ORIGINAL ARTICLE

PROVENANCE AND SITE EFFECTS ON PROGENY SAFFRON CORMS (*CROCUS SATIVUS*) PRODUCTIVITY

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ABSTRACT

Background: Saffron spice is the most expensive spice in the world. Its yield is determined by the effect of the genotype of mother corms, their environment and the interaction between both factors. Improving saffron yield and yield components could be accomplished through the evaluation of these effects on progeny corms asexual propagation. **Objectives**: The aim of the present study was then to evaluate progeny corm productivity of saffron (*Crocus sativus* L.) under the effects of the provenance of mother corms, the planting site and their interaction. **Methods:** A multifactorial design was started with four different corms provenances noted Sh, Am, Za and As that were planted in two different sites noted TAL and FSA. Progeny corm number, fresh weight and diameter average as well as total progeny biomass were measured at each harvest. **Results**: Results indicate morphological polymorphism among saffron mother corms between the provenances. Both tested factors had a significant effect on progeny corm productivity. In term of total useful progeny biomass able to flower with a diameter >2.5cm and mass >11g, Am accession was the highest performing at the FSA coastal site. Furthermore, this planting site showed the best results for other provenances in term of progeny corms quantitative traits. Useful progeny corms productivity was enhanced 7.38 times the starting biomass after only two years compared with Za accession planted in TAL (1.05). This last accession is marked by a described phenomenon characterized by the super-dominance of the first generated corm. Regardless the number of progeny corms, positive correlation was stated between the studied agro-morphometric traits and Am provenance in FSA planting site. **Conclusions**: This study offers preliminary information of saffron agro-morphometric variability under different plantation sites and allows a clonal selection with the planting conditions that show a clear superiority relating to progeny corms quantitative performance.
Keywords: *Saffron, Provenance, Planting site, Corms, Productivity.*

1. INTRODUCTION

Saffron describes both the *Crocus sativus* L. plant and the spice resulting from its dried stigmas. It is considered as the most expensive spice in the world and is highly appreciated for its color, taste and aroma. *C. sativus* is a geophyte species. It produces annual replacement progeny corms and is propagated solely from these corms. In autumn, saffron corms begin to blossom and put out their first green leaves. In spring, the leaves of saffron turn yellow and dry up. The plant stays dormant as an underground corm until the following autumn, when the growth cycle begins again by a new daughter corm [1]. The number, size and shape of these corms control the next generations' productivity. Based on biomolecular studies, two different hypotheses explain its origin: an autopolyploid from diploid *Crocus cartwrightianus* L. or an allopolyploid through the hybridization of *C. cartwrightianus* and *C. hadriaticus* [2]. The limited genetic background in cultivated saffron is said to be attributed to its asexual propagation and by successive selection [3] whereas molecular marker techniques were used to determine the genetic diversity among the natural population [4].

Due to the sterility barrier, improving saffron productivity through sexual approaches remains practically impossible. Therefore, the most efficient way is to select elite clones with mutations accumulated. Using many different provenances for saffron corms, it is possible to select superior corms through agro-morphological characterization. It's a direct promising way to improve this species [5]. This approach not only improves productivity but also eases its conservation. Morphological comparisons of *C. sativus* have revealed some differences in intensity of flower color, pollen size, number of style branches, stamens and viability [6]. A threshold corm size is required for flowers development [7], and size of the mother corm has a significant effect on vegetative development and the production of next generation daughter corms [8,9,10].

Saffron has been cultivated for centuries in the area of Taliouine province in Morocco. It is located in the massive of Siroua, the juncture of the High Atlas and Anti-Atlas mountains. It is grown over a total area of about 1650 ha. Over the last forty years, its cultivation spread to the southeast Taznakht to occupy an additional area of 450 ha and also to high altitudes in the region of Askaouen. Its productivity is merely three tons according to the National Agency for the Development of Oasis Zones and Argan (ANDZOA). It was extended and intensified in recent years. However, saffron characterization is still virgin and less attention is accorded to its morpho-physiology and genetic profile compared to the efforts provided to develop its marketing [11]. Therefore, the current work aims to (i) explore the variability within saffron through an agro-morphological corm characterization; (ii) evaluate the effect of the provenance of mother corms, their environments on progeny corms productivity; (iii) Lead a clonal selection of elite mother corms based on the agro-morphometric potentiality depending on the effects of the provenance and the planting site.

2. MATERIALS AND METHODS

2.1 Plant material and experimental sites

In order to conduct a comparison of saffron daughter corm productivity between different provenances, at different planting sites through two generations, a multifactorial design was carried out with four different mother corm provenances: Sidi Hssaine (Sh), Agadir Melloul (Am), Zagmouzen (Za) and Askaouen (As). These provenances were chosen from the most ancient cultivations in Morocco. These provenances show phenotypic differences according to previous surveys and each accession is considered as representative of its line among the different areas of saffron cultivation. Two experimental sites were prepared; the first one is from the traditional area of saffron production called Tallakht (TAL) in the South West. Its climate is arid with high elevation (1619m) cold in the winter and very hot in the summer's season. The second site is totally different and performed at a coastal low altitude (70m) with a temperate climate in Agadir Faculty of Science (FSA). By planting mother corms coming from different provenances in the same experimental site, it is exposed to the same environmental conditions. The second experimental site is performed for the same reason but also and mainly to study the different trends of these performances when the plantation occurs in another environment completely different than the usual planting area of saffron (Taliouine - Tazenakht). Tables 1 and 2 describe respectively, the provenances and the planting sites.

Table 1: The table presents the provenances of *Crocus sativus* mother corms.

Provenance	Code	Geographic description		
		Latitude (N)	Longitude (W)	Altitude (m)
Sidi Hssaine	Sh	30° 27'	7° 43'	1630
Agadir Melloul	Am	30° 45'	7° 22'	1663
Zagmouzen	Za	30° 38'	7° 49'	1846
Askouen	As	30° 44'	7° 46'	1947

Table 2: The table presents the climatic and topographic characteristics of the performed planting sites.

Parameter			TAL			FSA	
		2012	2013	2014	2012	2013	2014
Temperature* (°C)							
Maxima	Extrema	41.2	41.8	41.8	48.5	40.0	39.0
	Mean	27.3	28.0	27.9	27.0	26.8	26.6
Average		20.0	20.5	20.6	20.1	19.6	20.0
Minima	Mean	12.6	12.9	13.3	13.1	12.7	13.4
	Extrema	-3.7	-2.5	-1.4	2.1	0	0
Rainfall cumuli* (mm)		87.0	21.0	196.0	255.0	10.0	431.0
Ecosystem		Warm temperate, arid			Warm temperate, arid		
Topography							
Altitude		1619 m			70 m		
Longitude (N)		30° 26' 6.54"			30° 24' 30.67"		
Latitude (W)		7° 46' 1.70"			9° 32' 38.94"		
Slope		Flat terrain			Flat terrain		

(*) infoclimat data; TAL: Tallakht planting site; FSA: Agadir Faculty of Sciences planting site.

2.2 Experimental design

In order to compare agronomic and morphological divergences between the provenances in each site, a two-year field evaluation was conducted using complete randomized block design with three blocks and ten replications to insure individual mother corm investigations during the period 2012–2014. A total of thirty mother corms per provenance of *C. sativus* L. were planted in each site. The aim also was to track the multiplication behavior individually of these mother corms. They were chosen with a diameter strictly more than 2.5 cm weighting 14±0.5 g and presenting no significant differences between provenances. Sampled corms were cleared of external tunics, coded, measured, weighted and photographed. They were planted in each block in four furrows at a depth of 20cm, with 20 cm between plants and 50cm between furrows. At the end of each cycle, daughter corms from each mother corm were counted and characterized and immediately replanted in the same site. The cultivation practices used were those commonly used for this crop, respecting a bio process. The planting sites soils were virgins and no chemical fertilization was applied. Physical, chemical and biological characteristics of the planting sites soils are shown in Table 3.

Table 3: The table presents Soil characteristics of TAL and FSA planting sites.

Parameter	TAL	FSA
MO (%)	1.14	2.24
Total Nitrogen (%)	0.09	0.16
C/N	7	7.8
P2O5 (mg/Kg)	19	31
K2O (mg/kg)	231	335
(MgO) (mg/kg)	919	1252
Copper (mg/kg)	3.15	2.53
Zinc (mg/kg)	0.43	3.34
Manganese (mg/kg)	30.99	3.47
Iron (mg/kg)	11.90	2.41
pH (H2O)	8.5	8.4
Porosity	1.05	0.95
Useful water (mm/cm3)	1.2	1.60
Total limestone (%)	38.2	42.9
Texture	sandy	Fine silty – clay - sandy

TAL: Tallakht planting site; FSA: Agadir Faculty of Sciences planting site.

2.3 Agro-morphological progeny corm traits

Collected data were based on 7 characters in each site: number of progeny corms (NPC), fresh weight of progeny corm (FWPC), diameter (DPC), phenotypic coefficient of variation (PCV) percentage of useful progeny corms >2.5cm and >11g (PUPC, it is an important agronomic trait taking into account the critical size of corms able to flower), Total Progeny Biomass (TPB), including the flowering Total Useful Progeny Biomass (TUPB).

2.4 Statistical analysis

All statistical analysis was performed with SPSS V.19 and StataMP 13 softwares [12,13]. Analysis of variance was performed using general linear model procedure at different levels (year, provenance, site and their interactions). Differences between mean values were compared using the post hoc test given by Duncan Multiple Range Test (DMRT) at $P<0.05$ [14,15]. Values are presented as mean ± standard deviation (SD). The variation assessment was performed using univariate and multivariate analysis based on morphologic and agronomic traits. Principal component analysis (PCA) was performed using a matrix generated from the mean morphological data to better understand the correlations between all studied characters and factors affecting progeny corm productivity traits.

3. RESULTS

3.1 Provenance and site effects on progeny corms productivity

The collected corms from different provenances show phenotypic polymorphism at different levels mainly their color, shape and size (Fig. 1). The behavior of different provenances in the same site in terms of progeny corm productivity shows the same profile at the first year compared with the second one. Am provenance shows the best productivity in terms of the number of produced progeny corms especially in Tallakht site (Fig. 2). At this planting site, the multiplication factor in terms of progeny corms number was almost 5 times the initial number of mother corms at the first year and 4 times at the second year. During the second year, Am provenance produced 3 times more than Za in term of number of progeny corms in TAL site. Za provenance produces less in number but with a first super-dominant corm. This phenomenon enhances the phenotypic variation among this accession (Fig. 3).

Figure 1: The figure presents mother corms' aspects of Sidi Hssaine (Sh), Agadir Melloul (Am), Zagmouzen (Za) and Askaouen (As) provenances.

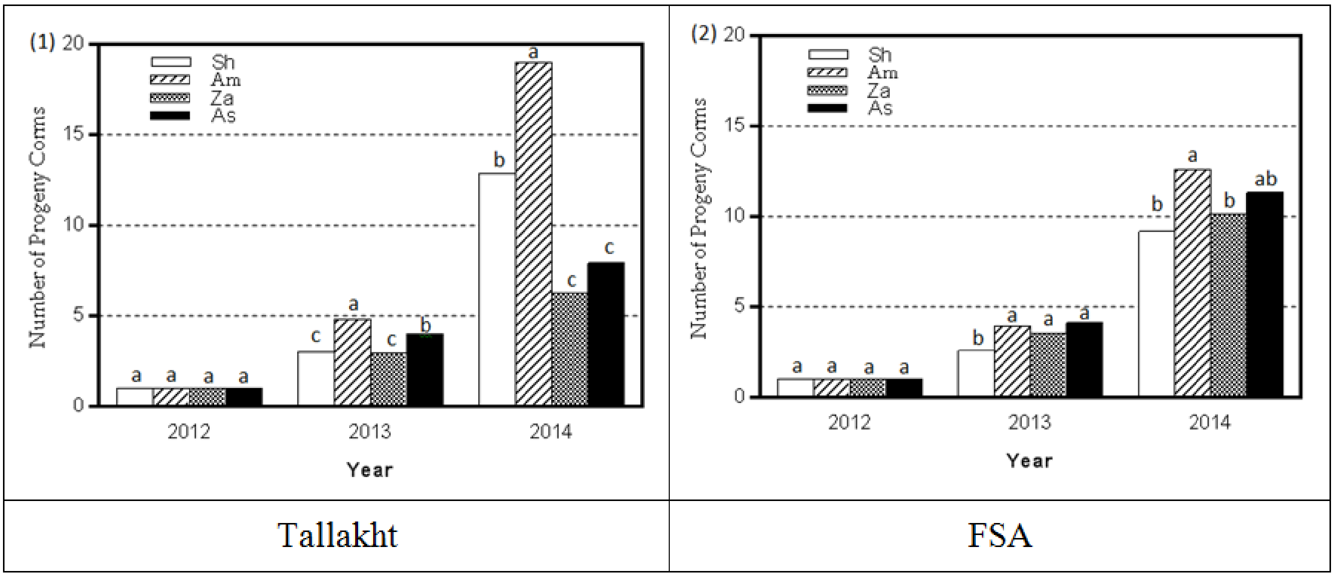

Figure 2: The figure presents the number of progeny corms of 4 provenances during the two years in Tallakht (1) and FSA (2) (Sh: Sidi Hssaine, Am: Agadir Melloul, Za: Zagmouzenand As: Askaouen). Different letters indicate significant differences for Duncan P<0.05.

Figure 3: The figure presents produced corms from the first year in Tallakht and FSA. Za with superdominant major corms (respectively C23 and H20) and Am without superdominance phenomenon (respectively B23 and G12).

The analysis of progeny corms fresh weight (Fig. 4) and diameter traits during the first and second years shows significant differences between the provenances (Fig. 5). Taking into account the first year corm weights and diameters obtained in Tallakht, Sh provenance showed significantly high number of progeny corms. This high number of progeny corms affected its weight and size (Fig. 2).

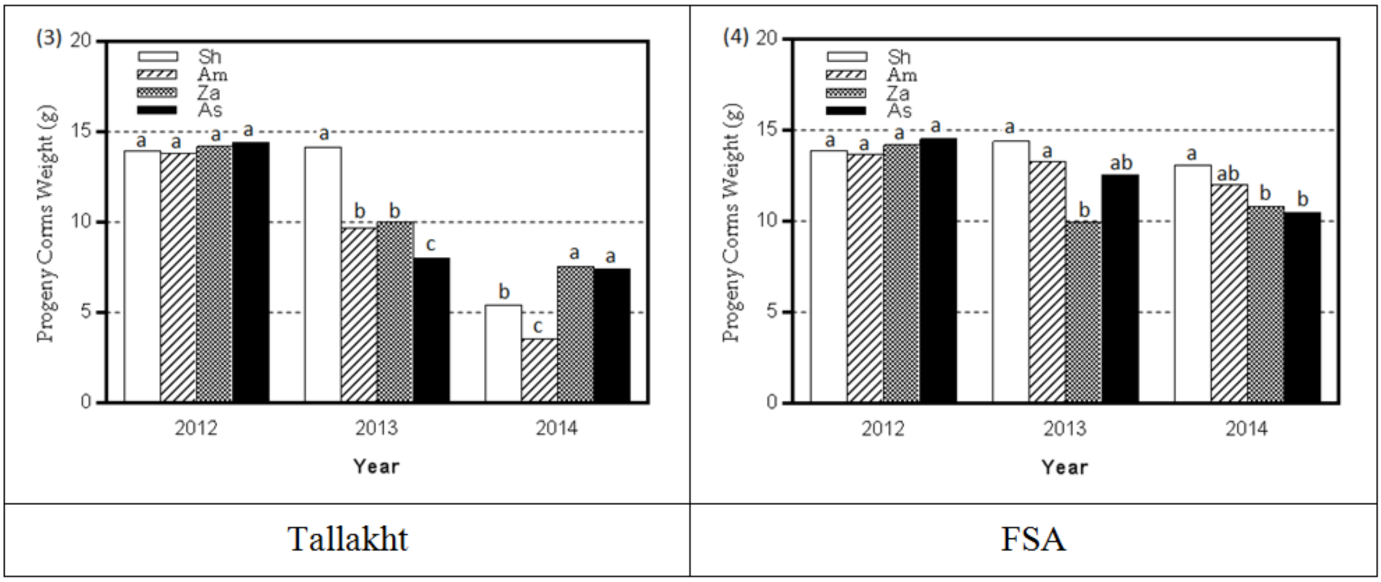

Figure 4: The figure presents progeny corms weight in two different planting sites TAL (3) and FSA (4) during the period 2012 - 2014 of four local provenances: Sh: Sidi Hssaine, Am: Agadir Melloul, Za: Zagmouzen and As: Askaouen. Different letters indicate significant differences for Duncan $P<0.05$.

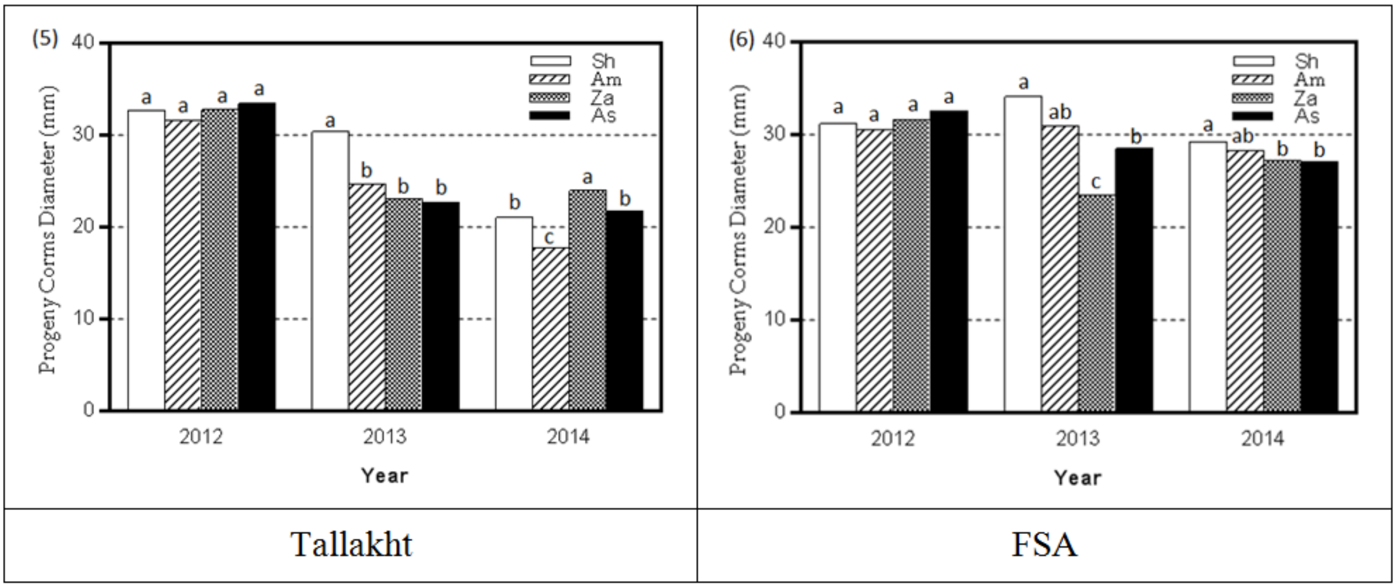

Figure 5: The figure presents progeny corms diameter in two different planting sites TAL (5) and FSA (6) during the period 2012 - 2014 of four local provenances: Sh: Sidi Hssaine, Am: Agadir Melloul, Za: Zagmouzen and As: Askaouen; Different letters indicate significant differences for Duncan $P<0.05$.

Corm size is an important trait for saffron plantation; it has to be greater than the critical value in order to generate more shoots, leaves and flowers. The recommended size for such corms qualified as useful corms is to be higher than a diameter of 2.5cm and or a weight of 11g. The multiplication rate was investigated among these provenances during two successive years and at two different sites. The obtained results show that Sh accession was performed best; it reached 80% of useful corms during the first harvest at the FSA planting site (Fig. 6). This performance was not maintained during the second year in TAL. This could be related to high number of progeny corms that negatively affected their size. It is noted that among the sampled provenances, the percentage of useful corms (>2.5cm and > 11g) bypassed 50% at the second year in all provenances.

Comparison of the number of progeny corms for all the provenances during two harvesting periods on both planting sites shows the same profile regardless the year. It is more amplified at the second harvesting period (Fig. 2). The site effect on NPC is not the same over all the provenances. It is different and depends of the provenance itself. It was not significant at the first year except for Am, and become more significant at the second year for all the provenances even it does not show a clear improvement regardless the used provenance.

Daughter corms weight of Am and As showed significant differences between plantation sites at the first year, and between all the provenances at the second year (Fig. 4). The mean weight values at TAL site were lower for the second year (3.5g to 7.5g) while for the FSA, these values were between 11g and 13g. They reflect the significant positive effect of site over the weight, diameter and percentage of useful corms traits.

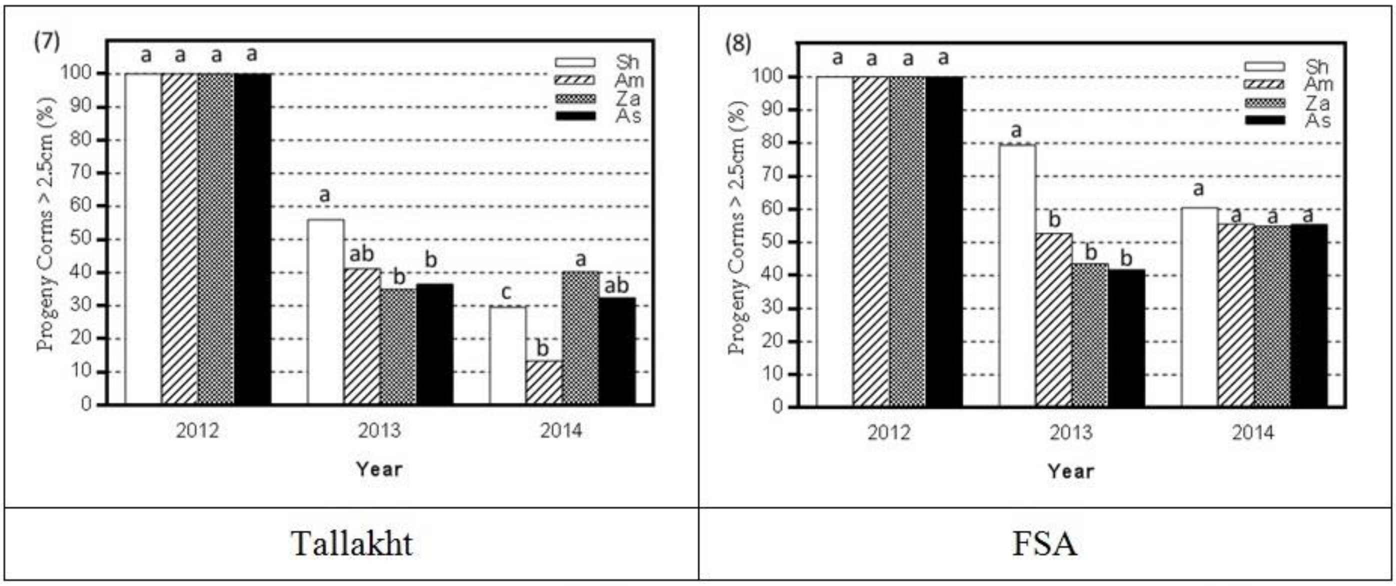

Figure 6: The figure presents superior useful corms rate distribution during two harvesting years in two different experimental sites TAL (7) and FSA (8) of four provenances (Sh: Sidi Hssaine, Am: Agadir Melloul, Za: Zagmouzen and As: Askaouen). Different letters indicate significant differences for Duncan P<0.05.

3.2 Total Progeny Biomass under provenance and site interaction effect

To clarify the comparison in progeny corms productivity under the dual effects of provenance and planting site, the number, size and weight traits were considered at once by performing an estimation of the total progeny biomass during the second harvest (Fig. 7). The combined effects of the provenance of mother corms and the planting site is clear and shows an important productivity of total progeny corms biomass at the FSA site ranged between 2935g to 4096g depending on the provenance. However, only 1236g to 1991g were obtained in Tallakht. The accession Am performed best especially in FSA planting site, followed by As and Sh with no statistically significant difference between them. Za accession in TAL site gave the lowest Total Progeny Biomass productivity. The same conclusions were noted on the total biomass of the corms >2.5cm and >11g (Fig. 7). The multiplication rate of total biomass after two cycles varied depending on the site and the provenance from 3 times to 9 times the starting mother corms' material in terms of total progeny biomass. The number of useful corms >2.5cm and >11g could be enhanced from 1.03 to 7.38 times after only 2 years by selecting Am provenance combined with FSA planting site effect.

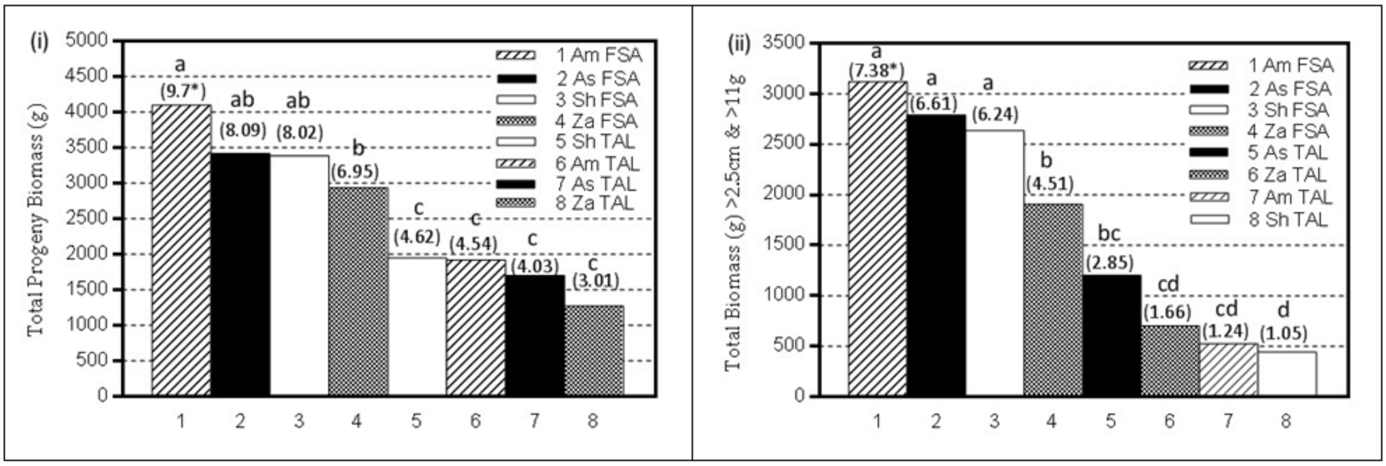

Figure 7: The figure presents the classification of four saffron provenances (Sh, Am, Za and As) planted in two different sites (FSA and TAL) according to two selection criteria (Total Progeny Biomass (i) and total useful biomass traits (ii)). Different letters indicate significant differences for Duncan with P<0.05.

3.3 Multivariate analysis and progeny corms productivity correlations

To search the correlations between descriptors associated with progeny corms productivity, their provenance and the planting site, principal component analysis was used. A matrix of mean value for the second harvesting period under the combined provenance and site interaction was subjected to analysis. Correlation between the characters studied and the first two principal components is shown in (Fig. 8). According to the current results, PCA explains by its two components 98.69% of the total observed variability. PC1 and PC2 absorbed 75.70% and 22.99% respectively. Each site's data is loaded differently. TAL planting site is projected at the right side of the first axis (PC1) formed by ShTAL, AmTAL, ZaTAL and AsTAL while FSA at the negative side. It shows the effect site over the agro-morphological traits of the progeny corms. AmFSA performed best at both sites in term of total progeny corms' biomass productivity and also the other interesting traits. Za accession is less correlated with number. It is reputed for its described super-dominance so as that it maintains a balanced state in terms of the rate of useful vs. useless progeny corms (Fig. 8).

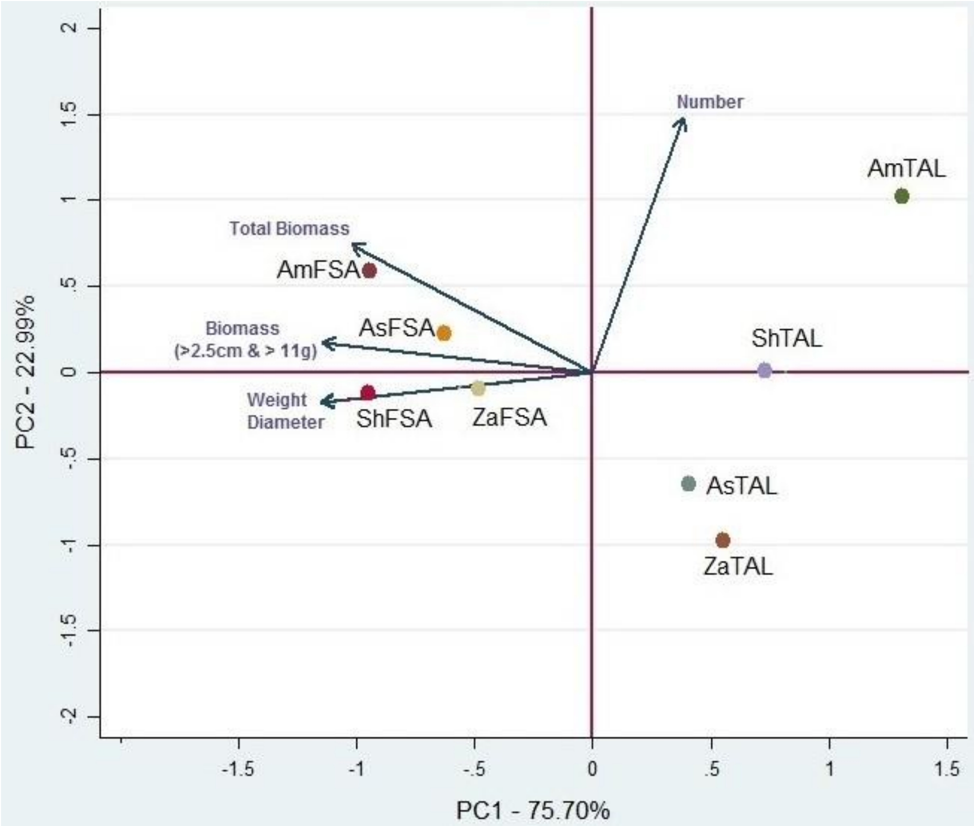

Figure 8: The figure presents biplot scatter diagram illustrating correlation matrix after a multivariate PCA analysis of four provenances (Sh, Am, Za and As), planted in two different sites (TAL and FSA). ShTAL, AmTAL, ZaTAL and AsTAL are clustered at the positive side of (PC1) while FSA data on the negative side. This last one correlates with total biomass, useful biomass (>2.5cm), weight and diameter mainly AmFSA and ShFSA observations.

4. DISCUSSION

Progeny corms productivity was affected by the planting site especially the coastal low altitudes, the provenance of mother corms and their interaction. Each provenance might potentially represent a high performing genotype. Mean comparisons according to Duncan multiple range test ($P<0.05$) revealed significant differences among the provenances and sites in term of progeny corms productivity over two harvesting periods. The correlated characters of fresh weight and major diameter traits showed higher levels of phenotypic coefficient of variation (PCV) indicating a high variation between provenances during the first harvest. It was more illustrated at Za provenance level, highlighting the highest variation related to the super-dominance of the first generated corm from each mother corm.

Mostly, previous studies about saffron mother corms and planting environment focused on evaluating the effect of size or density on flowering kinetic, stigma yield and the chemical quality [2-16,17,18,19,20,21]. Flowers, shoots, leaves and stigma yield of the next cycles of saffron cultivation are indirectly controlled by replacement progeny corms. Therefore, corms behavior along the multiplication process affects progeny corms quantitative and qualitative productivity. Therefore, less attention were accorded to the effect of mother corms provenances probably because of the sterility of saffron which is propagated asexually. Still, the hypothesis of the existence of variation is rejected under the molecular researches that have not yet demonstrated the existence of different clones [22,23,24].

Provenance or genotype effect of mother corms has been handled differently by several authors in completely different environments and accessions like in Iran, India and Turkey. Yau et al., (2006) worked with three strains of saffron (*C. sativus, C. sativus* var. 'cashmerianus' and *C. cartwrightianus*) [17]. It was planted in two different sites for two years. They pointed out that considering stigma productivity, the genus *Crocus* were more adapted especially at favorable coastal site. More recently, Amirnia et al., (2013) studied four Turkish ecotypes over three years but using new corms each year [25]. It is showed the existence of significant differences among the provenances for number and fresh weight of daughter corms. Moreover, daughter corm number and corm fresh weight had positive and direct effects on the stigma yield. Increased cultivation density and selecting bigger corms were useful factors for higher stigma yield. The current results from means comparison suggest a differential response depending on the site, between provenances due to NPC, DPC, FWPC and PUPC traits over the years. These traits could be used as selective traits for shoots, leaves and stigma yield for saffron. Therefore, taking in consideration the Total Progeny Biomass and mainly the Total Useful Progeny Biomass traits, Agadir Melloul and Askaouen provenances as assessed by the multivariate analysis were the best provenances. These two accessions are recommended mainly in the context of coastal planting sites, to be distributed to farmers and to be propagated *in vitro* as optimized in the context of tissue culture in an overall study [26].

FSA coastal site performance could be explained by soil fertility (mainly NPK), temperature and hygrometry also had substantial influence on the observed three-fold higher progeny corms size and fresh biomass differences after two planting cycles compared with TAL site. Useful corms>2.5cm and >11g were seven times higher. It was believed that mainly temperature accelerated photosynthesis then led to the accumulation of organic matters within progeny corms. The winter at the coastal FSA site was relatively mild and short. Thus, saffron could grow slowly in the winter and started to grow vigorously early in spring. In contrast, the winter at TAL was relatively cold and long, raised the chance for more buds to be generated. Although no saffron plants were killed at Tallakht in the two winters, frost damage was illustrated by short leaves and some yellow leaves. After the winter was over, temperature increased rapidly at the FSA site. Thus, the growth period was much shorter at TAL than at FSA, leading to the production of smaller corms in TAL but with high number and a concomitant increase in flower numbers. Saffron yield recorded at the following autumn supported this explanation (Ben El Caid, unpublished data).

In comparison with similar results reported in the literature with different accessions of saffron and environmental conditions, the number of produced corms had been normal at the first year and was higher than reported elsewhere for the second year mainly in TAL site. For example, the current 13.5g mother corm produced in TAL site a number of progeny corms depending on the accession and varied between 2.96 and 4.83 for the first year and 6.26 - 19.03 for the second one. Compared to Douglas et al., (2014), mean numbers of daughter corms obtained across two successive years from 3g to 15g corms class were 1 - 8, 1.4 - 10 and 1 - 6 respectively [27]. Compared to the FSA planting site, corms number was higher in Tal for all provenances. This could have two explanations: (i) in response to severe environment, more daughter corms are obtained but with small size. In the unfavorable conditions saffron plant opts for multiplication in number even by giving birth to small corms. FSA is an example where even with a rich soil and moderate climate, mother corms developed few daughter corms but with important accumulation of organic matter. This is also true for leaves where two different cases were stated: few long leaves or many but significantly short. (ii) The other explanation is the texture of soil, sandy porosity insures more physical plasticity in TAL planting site and eases corms multiplication especially when favored by the genotype or the provenance itself.

Data also shows that 51.07% and 35.36% of the total corm number produced in TAL and FSA respectively were below 11g for the first year; and 74.02% - 55,16% for the best provenances (Sh and As) for the second harvest. These results are important compared to Douglas *et al.* (2014) where it was reported that 77% and 57% progeny corms below 10g for the first and second year [9]. Furthermore, it was reported that 90% of the progeny corm yield was below 10g for the first harvest and 100% in the second year [28]. As for Douglas et al., (2014), it is also pointed out in the present study that the percentage of useful corms (with size >2.5 cm and >11g) were low over years [27]. 16 g corms class was less produced over years even though total weight was increasing. In the current study, a potentiality of 7.38 times the starting weight was obtained after only two years for Am provenance at FSA site.

Principal component analysis loaded each site data differently. It clustered the first site means at the positive side of the first axis with the costal site FSA on the negative side. For all the provenances, the coastal FSA site increased Total Useful Progeny Biomass, especially for Am and As. These characters are less correlated with progeny corm number which was associated with the same provenances but planted at the second less-favorable site TAL, mainly AmTAL and ShTAL. PCA shows that Am and As were the most highly performing genotypes especially in the FSA favorable coastal site. It was showed that the highest Total Progeny Biomass yield was recorded from the combination of Am provenance and FSA, starting with 422.25g the first year to reach 4096.09g at the second harvesting period. The combination of these favorable factors, according to statistically significant values, has led to higher total progeny biomass. However, Za provenance in TAL was the combination that gave less progeny corm's productivity with only 1272.51g of total biomass.

The present study is a contribution to assess saffron progeny corms productivity under the synergetic effects of the provenance of mother corms and the planting site across several growing years. Its outcomes have an impact on saffron nurseries and farmers practices conducted to optimize saffron asexual multiplication. The multiplication trend and its behavior affect directly the quality and quantity of the progeny corms which determines by its turn saffron spice yield. However, investing on this key factor requires more selections to be made among other performant clones with concerns on the associations to the molecular basis of their performances.

5. CONCLUSION

The present paper is a part of an overall study project that aims to evaluate the effect of mother corms provenance and the planting site effects on saffron progeny corms productivity. The study focused on individual analysis of corms and significant variations in the studied characters were shown from the provenance, site as well as their interaction effects. Using the agro-morpho-metrical approach, progeny corms productivity allowed selection of the provenance with the best Total Progeny Biomass and total useful progeny biomass. Furthermore, the higher amount of progeny corms from Agadir Melloul and Askaouen provenances coupled with the effect of the coastal site indicated an additive performance in term of useful replacement corms biomass that control stigma yield. Total Useful Progeny Biomass (TUPB) could be a good marker for early selection. Positive and significant correlation of TUPB with shoots, leaves, flowers and stigma is a useful criterion for clonal selection mainly when the performance of the concerned provenance is coupled with a coastal site. Moreover, the result has important practical implications for genetic management of saffron resources and for future selection activities of saffron plant.

Acknowledgment

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