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Salt stress effects on some morphological, physiological and biochemical parameters of saffron plant (*Crocus sativus* L.) in Eastern Morocco

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Abstract

Saffron (*Crocus sativus* L.) is the most expensive spice in the world. In Morocco, it is cultivated for centuries and used for culinary, medicinal and cosmetic purposes. This work aims to study salt stress effect on several morphological, physiological and biochemical parameters of Saffron plants. Four NaCl concentrations within water used for irrigation were tested: 0, 1, 3 and 5 $g.l^{-1}$. Comparing to controls (without NaCl treatment), results showed that at low concentration ($1g.l^{-1}$ NaCl), salinity had a positive effect on different studied parameters especially stigmata yield (+12.5%), number, length and area of leaves,number and weight of corms, proline and phenolic compounds contents. Increasing salt level in irrigation water influenced more or less negatively the majority of the studied morphological parameters. For example, with 5% NaCl treatement, the stigmata yield falls 12.5%, the height of the aerial part 45%, the number and weight of cormes 49 and 35% respectively. Salt stress would also lead to a decrease but generally more or less weak in leaf water potential, chlorophyll content and PSII quantum yield, and at the same time, it would lead to an accumulation in leaves of proline, soluble sugars and phenolic compounds. These last biochemical modifications could act to help the saffron plants against salt stress in order to keep relative water and the malondialdehyde compoud contents at levels comparable to those of the controls. Present study revealed morphological, physiological and biochemical adaptations of saffron plants to cope with the presence of salt in irrigation water and may be valuable for eastern Morocco with increasing salt stress concerns.

Abbreviations : MAD :Malondialdehyde, PSII:Photosystem II, RWC:Relative water content, ψF: Leaf water potential.

1. Introduction

Arid and semi-arid areas constitute two-thirds of the earth's surface [1]. In these areas often marked by severe drought, soil salinization is considered one of the main factors limiting crop production. In Morocco, agricultural soil salinization begins to grow with the expansion of irrigated areas, nearly 500 000 hectares of arable land are subject to an increasing salinization [2].

During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolisms are affected. The earliest response is a reduction in the rate of leaf surface expansion followed by cessation of expansion as the stress intensifies but growth resumes when the stress is relieved [3]. Plants survive under drought stress by using various morphological, bio-chemical and physiological responses [4]. One of the most common stress tolerance strategies in plants is the accumulation of osmolytes including soluble sugars, proline, sugar alcohols [5]. Overall, they contribute toward osmotic adjustment, detoxifcation of reactive oxygen species and stabilization of membranes [6]. In the context of these scenarios provided about the scarcity of water and soil salinization, irrigation is generally not a viable option for alleviating the problems of drought in irrigated agricultural systems. One of the possible solutions is the selection of plant material tolerant to salt, which will remain the most economic way for exploitation of land affected by salinity [7; 8].

Saffron, dried stigmas of the flower of *Crocus sativus*, is considered among the main local product of Morocco. In 2015, the saffron plantation was conducted in a surface area of around 1.600 ha with an average

yield of 3.5t, making Morocco the fourth saffron producer in the world [9]. From the point of view of climaticrequirements, saffron is characterized by its tolerance to salinity and saline water may be used for saffron production [10].
This study aims to elucidate the influence of salt stress on the agro-morphological and physiological behaviors of saffron plant under natural conditions of semi-arid climate of the eastern region of Morocco and under saline controlled conditions.

2.Material and Methods

2.1.Experimental site

Experiments were conducted in an open field at the Experimental Research Station of the Faculty of Sciences of Oujda (Eastern region of Morocco), located at 661 m altitude and 34 ° 39 '06-71" north and 01 ° 53 '58-80" West (GPS Back Track Bushnell) [11].

2.2.Plant material

Saffron (*Crocus sativus*) corms used come from Taliouine region, the main area of saffron production in Morocco. They were planted on 18 / 10 /2011 directly into the soil and field.

2.3.Treatments used

To evaluate salt stress effect, plants were irrigated for two successive years by one of the four NaCl solutions diluted in the same amount of water (100% of ET0),whose concentrations are 0 (controls who have been irrigated just with water having 0.7 $mS.cm^{-1}$ as electrical conductivity), 1, 3 or 5 $g.l^{-1}$.

2.4.Experimental design

The adopted experimental design is randomized complete block, includes 3 blocks with a total of 60 saffron plants, the blocks indicate the repeats and sub blocks represent treatments, including basic plot covers an area of 0.5m².

2.5.Measured parameters

The effect of the salt stess was studied onsaffron yield and on many morphological, physiological and biochemical parameters.

- *Stigmata yield*:the flowers were harvested early in the morning, then the stigmas are spread on flat receptacles in the shade for a few days, and the weight was obtained by weighing the dry stigmas.
- *Morphological parameters*: This study examined the evolution of the number, length and area of leaves, number, weight and size of the corms.Leaves number was counted each month for each tuft.Leaves area was estimated each month by using AUTOCAD 2010 software. The Growth of aerial partwas evaluated each month by measuring the height of each tuft of leaves.

For the number, weight and caliber of corms: At the end of the crop cycle, plants have been dug up, and corms rid of topsoil, cleaned and de-tunicated,and next their number, weight and caliberhave been determined. Calibers were determined by using a caliper. Three sizes are distinguished: large (Diameter > 2.5cm), medium(Diameter from 1.5 to 2.5 cm) and small caliber (Diameter <1.5cm).

- *Physiological parameters :* This point of study was devoted to the determination of the leaves relative water content (RWC), leaves water potential (LWP), chlorophyll pigment content, and quantum yield measurement (φ PSII).Relative water content ofleaves was determined by the method described by Barrs [12]. Leaf water potential which represents the force with which water is retained in the plant, is measured monthly using the method of Scholander *et al.* [13].Chlorophyll pigment content was determined according to the method of Tran *et al*. [14].Total chlorophyll concentration ($mg.g^{-1}FM$)was determined by following formula : $[chl\ (a+b)] = (7.15\times OD\ 663 + 18.71 \times OD\ 646) \times V/M$ with chl (a + b) which corresponds to the total chlorophyll, V: volume of total extract (ml) M: mass of fresh material (g) and OD: optical density at 646 or 663nm.Quantum yield (φ PSII) was measured using cell fluorometer FMS model (FMS 2 Pulse Modulated Chlorophyll Fluorescence Monitoring System, Hansatech).
- *Biochemical parameters* : The biochemical parmeters analyzed were proline, soluble sugars, and malondialdehyde (MDA). Leaves proline content was determined according to the method of Monneveux and Nemmar [15]. The content of proline in the leaves was calculated with reference to a proline standard curve. Leaves soluble sugars were determined according to the method of Yemn and Willis (1954) reported by Sidari *et al*. [16]. The content of soluble sugars in was calculated with reference to a glucose standard curve. Leaves MDA (membrane lipoperoxides) was determined according to the method of Heath and Paker [17]. The amount

of MDA is calculated using a molar extinction coefficient of 155 $nM^{-1}.cm^{-1}$, according to the Beer-Lambert law: Absorbance = ε x w x C (with ε: molar extinction coefficient, w: width of the tank (1 cm), and C: Concentration).For total phenol,extractionwas performed according to the method described by Ollivier *et al.* [18]. Results were expressed in µg of caffeic acid per g of saffron fresh material with reference to caffeic acid standard curve.

2.6.Statistical Analyses

The results were subjected to a descriptive statistical analysis and analysis of variance (ANOVA), with the "SPSS for Windows version 20" software and the comparison of means was made by the Tukey test the probability level of 5 %.

3. Results and Discussion

3.1. Effects of salinetraitment on saffron yield

The field monitoring showed that salt stress influenced the flowering parameters. The first flowers were observed in the controlfollowed by treatment 1g.l^{-1}NaCl. The number of flowers per plant is inversely proportional to the intensity of stress. The highest yield was recorded in the treatment 1g.l^{-1}NaCl (+12.5%) while the lowest one (-12.5%)has been registered with the treatment of 3 g.l^{-1} NaCl (Figure 1).

The delay flowering observed in our experiment confirms other previous worksthat had shown that saffron plants affected by salinity tend to delay their flowering time [19]. The same authors found that the yieldin stigmata increased following the increase of salinity in the soil up to 50 mM (3g.l^{-1}). The sameconclusion was found by Rajaei *et al*. [20] who showed that flowering and flower size were severely reduced when the salinity exceeds 100 mM which is in agreement with our results where the yield of stigmata has increased with treatment of 1 g.l^{-1} NaCl (+ 12.5%) and decreasedwith treatments 3 and 5 g.l^{-1} NaCl.

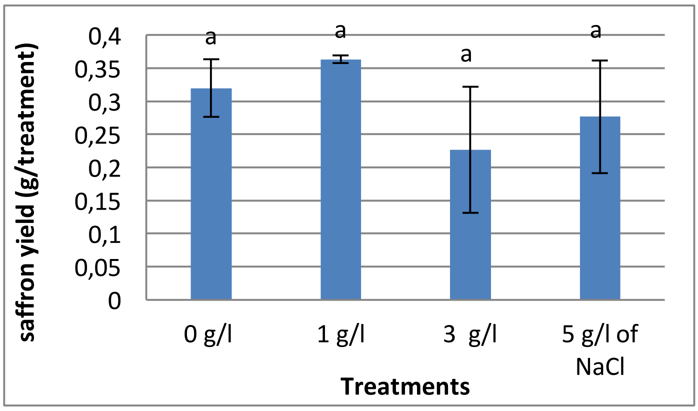

Figure 1. Effect of different levels of salt stress on saffron yield.

3.2. Effectsof saline treatment on some morphological parameterscharacterizing saffronplant

3.2.1.Effects onthe aerial part

Obtained results showed that the 1 g.l^{-1} NaCl treatment presented increases of 5,14and 20 % respectively for leaves number, leaves area andheight of aerial part of saffron plant (Table 2). However, treatments with 3 or 5 g.l^{-1}NaCl concentrations lead to a more or less important decrease in these morpholgical parameters. For example, treatment with 5 g.l^{-1} NaCl resulted inApril in reductions of 71 and 44% in the number of leaves and height of plants aerial partrespectively (Table 2).

The growth limitation observed in saffron plants has also influenced the dry biomass of the aerial part where water-irrigated treatments containing 3 and 5g / l record a very highly significant decrease (Figure2) . Our results are comparable to those found by several researchers in the same species [10, 20, 23] where the saffron plants remained alive and showed no signs of foliar damage even at 200 mM NaCl. However, the decrease in the growth of the aerial part (dry weight of leaves, number of leaves ... etc) is observed after increasing the concentration of NaCl. The same effects were observed on the argan tree seedlings [21] and Jojoba [22] which are reacted by reduction of aerial part in response to salt stress.In the case of 1g.l^{-1} NaCl treatment, the increase in the morphological parameters characterizing the aerial part of saffron plants can be explained by the fact that this low level of salinity had a nutritional positive effect on saffron plants. Indeed, Chloride (Cl) is responsible for oxygen availability to the photosystem II [23]. Sodium (Na),in turn, is involved in phosphoenolpyruvate regeneration in CAM and C4 plabts whose saffron belongs [24]. In the case of treatements with high concentrations of NaCl, the reduction in some studied morphological parameters of the aerial part could be explained by the fact that the NaCl acts by increasing the osmotic pressure of the medium which prevents the absorption of water by root system.This leads, therefore, to reduction in growth, whichwould be the result, at the cellular level, of a decrease in the number of cell divisions.

Table 2. Effect of different levels of salt stress levels on some morphological parameters characterizing the aerial part of saffron plants.

Month	NaCl treatment ($g.l^{-1}$)	Leaves number per tuft	Leaves area (cm²)	Height of plants (cm)
January	0	243^{a}	1049^{a}	29^{a}
	1	256^{a}	1051^{a}	29^{a}
	3	131^{b}	320^{b}	20^{ab}
	5	110^{b}	215^{b}	15^{b}
February	0	252^{a}	1559^{a}	33^{ab}
	1	260^{a}	1744^{a}	38^{a}
	3	123^{b}	528^{b}	23^{bc}
	5	100^{b}	321^{b}	17^{c}
March	0	224^{a}	1137^{a}	30^{ab}
	1	240^{a}	1464^{a}	36^{a}
	3	93^{b}	348^{b}	24^{bc}
	5	66^{c}	178^{b}	18^{c}
April	0	210^{a}	963^{a}	27^{ab}
	1	222^{a}	1116^{b}	35^{a}
	3	88^{b}	258^{c}	22^{bc}
	5	60^{c}	123^{c}	15^{c}

Significant differences in same column are shown by different letters (a,b,c); $p<0.05$.

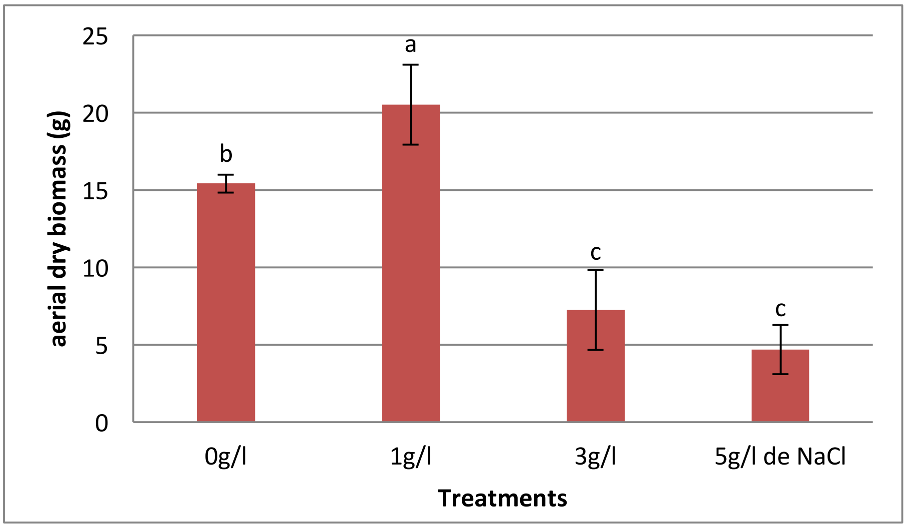

Figure 2: Effect of salt treatments (0, 1, 3, 5 g / l NaCl) on aerial dry biomass.

3.2.2.Effects on the underground part

Results showed that irrigation with salt water had opposite effects on both the number and weight of the daughter corms; positive effects at low concentrations and negative effects at high concentrations (Table 3).The highest weight value of daughter corms, 476 g, was recorded at plants irrigated with saline water containing 1 $g.l^{-1}$ NaCl representing an increase rate of 9% compared to control. However, irrigation water containing 3 or 5 $g.l^{-1}$ NaCl caused a significant reduction in the weight of corms. The latter would lose almost half their weight.In regards to number of daughter corms, applying moderate stress, 1 $g.l^{-1}$, gives more daughter corms (+7.5%) compared to the control.While, under 3 and 5 $g.l^{-1}$ NaCl treatments, the number decreased by 32 and 34 % respectively.

The size of the corms appears also dependent on the level of the applied salt stress. Control and 1 $g.l^{-1}$ NaCl treatment showed equal percentages for each rating category with a slight dominance of "big class" category. However, 3 and 5 $g.l^{-1}$ NaCl treatments marked high percentages of "medium" and "small" size (Table 3).
Although the low presence of salt in the irrigation water (1$g.l^{-1}$) could lead to a very slight improvement in these reserve organs, both in terms of number and weight, the application of relatively severe salt stress is generally manifested by a significant reduction in their weight and number compared to the control plants. Yarami and Sepaskhah [25] reported a similar trend who found that application of 1, 2, and 3 dS m^{-1} NaCl solutions causes a consecutive decline in the growth of saffron corm. The decrease in diametre of daughter corm under salt stress could be explained by the small amount of reserves stored in corms at the vegetative stage due to a reduction in photosynthates.Works of several authors worldwide [26,27,28] have shown that large caliber corms enhance precocity, flowering density and give large daughter corm for the next season.

Table 3. Effect of different levels of salt stress on some characteristics of daughter corms.

NaCl treatment (g/l^{-1})	Corms weight (g)	Corms number	Corms caliber (%)		
			Big	Medium	Small
0	436^{a}	121^{a}	39^{a}	31^{b}	30^{b}
1	476^{a}	130^{a}	38^{a}	37^{b}	24^{b}
3	226^{b}	81^{b}	15^{b}	36^{b}	49^{a}
5	233^{b}	79^{b}	8^{c}	47^{a}	45^{a}

Significant differences in same column are shown by different letters (a,b,c); $p<0.05$.

3.3. Effectsof saline treatment on water parameters of saffron plant

Results showed that the presence of NaCl induced a small reduction in relative water content (RWC) of leaves during the first months of this study (Table 3). However, a statistically significant decrease was observed on April month with5$g.l^{-1}$ NaCl treatment. Increasing the intensity of stress also causes lowering of leaf water potential (ψF) (Table 4). Same results were obtained by Rajaei *et al.* [20] who found that the RWC has decreased in a meaningful way when salinity increases by more than 100 mM NaCl (6 $g.l^{-1}$). Stability of water parameters may indicate a great capacity for osmotic adjustment [29]. It also could be due to other mechanisms independent of the decrease in leaf surface, involving regulation of leaf moisture content [30]. A sizable rainfall could also explain maintaining a high ψF and RWC during January and February, that has leached some of the salts accumulated at soil surface and thus reduce the osmotic stress.

Table 4. Effects of different levels of salt stress on water parameters of saffron plants.

Month	NaCl treatment ($g.l^{1}$)	Relative water content (%)	Leaf water potential (MPa)
January	**0**	58.32^{a}	-5.3^{ab}
	1	57.72^{a}	-4.7^{c}
	3	56.88^{a}	-5.5^{ab}
	5	57.33^{a}	-6.3^{a}
February	**0**	57.47^{a}	-7.0^{ab}
	1	60.83^{a}	-6.0^{c}
	3	56.50^{a}	-7.7^{ab}
	5	54.49^{a}	-8.7^{a}
March	**0**	60.79^{a}	-9.0^{b}
	1	57.23^{a}	-9.0^{b}
	3	55.22^{a}	-11.3^{a}
	5	53.81^{a}	-13.3^{a}
April	**0**	56.92^{a}	*
	1	55.95^{a}	*
	3	52.89^{ab}	*
	5	43.96^{b}	*

Significant differences in same column are shown by different letters (a,b,c); $p<0.05$.
*:Results of the April are not represented in the table because the measures have not yielded the results even

we apply a very high pressure

3.4. Effects of saline treatment on physiological and biochemical parameters.

3.4.1. Effects on chlorophyll content and photosynthetic activity

Results showed that irrigation with water containing 1 g.l^{-1} NaCl had virtually no effect on both chlorophyll pigment content and quantum yield of PSII (φ PSII). However, 3 and 5 g.l^{-1} NaCl treatments negatively affect these two parameters especially at the end of the season.At the highest level of salinity (5 g.l^{-1} NaCl), the lowest values of chlorophyll pigment and quantum yield of PSII were so recorded in the month of April where their reduction reach about 40 and 12% respectively (Table 5).

The decrease in photosynthesis under salt stress is cited by various authors as one of the causes of the reduction in vegetative growth and productivity [31]. However, this decrease is only 40% at highest level of salinity (5 g.l^{-1} NaCl) which is agree with the results of Yarami and Sepaskhah [25] who found that saffron photosynthesis has decreased only by 34% at highest level of salinity.

Table 5. Effects of different levels of salt stress on some physiological and biochemical parameters measured in leaves of saffron plants.

Month	NaCl Treatment (g.l^{-1})	Chlorophyll (mg.g^{-1}FM)	Quantum yield of PSII	Proline (µg.g^{-1}FM)	Soluble sugars (µg.g^{-1}FM)	Malondi-aldehyde (nmol.g^{-1}FM)	Phenols (µg.g^{-1}FM)
Jan	0	0.86^{a}	0.77^{a}	591^{c}	3136^{c}	0.00103^{a}	235^{b}
	1	0.89^{a}	0.71^{a}	717^{b}	3528^{bc}	0.00101^{a}	329^{b}
	3	0.74^{a}	0.69^{a}	1273^{a}	4101^{ab}	0.00108^{a}	308^{ab}
	5	0.71^{a}	0.66^{a}	1606^{a}	4443^{a}	0.00120^{a}	389^{a}
Feb	0	0.99^{a}	0.76^{a}	846^{b}	3389^{a}	0.00109^{a}	244^{b}
	1	1.09^{a}	0.71^{a}	1530^{a}	3959^{a}	0.00105^{a}	335^{b}
	3	0.91^{a}	0.67^{a}	1679^{a}	4419^{a}	0.00150^{a}	355^{ab}
	5	0.87^{a}	0.66^{a}	1940^{a}	4409^{a}	0.00160^{a}	420^{a}
March	0	0.96^{a}	0.78^{a}	560^{c}	2675^{b}	0.00117^{a}	377^{b}
	1	1.01^{a}	0.77^{a}	631^{bc}	2810^{b}	0.00126^{a}	399^{b}
	3	0.76^{ab}	0.75^{a}	840^{ab}	3864^{ab}	0.00123^{a}	484^{ab}
	5	0.59^{b}	0.70^{b}	939^{a}	4274^{a}	0.00166^{a}	590^{a}
April	0	0.91^{a}	0.74^{a}	562^{c}	1151^{b}	0.00182^{b}	430^{a}
	1	0.93^{a}	0.75^{a}	716^{c}	1049^{b}	0.00188^{b}	473^{a}
	3	0.69^{b}	0.66^{b}	834^{b}	1486^{b}	0.00221^{b}	406^{a}
	5	0.55^{c}	0.65^{b}	990^{a}	2020^{a}	0.00278^{a}	375^{b}

Significant differences in same column are shown by different letters (a,b,c); $p<0.05$.

3.4.2.Effects on foliar concentrations of proline and soluble sugars

High concentrations of NaCl induced a more or less accumulation of proline and soluble sugars in saffron leaves. The highest values were recorded in the treatment 5 g.l^{-1}during February month: +129% for proline and +30% for soluble sugars. However, during the last two months before dormancy (March and April), the content of these tow osmoregulators was dramatically decreased at all treatments.

In the case of osmotic stress, several studies have already mentioned the important role of osmoticums accumulation, including proline and soluble sugars [32]. In stressed saffron plants, leaf proline concentration changes with the increase in salt concentration. The same results were observed on the saffron [20] and on the corn [33]. The decrease during last two months before dormancy, causing a significant decrease in relative water content, could be explained by thedecrease in photosynthetic activity following the senescence of leaves, as it could be due to a translocation of reserve products (proteins, sugars ...) to the storage organs (Table 5).

3.4.3. Effects on leavescontent of malondialdehyde and phenolic compounds

According to the results, content of malondialdehyde (MDA) increased with intensity of stress. The largest increase was recorded in the 5 g.l^{-1} NaCl treatment and specialy in April month. In addition, stressed plants accumulate higher levels of phenolic compounds than control plants (Table 5).

The observed increase in the content of MDA could be due, on one hand, to the presence of high levels of sodium and chloride in the cells, having a direct toxic effect on the cellular membranes and enzyme systems [34], and on the other hand,it could be due to a reduction in the activity of the plant antioxidant system. The significant reduction, recorded during the month of April, of the phenolic compounds, which are considered as cellular antioxidants,could confirm the latter assumption.

3.4.4.Principal Component Analysis

The obtained results show that, with Axis 1 correlates all parameters studied which describe the physiological and agronomic be-havior of the aerial part of saffron plant. On this axis, there is a very close correlation between the vegetative growth parameters (LA, LN, LH), water parameters of the plant (RWC,LWP) and the chlorophyll content (CHLT, PSII). In addition, thereis a negative correlation between the parameters involvedinosmotic regulation (PC, SSC) and the other parameters studied. In other words the increase in the production of osmoregulatorsleads to a decrease in the growth parameters which is confrmedby the decrease of these parameters in the sever treatment (Figure3).

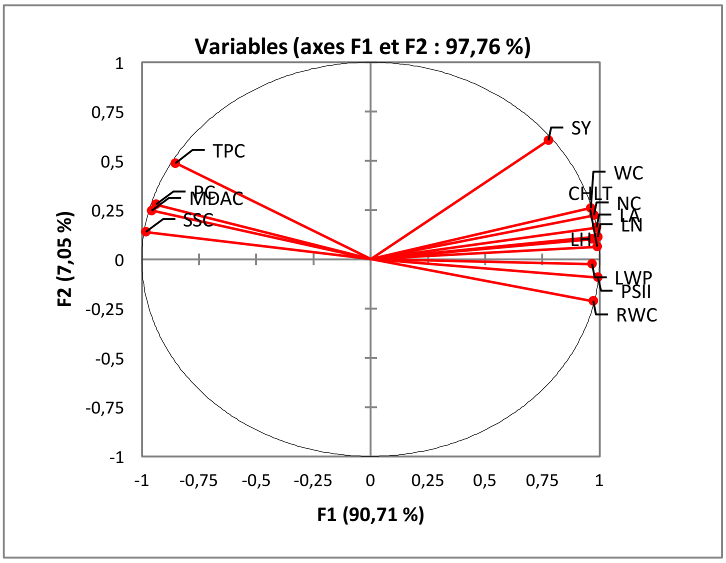

Figure 3. Representation of the studied characters in the plane (1-2).Principal Component Analysis. LA: Leaf area, LN: Leaf number, LH: Leafheight, SY: Stigma yield, RWC: Relative water content, LWP: Leaf water potential, CHLT: Total chlorophyll content, PSII: Quantum Effciencymeasurement, WC: Weight of corms, NC: Number of corms, PC: Prolinecontent, SSC: Soluble sugars content, TPC: Total phenol content, MDAC:Malondialdehyde content.

Conclusion

Obtained results in this study are consistent with several studies that have been done on saffron, where low NaCl concentration (1 g.l^{-1}) has positively affected growth and development of saffron plants. However, the levels of 3 and 5 g.l^{-1} NaCl caused a decrease of morphological parameters which is manifested by a decrease in number, length and surface of leaves and diameter of daughter corms.

In general, the morphophysiological adaptation traits were observed even at severe level of salt stress which resulted in an acceptable decrease in stigmata yield. Maintaining physiological and biochemical parameters at a high level could indicate a large capacity for osmotic adjustment allowing plants to achieve acceptable performance. This result confirms saffron tolerance to salt stress despite the decreaseassignmentof the vegetative apparatus. Encouraging saffron cultivation in arid and semi-arid regions will value those that are currently difficult to growbecause of the salinity and help support farming systems with low inputs, to improving the income of small producers and limiting the rural exodus.

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