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EFFECT OF SILICON APPLICATION ONTALIOUINE CROCUS SATIVUS (L) CULTIVATION UNDERSALT STRESS

FahimiJamila [1, 2], Bouzoubaâ Zakia [*2], AchemchemFouad [1], SaffajNabil [1], Mamouni Rachid [1]

[1] Laboratories for Agrophysiology and Post Harvest - Research Unit for Natural Resources and Terroir Products (UR RN & PDT) INRA- CRRA- Agadir

[2] Team: Materials, Catalysis and Valorization of Natural Resources, Ibn-Zohr University; Department of Chemistry, FSA. Agadir

Abstract

This study investigates the effect of silicon (Si) application on saffron plantgrown under salt stress. Therefore; Saffron, Crocus sativus L. was grown in different treatments of NaCl in presence and absence of 1 mM of silicon in its soluble form, orthosilicic acid(H4SiO4). Our results exhibited that the application of silicon enhanced the physiologicalstudied parameters and morphological attributes of saffron stigmas; the length of stigma improvement was 29% and 41,4% in saline treatments of 50 mM and 100mM respectively in presence of silicon compared to the same treatments without silicon, the dry weight of the stigma boosted by 40% for the treatment of 50mM of NaCl and 20% for 100 mM treatments compared to the same treatments in absence ofsilicon. Siliconaddition ameliorated RWC, total phenolic,anti-radical leaves activity and K+ contents and K+/Na+ ration in both roots and leaves. These results suggested that Si application enhanced saffron plant and improved the weight and length of saffron stigma.

Keywords: Saffron Crocus Sativus L; Salinity; Silicon; Stigma;Anti-Radical Activity.

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1. Introduction

Salinity is the most calamitous abiotic stress which limits the growth and yield of agricultural and horticultural crops worldwide(Charu et al. 2014; Munns, 2005; Shahbaz and Ashraf, 2013). Salt stress causes a major reduction in plants growth(Paul, 2013), it alters almost the physiological and biochemical pathways in the plants(Nabati et al., 2011; Pitman and Läuchli, 2002). According to Jamil et al. (2011), it has been predicted that more than 50% of the arable land would be salinized by the year 2050. Therefore, an alternative strategy should be adopted to mitigate the negative

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effects of salinity on the plant growth and yield; the application of silicon is declared one of the most appropriate solutions(Liang et al. 2007).

Silicon (Si)is the second most abundant element in the Earth's crust after oxygen. It has been widely reported about the Si functions in the alleviation of stress and enhancement of plant growth(Bouzoubaâ, 1991, 2007; Liang et al., 2007; Ouzounidou et al. 2016). The plant absorbs Si under its form monosilicic acid or orthosilicic acid (H_4SiO_4)(Matichenkov and Bocharnikova, 2004). Silica accumulating under the cuticle of leaves reduce water loss by transpiration under drought stress(Hodson et al. 2005). Same as in cell vacuoles, it enhances light capture by lodging which promotes photosynthetic activity(Ma et al.,2011). The absorbed siliconboosts plant resistance against salt stress and metal ions such as Zn, Al, Mn, and Cd. It also improves activities of non-enzymatic antioxidant and decreasesthe rate of lipid peroxidation(Neumann and Nieden, 2001; Moussa, 2006; Zhu et al., 2004).

Saffron *Crocus sativus* (L), belonging to Iridaceae family, is traditionally exploited to obtainthe dried stigmas of the flower, which is declared the most expensive spice of the world(Sánchez-Vioque et al., 2012). in traditional medicine, it is used for several effectssuch as cardiotonic, stomachic, antispasmodic(Hosseinzadeh et al., 2009). Moreover, saffron spice shows many pharmacological properties among which, anticonvulsant(Hosseinzadeh and Younesi, 2002), antidepressants, antinociceptive and anti-inflammatory, antioxidant, antitussive, reducing withdrawal syndrome, improving male erectile dysfunction(Razavi and Hosseinzadeh, 2015),acetylcholinesterase inhibiting(Geromichalos et al., 2012), enhancing spatial cognitive abilities after chronic cerebral hypoperfusion(Hosseinzadeh et al., 2012)hypotensive(Kianbakht and Hajiaghaee, 2011) , and significant antiproliferative effects on humancells of colorectal cancer(Aung et al., 2009). Morocco products about 3 tons; it presents 1,5% of the world production, restricted toTaliouine region and it reflects a great reputation nationally and internationally(Lage and Cantrell, 2009). An estimated 75000 blossoms or 225000 hand-picked stigmas are needed to make one pound of saffron, this can explain its market price(Aytekin and Acikgoz, 2008), that makes it a rare spice of great commercial value(Aït Oubahou and El Otmani, 2002)andso, granted to this cultivation a high-value resource for job creation and improvement of family income, especiallyin rural area.

Crocus sativus L. is an important cultivation, it isthe most expensive spice in the globe. On the other hand; salinity is a worrying problem for agriculture which is getting worse, affecting more areas and so causing aserious and continuous progress of saline lands. Therefore, the application of silicate fertilizer may, in fact,mitigate drought stress (Ali et al., 2008), reduce the high demand for irrigation, which in turn would diminish salinization of cropland. Moreover, silicon is not corrosive and pollution-free,accordingly, silicon fertilizer is a high-quality fertilizer for developing ecologically green agriculture (Zhu and Gong, 2014). Due to those facts, the main objective of the proposed study was to evaluate the effects of silicon on biochemical aspects of saffron and morphological attributes of its stigma under salt conditions.

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2. Materials and Methods

Plant Material and Growth Conditions

In order to evaluate the silicon effect on saffron *Crocus sativus* L. under salinity stress conditions, an experiment as RCBD (Randomize Complete Block Design) in three replications was performed in the greenhouse at The National Institute for Agricultural Research –INRA of Agadir. The experimental treatments concluded: salinityat 2 levels 50 mM NaCl, 100 Mm NaCl with1 mM calcium silicate. The pots were prepared for the cultivation of saffron corms filling with sand and peat with the rate of 1:1; then all pots were irrigated with water. 24 hours later three saffron corms were planted in each pot, then each pot was uniformly irrigated with the water treatments. All agricultural operations such as weeds removal, irrigation with salt water, were done until the end of the growth period (about 4 months)(Fahimi et al., 2017). Some parameters of saffron were measured and recorded during experiment time.

Extraction and Analyze of Mineral Elements

After drying, the samples (leavesand roots) were reduced to fine powder by means of a ball mill. Known quantities of vegetable powder previously dried in an oven are placed in the pellets in the presence of a known volume of 0.5% nitric acid. The dry matter/volume ratio is 20 mg of dry matter for 50 ml of acid. Hermetically closed pills to avoid concentration of the extracts by evaporation are stirred periodically 4 to 5 times per day. The extracts were then filtered on filter paper without ash and are therefore ready for the determination of the mineral elements(Hamrouni et al., 2011). Na and K in the sample solution were analyzed using a flame photometer.

Relative Water Content

The Relative water content (RWC) measurement allows knowing the relative water content of the plant. The calculation of this value requires the measurement of 3 parameters: fresh mass (FM), imbibedmass(IM) and dry mass (DM). The fresh mass is weighed immediately after harvesting; the imbibed mass is measured after 24h of immersion in distilled water at 4 °C. and the dry mass (DM) is determined after 24 hours of drying the sample in an oven at 80 °C. The RWC is calculated by applying the following equation (Dany, 2013)

RWC = 100 * (FM-DM) / (IM-DM)

The number of plants is fifteen samples for each treatment.

Total Phenolic Content

To extract the polyphenols from different parts of our plant by maceration, we opted for the protocol described by Romani et al. (2006)with some modifications: 1g of leaves powder is macerated at room temperature for 12h with 10 ml of aqueous solutions of the methanol 70%. After filtration on a muslin tissue, the filtrates are centrifuged for 20 min at 4000 rpm at room temperature, filtered on filter paper N°1 and stored at 4 °C until use. The total phenolic of the extracts were determined using the Folin and Ciocalteu reagent, following the method described by Singleton and Rossi with slight modifications.100 µl of saffron leaves extract are mixed with 500 µl of the FolinCiocalteu reagent, after 5 min is added 400 µl of Na_2CO_3 with 7.5 % (w/v)) and the volume was made up to 3 ml with distilled water. The reaction was kept in the dark for 30 min and after centrifuging the absorbance of blue color from different samples was measured at

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765 nm. The phenolic content was calculated as gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of gallic acid. All determinations were carried out in triplicate. Sample and standard readings were made using a spectrophotometer at 765 nm against the reagent blank.

Antioxidant Activity Assay

The antioxidant activity of the prepared leave extracts from saffronwas assessed by measuring the scavenging power of free radical 2,2-diphenyl-1-picrylhydrazyl(DPPH) according to the method of Hossain et al., (2014). All extracts were dissolved in methanol and assayed in a quad. The crude extract such was taken in a test tube and dissolved with 10 mL of methanol. Five concentrations such as 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/ mL were prepared through dilution method. DPPH (3.3 mg) was taken in a 100 mlvolumetric flask and dissolved with methanol. Each prepared concentration (1.5 mL) was taken in a test tube and added 2.5 mL of DPPH solution. The prepared mixture was shaken gently by hand and kept in dark place for 90 min. The absorbance of the samples was measured by using UV-visible spectrophotometer at wavelength 517 nm. Finally, the inhibition of free radical DPPH percentage I (%) is calculated using the following formula:

$$\text{Inhibition (\%)} = 100 \times (\text{Acontrol} - \text{Atest})/\text{Acontrol}$$

Where Acontrol is the absorbance of the control (containing all reagents without the test product) and Atest is the absorbance of the test compound (containing all reagents and the test product). The value IC50 (µg/ml) is calculated from the graph of the DPPH scavenging effect percentage against the sample concentration and it is used to characterize the antioxidant activity of different examined extracts, the extract concentration required to cause a 50% inhibition. A lower IC50 value corresponds to a higher antioxidant activity of the plant extract. All tests were performed in quadruplicate for each concentration.

Length and Weight of Saffron Stigma

After saffron stigma harvest, we measure its length using a ruler. The weight is measured by precision scale after its dry.

Statistical Analysis

Data were analyzed using the statistical software (Minitab 17); mean data were compared by Tukey's test ($p \leq 0.05$).

3. Results and Discussion

The higher dry weight of saffron stigma was obtained in the treatment of silicon; the addition of silicon increased markedly the dry weight of the stigma by 40% for the treatment of 50 mM of NaCl and 20% for 100mM treatments compared to the same treatments without silicon. Same as the length of stigma, the value was 6,35 cm in the treatment of silicon, the percentage of its improvement is 52,3% compared to the same treatment without Si,29% and 41,4% in saline treatments of 50 mMand 100 mM respectively (Table 1). Thus, the supply of silicon augmented the stigma of *Crocus sativus*, those results are in agreement with many studies that reported the

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role of silicon in enhancing the agricultural crop quality, growth and yield(Ahmed et al., Khurshid, 2011; Balakhnina and Borkowska, 2013; Korndörfer and Lepsch, 2001).

Table 1: Dry weight and length of saffron stigma of plants grown under salt condition in absence and presence of Silicon

Treatment	Dry weight of the stigma (mg)	Length of the stigma (cm)
0mM NaCl	0,006±0,0008^{ab}	4,17±0,47^{c}
50mM NaCl	0,005±0,002^{ab}	5,72±1,07^{b}
100mM NaCl	0,005±0,0012^{b}	5,15±0,43^{bc}
0 NaCl/ + Si	0,007±0,0011^{a}	6,35±1,04^{ab}
50mM NaCl/ +Si	0,007±0,0012^{a}	7,38±1,23^{a}
100mM NaCl/ +Si	0,006±0,002^{ab}	7,28±1,93^{a}

Data are expressed as mean values ±SD. Values followed by different letters (a, b,c) in the same column are significantly different at P≤0,05

Plant growth is greatly combined with many physiological factors such as RWC; our present data showed that the lower percentages of RWC were obtained in the control treatment also under salt conditions (fig 1), while the application of silicon promoted the RWC in stressed conditions, it raised the RWC by 6% and 8% in the treatments of 50 mM and 100 mM of NaCl respectively, Hodson et al., (2005) reported the same thing, which it may due to the accumulation of silicon under the cuticle of leaves that leads to reducing water loss by transpiration under drought stress. Moreover, Ma reported that silicon can strengthen xylem vessels (Ma, 2004), that are structures responsible for water transport into the plant (McElrone et al., 2004). Consequently, this firm xylem vessels can just allow to plants a resistance in stressful environments, likewise enhancing water absorbed by plants (Sperry et al., 2002). Abbas et al., (2015)as well demonstrated that the application of silicon enhanced the RWC of okra (*Abelmoschus esculentus*) species.

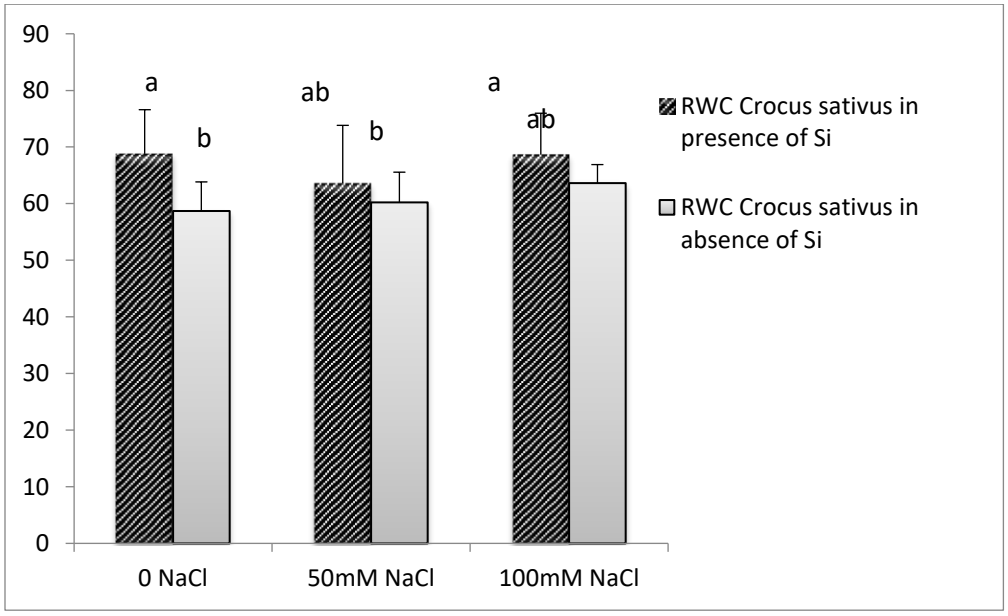

Figure 1: Relative water contents in *Crocus sativus* plant under different treatment of NaCl with or without Silicon

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Data are expressed as mean values ±SD. Values followed by different letters (a, b) are significantly different at P≤0,05

Table 2: Leaf polyphenol concentrations and anti-radical activities in Taliouine*Crocus sativus* L. leaves under different treatments of NaCl with or without Silicon

Treatment	Leaves total phenol contents µg GAA g−1 DW	IC50 µg/ml
0 NaCl/ 0 Si	83,26±2,51[a]	195.13±5.39[bc]
50 mM NaCl/ -Si	93,02±5,91[a]	227.91±41.80[ab]
100 mM NaCl/ -Si	80,00±28[a]	266.22±25.10[a]
0 NaCl/ + Si	94,22±2,13[a]	181.58±13.71[bc]
50 mM NaCl/ +Si	94,07±2,92[a]	175.80±9.70[c]
100 mM NaCl/ +Si	99,15±2,92[a]	156.65±12.64[c]

Data are expressed as mean values ±SD. Values followed by different letters (a, b, c and d) in the same column are significantly different at P≤0,05

Table 2: Na, K concentrations (ppm) and K+/Na+ ratio in leaves and roots of saffron grown under different treatments of NaCl in absence and presence of Silicon.

Treatment	Na^+ contents in leaves(ppm)	K^+ contents in leaves (ppm)	K^+/Na^+Ratio in leaves	Na^+ contents in roots (ppm)	K^+ contents in roots (ppm)	K^+/Na^+ ratio in roots
0 NaCl/ 0 Si	15,67±0,91ab	9,00±2,65b	0,58±0,19b	43,93±4,03a	22,63±3,32b	0,52±0,09c
50 mM NaCl/ -Si	16,17±0,47a	6,20±0,72b	0,38±0,05b	45,83±1,79a	12,70±4,21c	0,28±0,08d
100 mM NaCl/ -Si	17,53±0,5a	6,47±2,23b	0,37±0,12b	48,83±2,93a	7,10±0,87c	0,15±0,02d
0 NaCl/ +Si	12,93±1,62c	15,00±0,90a	1,17±0,16a	21,50±0,35c	41,17±3,35a	1,92±0,16a
50 mM NaCl/ +Si	13,57±0,45bc	17,07±0,95a	1,26±0,10a	31,43±1,5b	40,43±2,91a	1,29±0,03b
100 mM NaCl/ +Si	15,70±0,44ab	18,00±1,90a	1,15±0,15a	35,87±3,72b	40,97±2,73a	1,15±0,05b

Data are expressed as mean values ±SD. Values followed by different letters (a, b, c and d) in the same column are significantly different at P≤0,05

In stressful environments, plants act by producing different osmolytes and antioxidant compounds as total phenolic (Ashraf and Foolad, 2007).In the previous study, our results exhibited proline was accumulated in leaves of the saffron plant grown at the high concentration of salinity, same as the contents of total sugar in leaves (Fahimi et al., 2017). Total phenolics are cellular solutes which minimize environment stress (Singh, 2004); in table 2, salt stress didn't affect total phenolic contents. Whereas, the amount of silicon application augmented leaves total phenol contents of saffron but not significantly, it is 13% in absence of NaCl and 4% in presence of NaCl for both concentrations 50 mM NaCl and 100 mM NaCl. Leaves total phenol contents and leaves anti-radical activity showed a strong negative correlation (-0.76), silicon-treated plants showed

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higheranti-radicalactivity. The present data are in agreement with what Abbas et al., (2015)found in his work on Okra-7080, it presented maximum total phenolics when treated with Si. Therefore, they concluded that the application of silicon increased antioxidant activities and level of certain osmolytes and reduction in toxic ions (Na and Cl) which led to enhance plant biomass, total chlorophyll contents, photosynthetic activity, RWC and total soluble proteins under NaCl stress. Hence, it is suggested that Si is an excellent stress-mitigating agent for protecting plants from NaCl induced toxicity(Abbas et al., 2015). Moreover,Sahebi et al.,(2015)reviewed that supply of Si mitigates stress and boosts plant growth under abiotic stress such as salinity. The apoplastic transport of Na+ and Cl− ions was decreased by Si deposition under salt conditions. Zhu and Gong reported that silicon mechanisms under salt conditions comprise the aspects of upkeeping optimal water content, ameliorating photosynthesis activity and curbing transpiration rate, mitigating ion toxicity thus reducing oxidative stress, and biosynthetic regulation of solutes and plant hormones (Zhu and Gong, 2014). Likewise, in their work Manivannan et al., (2016)revealed that Si protected *Capsicum* from salinity stress by alleviating oxidative stress and enhancing growth by regulating photosynthesis, nutrient management, and antioxidant enzyme metabolism.In this study, Na^+ concentrations in both leaves and roots of *Crocus sativus* were higher under salinity conditions, but silicon application decreased significantly sodium concentration in both leaves and roots in salt conditions especially in roots, the percentages of decrease of sodium contents in leaves were 16,17% and 10,53% for the salt treatments of 50mM and 100mM of NaCl respectively in presence of Si compared to the same treatments in absence of Si. However, the percentages were more important in roots, 31,4% and 26,5% in 50mM and 100 mM NaCl treatments with Si application respectively compared to the same treatments in absence of Si. Moreover, stress conditions reduced noticeably K^+ concentrations in both leaves and roots tissues of *Crocus sativus* compared with the treatments without silicon. Leaf and root potassium concentrations were significantly increased when Si was added specially to salt treatments. In leaves, the contents were more than twice for both salt treatments 50mM and 100mM with Si supply compared to the same treatments without Si. In roots as well, the K^+ concentrations were three times more important for salt treatment of 50mM with Si than its concentrations in absence of Si, and five times for salt treatment 100 mMin presence of Si. In addition to that, the addition of Si increased K^+/ Na^+ ratio in leaves and roots as well(Table 3).Various studies reported that Si may mitigate salt stress by affecting Na^+ and K^+ concentrations(Ashraf et al., 2010; Garg and Bhandari, 2016). For example, in their work on *Cicer arietinum* L., Garg and Bhandari (2016) found that the addition of silicon under salt conditions reduced the Na^+ uptake in roots and in leaves but it enhanced K^+ concentration as well as the K+/Na+ ratio.

4. Conclusion

Regarding our results, the inclusion of silicon to *Crocus sativus* L. improved biochemical parameters of Taliouine *Croccus sativus* (L) plant and morphological attributes of its stigma grown under salt stress through increasing RWC, decreasing the uptake of Na^+ as a toxic ion in roots and its translocation to leaves while promoting K^+ uptake in both roots and leaves, slight enhancement of leaves anti-radical activity and total phenolic which lead to promote the stigmas of the crop.

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*Corresponding author.
E-mail address: bouzoubaa_zakia@ yahoo.fr