, 115, 116, 118, 120, 126, 135,136, 137.
Grain yield per hectare (t/ha)	1.3	> 3	Pool-5 : 1, 15, 32, 100, 102, 106, 111, 113, 115, 116, 118, 120, 122, 123, 124, 126, 127, 132.
Seed oil content (%)	24	> 35	Pool-6 : 1, 75, 78, 84, 107, 133, 134, 142, 156, 163.

### Conclusions:-

In light of the results obtained, a large genetic diversity was noticed among the accessions evaluated for all the parameters studied. Also, there was a significant effect of the interaction environment (year) × accession on all the parameters, except TSY and NHP. This finding means that accessions were stable across both environments for these two traits. Thus, there will be a possibility to identify and select some stable and widely adapted accessions, with higher TSY and NHP than the check variety. This is very interesting since TSY is the most important breeding objective in safflower. Furthermore, it was possible to conceive and establish six genetic pools on the basis of single and economically important traits. These traits are spinelessness, early flowering, rust resistance, broomrape resistance, seed oil content and seed yield. The accessions comprised in each pool showed an interesting level of performance and stability in terms of each corresponding trait. The pools thus obtained will be multiplied, conserved and then used as valuable genetic resources in crossing and interbreeding for safflower breeding programs in Morocco as well as in other safflower cultivation areas in the world.

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