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Cross Pollination of Safflower (*Carthamus tinctorius* L.) under Moroccan Environmental Conditions

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ABSTRACT

Safflower crop has a good potential in semiarid areas of Morocco. The knowledge of cross pollination rate in our local environment is of a great interest for designing optimal breeding strategies. No investigation has been carried out before neither in Morocco nor in other north-African countries. Thus, a study was carried out in 2009 at Allal Tazi (Kenitra province) to estimate the rate of cross pollination in safflower using high oleic acid as a biochemical marker. Plants of the high oleic acid cultivar 'CR-6' were surrounded by plants of the cultivar 'Rancho' with standard oleic acid content. The observed average outcrossing rate was 26.6%, ranging from 8.3 to 53.0% at the plant level, and from 0 to 79.2% at single-head level. According to our results, bagging safflower heads before flowering is of paramount importance to ensure self-fertilization in genetic studies and pedigree breeding.

Keywords: bagging heads, between-plant variation, biochemical marker, Outcrossing rate, within-plant variation

INTRODUCTION

The importance of oil crops such as safflower (*Carthamus tinctorius* L.) has increased in recent years with an increasing demand for vegetable oil (Pahlavani *et al*. 2007) and, especially, with the interest in production of biofuels (Dordas and Sioulas 2008). Safflower is, in general, produced on marginal lands relatively dry and relatively deprived from the benefits of fertilizer inputs or irrigation. Attempts to improve seed yield and quality by developing new genotypes and agronomic practices have gained importance throughout the world. Breeding strategies for developing efficient and adapted cultivars depend on pollination system of the crop, target environment for which the cultivar is developed and available resources. Safflower has been considered a predominantly autogamous plant, with a low outcrossing rate of less than 10% (Knowles 1989; Patil *et al*. 1991). However, there is a significant influence of environmental conditions on this rate. It was reported that cross pollination rate could exceed 50% in various agro-climatic environments (Dajue and Mündel 1996; Singh and Nimbkar 2007; Rudolphi *et al*. 2008). The main factors affecting this rate are temperature, humidity and pollinating insects abundance. Bees are the major pollinators, and species of *Apis* genera are the predominant visitors and foragers on safflower (Kumar and Singh 2008; Shao *et al*. 2012).

Safflower is not currently cultivated in Morocco and the crop was abandoned since 1992 due to industrial and commercial constraints. However, it has a good seed yield potential in Morocco semiarid areas, exceeding 2.5 t/ha (Nabloussi and Boujghagh 2006). Thus, the Ministry of Agriculture has called for restarting this crop through a global action plan including agricultural research. It is suggested as winter crop for all areas of this country, including those of low rainfall, where the potential of other oilseed crops is more limited (Zraibi *et al*. 2012). Current and future research would lead to the development of safflower as a major oilseed crop in Morocco (Nabloussi and Boujghagh 2006). Assuming that safflower is essentially a self-pollinated crop, a breeding program was aimed at releasing elite inbred lines as new cultivars for Moroccan conditions, without bagging heads of selected plants among segregating and heterogeneous germplasm. Nevertheless, in such open-pollination conditions, we observed high heterogeneity level in the progenies of spineless germplasm, indicating that natural cross-pollination with spiny material has occurred to some extent. It was reported that the spiny trait is dominant over the spineless trait (Narkhede and Deokar 1990; Pahlavani *et al*. 2004). No research has been carried out before to estimate the outcrossing rate of safflower under Moroccan environmental conditions, nor in other north-African countries having close environmental conditions. The knowledge of this rate is of a great interest for designing proper safflower breeding strategies. Moreover, the rate of outcrossing is also crucial for the production, at a commercial scale, of oil with specific characteristics such as high oleic content. Thus, the objective of the present study was to estimate the cross-pollination rate in safflower under a Moroccan environment, with a high potential for this crop cultivation.

MATERIALS AND METHODS

In this study, the oleic acid character was used as a biochemical marker to estimate outcrossing rate between two cultivars, 'Rancho' with standard oleic acid content (20-35%) and 'CR-6', a cultivar derived from Oleic-Leed, with high oleic acid content (75-82%). Both cultivars were developed at the Institute for Sustainable Agriculture (IAS-CSIC), Cordoba, Spain. The design used is similar to that adopted by Velasco *et al*. (2012), consisting of surrounding each plant of 36 plants of the cultivar 'CR-6' by 24 plants of the cultivar 'Rancho' (**Fig. 1**). Seeds of both cultivars were sown on 4 February 2009 in the INRA experimental farm of Allal Tazi, located at 30 km from Kenitra city (34°31'16"N, 6°19'19"W) at an elevation of 10.5 m and with a rainfall average of 550 mm. Inter and intra row spacing were 1.5 m and 1 m, respectively. At maturity, 12 random heads of each high oleic plant were harvested to analyse the oleic acid content of individual seeds. For each capitulum, six to 12 seeds were analysed. High

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Short Communication

R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	**O_1**	R	R	**O_2**	R	R	**O_3**	R	R	**O_4**	R	R	**O_5**	R	R	**O_6**	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	**O_7**	R	R	**O_8**	R	R	**O_9**	R	R	**O_{10}**	R	R	**O_{11}**	R	R	**O_{12}**	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	**O_{13}**	R	R	**O_{14}**	R	R	**O_{15}**	R	R	**O_{16}**	R	R	**O_{17}**	R	R	**O_{18}**	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	**O_{19}**	R	R	**O_{20}**	R	R	**O_{21}**	R	R	**O_{22}**	R	R	**O_{23}**	R	R	**O_{24}**	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	**O_{25}**	R	R	**O_{26}**	R	R	**O_{27}**	R	R	**O_{28}**	R	R	**O_{29}**	R	R	**O_{30}**	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	**O_{31}**	R	R	**O_{32}**	R	R	**O_{33}**	R	R	**O_{34}**	R	R	**O_{35}**	R	R	**O_{36}**	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Fig. 1 Experimental design used for the study of outcrossing rate in safflower. R represents plants of 'Rancho' and **O_i** represents plant i of 'CR-6'.

Table 1 Average outcrossing rate (%), variation and mean square estimated on high oleic safflower plants grown under Moroccan conditions in 2009.

Plant	Average rate	Range	Plant	Average rate	Range
1	14.8	0-33.3	20	26.4	0-50.0
2	25.0	0-50.0	21	22.2	0-50.0
3	33.3	0-56.5	22	35.2	16.7-50.0
4	11.1	0-33.3	23	50.0	33.3-75.0
5	11.1	0-16.7	24	40.9	16.7-66.7
6	11.7	0-33.3	25	48.3	16.7-66.7
7	45.8	0-66.7	26	11.1	0-33.3
8	36.4	16.7-50.0	27	30.3	16.7-60.0
9	15.2	0-50.0	28	19.4	0-50.0
10	20.8	0-50.0	29	26.7	0-50.0
11	12.5	0-33.3	30	10.2	0-20.0
12	53.0	33.3-79.2	31	52.1	20-75.0
13	24.2	0-33.3	32	10.1	0-16.7
14	21.2	0-50.0	33	8.3	0-16.7
15	37.9	20.0-50.0	34	39.0	16.7-50.0
16	34.7	16.7-50.0	36	30.8	0-50.0
19	9.7	0-20.0	Overall mean	26.6	0-79.2
			Mean square	12744.1***[a]	----

[a] Significant differences at 1‰ probability level

oleic acid content in 'CR-6' seeds is controlled by one partially recessive gene and by the genotype of the embryo and consequently, the percentage of oleic in the seed oil is influenced by the pollination of foreign pollen (Hamdan *et al*. 2009). Thus, the outcrossing rate was estimated as the percentage of low oleic acid seeds (< 50%) collected in high oleic acid plants. An analysis of variance was performed to compare the high oleic acid plants for outcrossing rate. Duncan's multiple range test was applied to measure significant differences between means.

RESULTS AND DISCUSSION

Before flowering, because of a wilt disease attack, three plants of 'CR-6' (17, 18 and 35) and 41 plants of 'Rancho' were lost, corresponding respectively to 8 and 11% of the initially grown plants. The average outcrossing rate observed in the 33 remaining 'CR-6' plants was 26.6%, ranging from 8.3%, for the plant 33 to 53.0%, for the plant 12 (**Table 1**). Differences between plants were highly significant ($p<0.001$). In previous outcrossing studies in India, based on using flower colour as a marker, the estimated cross-pollination percentage ranged from 6.0 to 21.9%, depending on the planted cultivars (Raghunatam and Dutt 1986), and from 2.4 to 6.3%, depending on spacing between plots (Patil *et al*. 1991). However, in recent studies in Germany, based on the spineless character as a morphological marker, the outcrossing percentage varied from 6.5 to 18.1% between plots, and from 29.9 to 63.1% within plots (Rudolphi *et al*. 2008). In Spain, using the same safflower cultivars and experimental design as in our study, the estimated mean cross-pollination rate was 10.3% in three experiments (Velasco *et al*. 2012). This value is lower than that found in the present research. This suggests that the environmental conditions under which the present experiment was conducted were probably more suitable and favourable to bees' abundance and activity. Three plants (12, 31, 23) out of 33 exhibited cross-fertilization above 50% whilst 10 plants (1, 5, 6, 11, 4, 26, 30, 32, 33, 19) exhibited cross-pollination below 15% (**Table 1**). This indicates large spatial heterogeneity in foraging activity of pollinators. By observing the variation of outcrossing rate between high oleic plants (**Table 1**) and their distribution in the field (**Fig. 1**), it seems apparent that bees (and probably others insects) visitation occurred in a random and unbalanced way. This is in agreement with the results of McPherson *et al*. (2009), who also found that outcrossing rate in safflower was spatially heterogeneous. At the single-head level, the cross-pollination rate varied from 0 to 79%. This latter was found in a single capitulum of plant 12, which also exhibited the higher average cross-pollination rate at the single-plant level. It is noteworthy that no significant differences for outcrossing rate were observed between heads of the same plant ($p>0.05$), indicating that the heads of each plant have similar chance to be foraged by bees.

In conclusion, the estimated outcrossing rate for safflower under the environmental conditions of our study was close to 27%, which is much higher than previous estimations in most of the previous studies conducted thus far. These results have important implications for genetic studies and breeding programs. Thus, bagging safflower capitula before flowering is absolutely necessary to ensure self-fertilization in genetic studies and pedigree breeding for developing elite lines as promising cultivars in Morocco.

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REFERENCES

Dajue L, 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, 83 pp

Dordas CA, Sioulas C (2008) Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. *Industrial Crops and Products* **27**, 75-85

Hamdan YAS, Perez-Vich B, Velasco L, Fernandez-Martinez JM (2009) Inheritance of high oleic acid content in safflower. *Euphytica* **168**, 1-9

Knowles PF (1989) Safflower. In: Röbbelen G, Downey RK, Ashri A (Eds) *Oil Crops of the World*, McGraw-Hill, NY, pp 363-374

Kumar N, Singh R (2008) Relative abundance of honey bee foragers visiting safflower (*Carthamus tinctorius* L.) and nectar-sugar concentration in bloom. *Pest Management and Economic Zoology* **16**, 135-141

McPherson MA, Good AG, Topinka AKC, Yang RC, McKenzie RH, Cathcart RJ, Christianson JA, Strobeck C, Hall LM (2009) Pollen-mediated gene flow from transgenic safflower (*Carthamus tinctorius* L.) intended for plant molecular farming to conventional safflower. *Environmental Biosafety Research* **8**, 19-32

Nabloussi A, Boujghagh M (2006) Safflower breeding in Morocco: history and current situation. *Sesame and Safflower Newsletter* **21**, 84-87

Narkhede BN, Deokar AB (1990) Inheritance of spininess and pericarp types in safflower. *Journal of Maharashtra Agricultural Universities* **15**, 279-281

Pahlavani MH, Razavi SE, Mirizadeh I, Vakili S (2007) Field screening of safflower genotypes for resistance to charcoal rot disease. *International Journal of Plant Production* **1**, 45-52

Pahlavani MH, Saeidi G, Mirlohi AF (2004) Inheritance of flower color and spininess in safflower (*Carthamus tinctorius* L.). *Journal of Heredity* **95**, 265-267

Patil RB, Patil PS, Bharud RW, Daviskar AP (1991) Natural crossing in safflower. *Journal of Maharashtra Agricultural Universities* **16**, 296-297

Raghunatham G, Dutt KVLN (1986) Outcrossing studies in safflower. *Journal of Oilseeds Research* **3**, 132-134

Rudolphi S, Becker HC, Von Witzke-Ehbrecht S (2008) Outcrossing rate of safflower (*Carthamus tinctorius* L.) under the agro climatic conditions of Northern Germany. *Proceedings of the 7th International Safflower Conference*, Wagga Wagga, Australia, November 3-6

Shao JF, Quan QM, Cai WG, Guan LL, Wu W (2012) The effect of floral morphology on seed set in *Carthamus tinctorius* Linnaeus (Asteraceae) clones of Sichuan province in China. *Plant Systematics and Evolution* **298**, 59-68

Singh V, Nimbkar N (2007) Safflower (*Carthamus tinctorius* L.). In: Singh RJ (Ed) *Oilseed Crops (Vol 4) Genetic Resources, Chromosome Engineering, and Crop Improvement*, CRC Press, Boca Raton, FL, USA, pp 167-194

Velasco L, Fischer M, Fernandez-Martinez JM (2012) Estimation of cross-fertilization rate in safflower (*Carthamus tinctorius* L.). *Spanish Journal of Agricultural Research* **10**, 155-159

Zraibi L, Nabloussi A, Merimi J, Kajeiou M, El-Amrani A, Mihamou A, Abid M, Serghini Caid H (2012) Effect of moisture stress on morphological, physiological and agronomic parameters of different safflower (*Carthamus tinctorius*) varieties. *Journal of Oilseeds Research* **29**, 210-214