Higher activity of PSI compared to PSII accounts for the beneficial effect of silicon on barley (*Hordeum vulgare* L.) plants challenged with salinity

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Abstract

This study was conducted to assess whether silicon (Si) supply can alleviate the harmful effects of severe salinity in barley (*Hordeum vulgare*). Plants were grown on non-saline (0 mM NaCl) or saline (200 mM NaCl) nutrient media supplemented or not with 0.5 mM Si. Salinity impacted plant morphology and induced sodium and chloride accumulation within plant tissues. It significantly affected almost all measured parameters. Interestingly, Si supply alleviated salt stress effects on plant morphology, growth (up to +59%), water status (up to +74%), membrane integrity (up to +35%), pigment contents (up to +121%), and the activity of the two photosystems (PSI and PSII) by improving their yields, and by reducing their energy dissipation. Si beneficial effect was more pronounced on PSI as compared to PSII. As a whole, data inferred from the present study further confirmed that silicon application is an effective approach to cope with salinity.

Introduction

Climate change severely affects agricultural productivity since cultivated plants are increasingly confronted with various abiotic stresses, such as salinity, drought, nutritional deficiencies, *etc.* (Tomaz *et al.* 2020, Jiao *et al.* 2021). Salt stress is a major environmental problem worldwide. The total land area of the world that is severely affected by salinity is around 1.5 billion hectares (Abdelly *et al.* 2010). Many countries will face more and more

Highlights

- Under salt stress, silicon enhances growth, water status, and photosynthetic activity
- Silicon enhanced all parameters related to both PSI and PSII
- PSI was more altered by salt and responded better to Si supply compared to PSII

problems related to salinization than they are currently encountering, in particular, countries in arid and semiarid regions. This increase in salinization has been even faster especially in the last 20–30 years because global climate changes have begun to come into the agenda and there are significant land deterioration and salinization (Okur and Örçen 2020).

Salinity affects plant morphology as well as physiological and biochemical processes. Indeed, in addition to the reduction of plant growth, photosynthesis, mineral nutrition, and water-use efficiency, high salinity leads also to physiological drought and ionic toxicity in plant tissues, reducing crop productivity and agricultural yields (Khan *et al.* 2019). These deleterious effects of salt stress on plant growth are related to (*1*) low osmotic potential of the soil solution (water osmotic stress), (*2*) nutritional imbalance, (*3*) specific ion effect (salt stress), or (*4*) a combination of these factors (Ashraf 2004). The osmotic stress is due to the loss of cellular water content, caused by Na^+ and Cl^- ions accumulation (ion toxicity). These ions are the main contributors to the cellular osmotic potential, and they have to be compartmentalized and sequestrated into vacuoles or endosomal compartments by ion exchangers and the H^+ pumps localized in the tonoplast or endosomal membranes (Isayenkov *et al.* 2020). This subsequently limits nutrient uptake and photosynthetic assimilation by crops to increase productivity (Xue *et al.* 2019). Salinity has been also reported to induce an overaccumulation of reactive oxygen species (ROS) (Zhu 2001, Challabathula *et al.* 2022), leading to oxidative stress that is responsible for pigment degradation, lipid peroxidation, membrane alteration, protein denaturation, and DNA mutations (Mittler 2002, Noctor *et al.* 2014). As regards photosynthesis alteration, it can be due to stomatal and/or nonstomatal effects (Pan *et al.* 2021). Stomatal photosynthesis limitation is the consequence of stomatal closure which limits leaf gas exchange (Fricke *et al.* 2004). Chloroplasts that are the main source of salt-induced ROS accumulation are themselves targets of damages caused by ROS, which leads to photoinhibition as a nonstomatal limitation of photosynthesis (Mittler 2002, Pottosin and Shabala 2016).

One of the various mitigation and adaptation approaches used to overcome these negative effects of high soil salinity is the exogenous application of silicon (Si), which has been recognized as a recent ecological approach for enhancing salinity stress responses in plants (Khan *et al.* 2019). Silicon is ranked second after oxygen, among

the eight most abundant elements on the Earth's crust. It was shown to have beneficial roles in plants against abiotic and biotic stresses. In general, Si is not considered essential for plant growth due to the lack of evidence for its involvement in plant metabolism (Dhiman *et al.* 2021). Nevertheless, its application to plants provides them with better tolerance to various environmental stresses, such as salinity (Akhter *et al.* 2022, El Moukhtari *et al.* 2021a,b; Laifa *et al.* 2021), drought (Thorne *et al.* 2020), and nutritional disorders (Benslima *et al.* 2021, 2022; Ksiaa *et al.* 2022).

The molecular mechanism of Si uptake varies amongst species. The mechanism of root uptake of Si and subsequent transport to shoots can be active, passive, or rejective (Takahashi *et al.* 1990). In active mode, plants absorb Si faster than water, resulting in a lack of Si in the nutrient solution. In contrast, the passive mode of Si uptake occurs at the same rate as water. Plants that tend to eliminate Si show an increase in Si concentration in the uptake solution, indicating a mechanism of rejection (Mitani and Ma 2005). Due to different accumulation mechanisms, plants are classified as high, medium, and non-accumulators or excluders. It has been reported that most monocots have both active and passive xylem load of Si, such as rice, a typical Si accumulator (Takahashi *et al.* 1990, Mitani and Ma 2005), barley (*Hordeum vulgare*) (Nikolic *et al.* 2007), and wheat (*Triticum aestivum*) (Jarvis 1987, Rains *et al.* 2006). On the contrary, most dicots take up Si passively (Takahashi *et al.* 1990). Some dicots such as cucumbers (*Cucumis sativus*) with moderate Si accumulation show an active mode of absorption (Liang *et al.* 2005), in addition to the passive mode (Faisal *et al.* 2012). Some other dicots such as tomatoes restrict Si transport from root to shoot, thus having low Si contents (Takahashi *et al.* 1990, Mitani and Ma 2005).

Numerous research studies have shown the advantageous effects of silicon against salinity since it reduces the harmful salt-induced effects in plants (Coskun *et al.* 2019). Its supply has a beneficial impact on plant growth and development as well as on salt ion distribution by decreasing the apoplastic transportation of Na^+ and Cl^- and by adjusting their absorption and accumulation. Some studies indicated that Si acts as a mechanical barrier preventing Na^+ uptake by roots and that Si deposition strengthens cell walls through suberization and silicification (Akhter *et al.* 2022). Other possible mechanisms include the reduction of oxidative stress (Mahmoud *et al.* 2020), the enhancement of photosynthesis (Abdelaal *et al.*

Abbreviations: Car – carotenoids; Chl – chlorophyll; DM – dry mass; ETR – electron transfer rate; F – fluorescence yield measured briefly before application of a saturation pulse; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; F_m' – maximal fluorescence yield of the light-adapted state; F_v/F_0 – maximum quantum yield of PSII photochemistry (more sensitive than F_v/F_m); F_v/F_m – maximal quantum yield of PSII photochemistry; MDA – malondialdehyde; NPQ – nonphotochemical quenching; $P700_{ox}$ – oxidized PSI; $P700_m$ – maximal fluorescence yield of dark-adapted sample with all PSI centres closed; $P700_m'$ – maximal fluorescence yield of illuminated sample with all PSI centres closed; PAM – pulse-amplitude modulation; ROS – reactive oxygen species; $Y_{(I)}$ – quantum yield of photochemical energy conversion in PSI; $Y_{(II)}$ – quantum yield of photochemical energy conversion in PSII; $Y_{(NA)}$ – quantum yield of nonphotochemical energy dissipation in reaction centres limited by acceptor side; $Y_{(ND)}$ – quantum yield of nonphotochemical energy dissipation in reaction centres limited by donor side; $Y_{(NO)}$ – quantum yield of nonregulated nonphotochemical energy dissipation in PSII; $Y_{(NPQ)}$ – quantum yield of regulated nonphotochemical energy dissipation in PSII.

2020), the regulation of osmolarity (Yan *et al.* 2020), mineral uptake and deposition in tissues (Al Murad *et al.* 2020), and the antioxidant defense system (Farouk *et al.* 2020). For instance, the addition of Si improves plant growth and yield and grain quality in rice (Ma and Takahashi 2002). Similar beneficial effects were also observed in other cereals such as wheat (Tuna *et al.* 2008) and sea barley (*Hordeum marinum*) (Laifa *et al.* 2021). Moreover, the application of silicon increases transpiration rate and net CO_2 assimilation under various stresses (Shen *et al.* 2010). The use of Si effectively prevented the degradation of photosynthetic pigments. Photosynthesis-related proteins, such as PSI, PSII, Rubisco, and other chloroplast-related proteins were regulated by Si under saline and hyperhydric conditions (Liu *et al.* 2019).

It has been reported also that Si application can improve the expression of key genes related to photosynthesis, increase the contents of photosynthetic pigments, and improve CO_2 uptake (Li *et al.* 2018). It has been reported by Khan *et al.* (2017) that, under salt stress, the application of silicon improved photosynthetic seedling behaviour *via* the enhancement of chlorophyll (Chl) content and the maximum photochemical yield of PSII (F_v/F_m).

The main objective of this work was to study the beneficial effects of Si on growth, water status, and mineral nutrition, and to evaluate the photosynthetic performance, in barley (*Hordeum vulgare*), under severe salt stress (200 mM NaCl). These parameters are generally affected by salinity, therefore, measuring them can provide us with the state of the plants and their tolerance to such abiotic stress. This plant is an important nutritional cereal crop grown worldwide and has an interesting economic potential. Moreover, it is recognized for certain salt tolerance and is also known for its large capacity to accumulate silicon. We hypothesized that Si improves all mentioned parameters in *H. vulgare* and enhances the photosynthetic performance even under severe salinity.

Materials and methods

Plant material and growth conditions: *Hordeum vulgare* seeds of the local cultivar Manel were sterilized with 150 mL of distilled water containing 200 µL of bleach for 5 min and then rinsed 3 times with distilled water. The disinfected seeds were germinated in perlite and moistened with distilled water for 6 d. The obtained seedlings were transplanted into dark plastic buckets of 5.75 L each (20 seedlings per bucket) and hydroponically grown in a half-strength Hewitt's nutrient medium (Hewitt 1966) continuously aerated by aquarium pumps. After seven days of acclimatization, plants were subdivided into four lots, two buckets per lot, each bucket containing 20 plants. Plants in the first two lots were cultivated under nonsaline conditions: one nontreated with silicon (Control) and the other added with 0.5 mM of Si (Si treatment) in the form of sodium silicate Na_2SiO_3 (*Sigma-Aldrich*). The remaining two lots receiving the same silicon concentrations (0 and 0.5 mM Si) were grown in the presence of 200 mM NaCl. The experimental design was a factorial experiment in a randomized complete block design. According to Debez *et al.* (2020), the local cultivar Manel is considered moderately tolerant to salinity compared to other cultivars.

The choice of the silicon concentration (0.5 mM Si) was based on a preliminary study (data not shown). The experiment was conducted in a greenhouse, in the Centre of Biotechnology of Borj-Cedria (North-East Tunisia, 36°42'32.9"N, 10°25'40.9"E), under sunlight conditions at 23/25°C. Relative humidity ranged from 60 to 80% and light intensity varied from 400 to 1,200 µmol(photon) $m^{-2} s^{-1}$.

Harvest and growth measurements: After 7 d of treatment, plants were harvested and cut into shoots and roots. Roots were washed once with HCl cold solution (0.01 M) to remove extracellular nutrients, then twice with cold distilled water (maintained at 4°C), and finally dried with filter paper. Shoot and root samples were immediately weighed for fresh mass (FM) determination and then oven-dried at 60°C for 5 d for dry mass (DM) determination. Plants reserved for biochemical tests were stored in the freezer at –20°C.

Water content: Water contents (WC) of both shoots and roots were calculated using the following equation: WC [ml(H_2O) g^{-1}(DM)] = (FM – DM)/DM.

Photosynthetic pigment concentrations: The extraction of photosynthetic pigments was done using the method of Lichtenthaler (1987). Fresh leaf discs (50 mg) were introduced into vials containing 3 ml of 80% acetone. The vials were then placed in the dark at 4°C for 7 d. The absorbance of the extracts was determined at 470, 646, and 663 nm for Chl *a*, Chl *b*, and total carotenoid assays, using a UV-visible spectrophotometer (*Specord 210 Plus*, *Analytik Jena*, Germany). Chl (*a* and *b*) and carotenoid contents were calculated according to the following formulas: Chl *a* [µg ml^{-1}] = (12.21 × A_{663}) – (2.81 × A_{646}), Chl *b* [µg ml^{-1}] = (20.13 × A_{646}) – (5.03 × A_{663}), carotenoids [µg ml^{-1}] = (1,000 × A_{470} – 3.27 [Chl *a*] – 104 [Chl *b*])/229, where A_{470}, A_{646}, and A_{663} represent extract absorbance at 470, 646, and 663 nm, respectively.

PSI and PSII activities: The measurement of PSI and PSII activities was performed as described by Klughammer and Schreiber (2008a,b). The measurements were carried out using *Dual-PAM-100* (*Heinz Walz*, Effeltrich, Germany) on *H. vulgare* leaves preadapted to the dark (stored before measurements for 30 min in the dark).

Measurements of the operational photochemical efficiency and energy dissipation of PSII were also made. First, the minimum level of fluorescence (F_0) was determined by applying very low-intensity light. Under these conditions, the reaction centres of PSII were all open and fluorescence was minimal. Then, a maximum level of fluorescence (F_m) was obtained thanks to a flash of saturating light, which caused the closure of all reaction centres of PSII and led to an overall reduction in photochemical efficiency and therefore to a maximum increase in fluorescence (F_m).

Then, leaves were exposed to an actinic light (0, 6, 12, 21, 56, 107, 146, 257, 412, 652; and 1,017 μmol(photon) m^{-2} s^{-1}) that initiated electron transport between photosystems to record the yield of photochemical energy conversion in PSII [Y$_{(II)}$], the yield of regulated nonphotochemical energy dissipation in PSII (NPQ), and the yield of nonregulated nonphotochemical energy dissipation in PSII [Y$_{(NO)}$].

PSI absorbance was measured by P700 dual-wavelength emitter-detector units (830 and 875 nm) according to Klughammer and Schreiber (2008b). For this photosystem, the yield of photochemical energy conversion in PSI [Y$_{(I)}$], the yield of nonphotochemical energy dissipation in reaction centres limited by the acceptor side [Y$_{(NA)}$], and the yield of nonphotochemical energy dissipation in reaction centres limited by donor side [Y$_{(ND)}$] were measured.

Lipid peroxidation: Membrane lipid peroxidation was assessed by measuring leaf malondialdehyde (MDA) concentration. Fresh leaves of *H. vulgare* were homogenized at 4°C in a 5% (w/v) trichloroacetic acid (TCA) solution. Then, the homogenate was centrifuged for 15 min at 13,000 × *g* at 4°C. An aliquot of the supernatant (1 ml) was added to 0.5% thiobarbituric acid (TBA) prepared in 20% TCA. The mixture was incubated at 95°C for 30 min in a shaking water bath and then cooled in an ice bath. After that, centrifugation was performed at 13,000 × *g* for 5 min and the supernatant was collected for a colorimetric assay at 532 and 600 nm.

Nutrient extraction and analysis: To extract ions, obtained dry matter (after 15 d at 60°C) from roots (15 mg) and shoots (25 mg) was incubated in 1 N H$_2$SO$_4$ (15 ml) at 80°C for 1 h (Zorrig *et al.* 2010, 2019). K$^+$ and Na$^+$ contents were determined by flame photometry (*BWB flame photometer*, *BWB Technologies*, United Kingdom), and those of Cl$^-$ ions were determined by a chloride meter (*Model 926, Sherwood Scientific Ltd.*, Cambridge, United Kingdom).

Statistical analysis: All statistical analyses were performed with *XLSTAT* software version 2014 (https://www.xlstat.com/en/). Two types of statistical analysis were performed throughout this work: a comparison between two independent sample groups (according to the *Student*'s *t*-test at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$) and a Principal Component Analysis (PCA) considering variables centered on their means and normalized with a standard deviation of 1.

Results

Morphological aspect: When subjected to severe salt stress plants showed an obvious reduction in their size as compared to the control although Si presence in the saline medium improved plant vigour (Fig. 1).

Root and shoot fresh masses: In control plants, root FM was approximately 0.43 g (Fig. 2A). This parameter was reduced to 0.19 g under salt stress. Shoot FM also decreased from 1.3 g to 0.27 g (Fig. 2B). The addition of 0.5 mM Si under salinity stress showed an improvement in shoot and whole plant FM by 59 and 40%, respectively, but no improvement in root FM.

Water content: Salt stress showed a reduction of 65% in shoot water content compared to the control (Fig. 3B). However, any significant reduction was observed in root water content (Fig. 3A). The enhancing effect of silicon on tissue hydration was only observed in shoots under both non-saline (+11%), and saline (+74%) conditions.

Photosynthetic pigment concentrations: Salt stress caused a statistically significant reduction in Chl *a*, Chl *b*, and carotenoid contents by 73, 74, and 48%, respectively (Fig. 4A–C). Silicon supply induced a statistically significant amelioration only for Chl *b* content under saline conditions (*ca.* +121%).

An increase in the ratio of carotenoid content to Chl (*a+b*) contents was recorded under saline conditions,
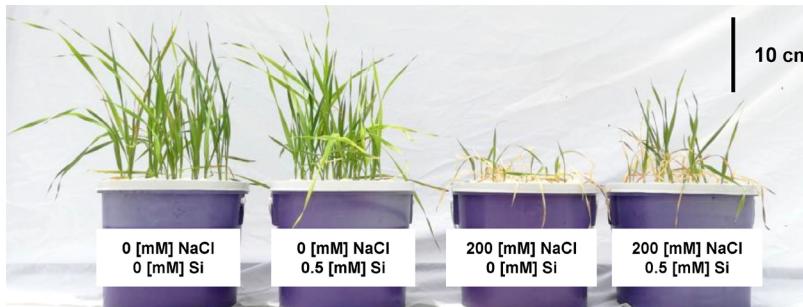
Fig. 1. Morphological aspect of *Hordeum vulgare* plants, at the age of 20 d, exposed during the last 7 d of culture to two concentrations of silicon (Na$_2$SiO$_3$) and NaCl. The two buckets on the left represent plants with two concentrations of silicon (0 and 0.5 mM Si) in the absence of NaCl. The two buckets on the right represent plants with two silicon concentrations (0 and 0.5 mM Si) in the presence of NaCl (200 mM). This photograph corresponds to only one of three cultures carried out separately under similar conditions and shows reproducible results. For the three performed cultures, we showed a depressive effect of the NaCl against a beneficial effect of silicon.
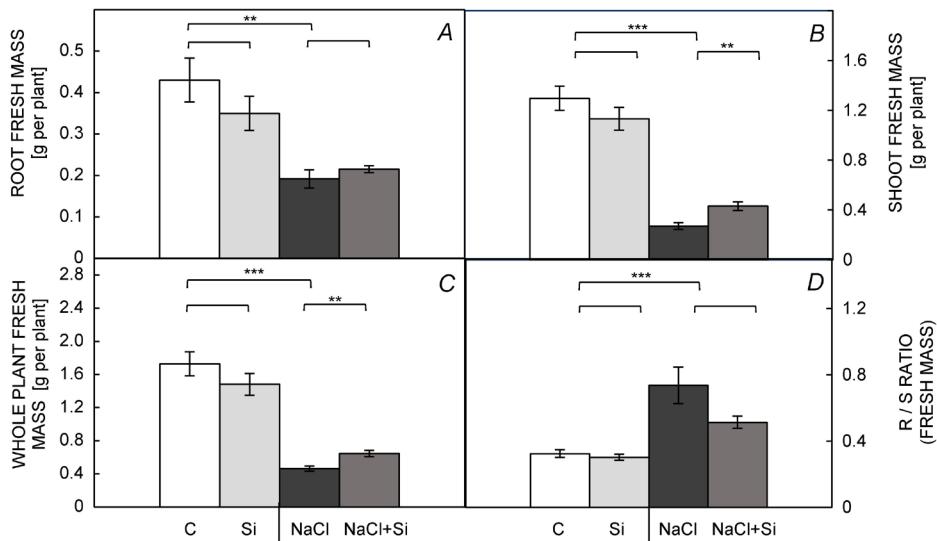
Fig. 2. Effect of silicon on the root (*A*), shoot (*B*), and whole plant (*C*) fresh mass and the root/shoot ratio (*D*) in salt-treated and non-salt-treated plants. *Hordeum vulgare* plants were exposed to two concentrations of silicon (0 and 0.5 mM) in the absence or presence of NaCl (200 mM) during the last 7 d of culture. C – control plants. The values are averages of seven replicates. Error bars correspond to standard errors. *Asterisks* mark the statistically significant differences according to *Student*'s *t*-test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).
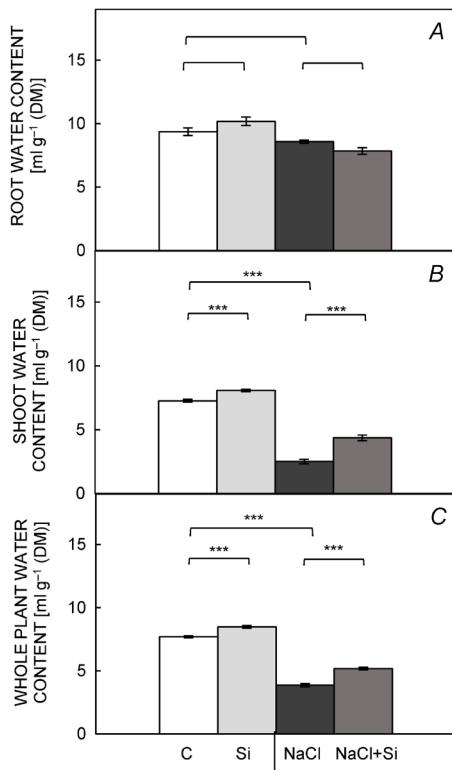
Fig. 3. Effect of silicon on the root (*A*), shoot (*B*), and whole plant (*C*) water content in salt-treated and non-salt-treated plants. *Hordeum vulgare* plants were exposed to two concentrations of silicon (0 and 0.5 mM) in the absence or presence of NaCl (200 mM) during the last 7 d of culture. The values are averages of seven replicates. Error bars correspond to standard errors. *Asterisks* mark the statistically significant differences according to *Student*'s *t*-test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

showing that carotenoids were less sensitive to salt stress than chlorophylls (Fig. 4*F*).

PSI and PSII activities: PSI and PSII activities of light-adapted leaves obtained in plants grown under non-saline conditions and supplied with 0.5 mM Si (Si treatment) were not presented. Indeed, for this analysis, Si treatment did not show any effect of silicon under control conditions (data not shown). Hence, this treatment was not considered with the aim to focus on the main purpose of this research (to investigate the effects of silicon under salt stress).

Salt stress induced a significant reduction in maximal fluorescence (F_m) compared to the control. The exposure of salt-treated plants to silicon treatment resulted in a significant increase of F_m by 68% (Fig. 5*B*).

As regards the light test, the overall analysis of our results showed that salt stress induced a more pronounced reduction of the operational photochemical yield (Y) and the electron transfer rate (ETR) in PSI compared to those of PSII (Figs. 5*E,F*; 6*A,B*). Si protective effects were more obvious in PSI. Indeed, these results showed that PSI was more altered by salt stress and responded better to silicon supply.

The quantum yield of regulated nonphotochemical energy dissipation [$Y_{(NPQ)}$] increased in proportion to the increase in PAR. Under salt stress, and up to a PAR value of about 300 μmol(photon) m^{-2} s^{-1}, $Y_{(NPQ)}$ values were higher than those of the control. An antagonistic effect was recorded in the presence of silicon, which caused a decrease in $Y_{(NPQ)}$ under low PAR, up to a value of about 400 μmol(photon) m^{-2} s^{-1}. Salt stress also increased the quantum yield of nonregulated nonphotochemical energy dissipation [$Y_{(NO)}$], but silicon addition reduced this unregulated energy loss, values being even lower than the level of the control (Fig. 5*G,H*).
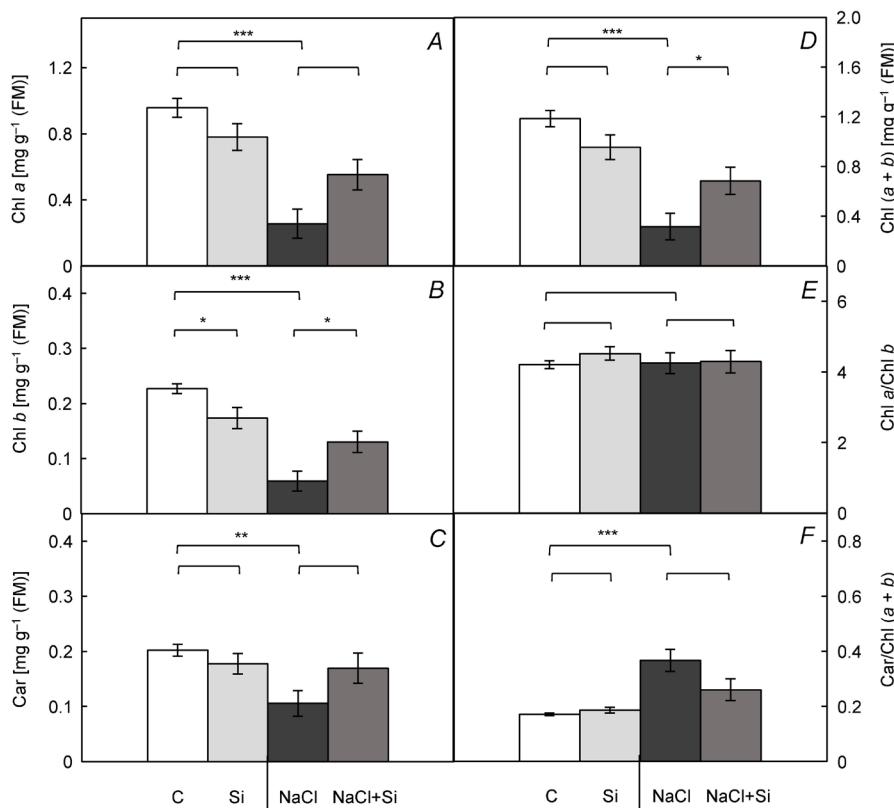
Fig. 4. Effect of silicon on photosynthetic pigment contents of leaves in salt-treated and non-salt-treated plants. Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Car – carotenoids. *Hordeum vulgare* plants were exposed to two concentrations of silicon (0 and 0.5 mM) in the absence or presence of NaCl (200 mM) during the last 7 d of culture. The values are averages of five replicates. Error bars correspond to standard errors. *Asterisks* mark the statistically significant differences according to *Student*'s *t*-test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

The quantum yield of nonphotochemical energy dissipation in reaction centres limited by the acceptor side [$Y_{(NA)}$] increased in plants subjected to salt stress only (Fig. 6C). By contrast, a decrease in the quantum yield of nonphotochemical energy dissipation in reaction centres limited by donor side [$Y_{(ND)}$] was recorded in these plants (Fig. 6D). The treatment with silicon adjusted the yields of both types of energy dissipation to levels even better [higher $Y_{(ND)}$ and lower $Y_{(NO)}$] than in control plants.

Salt stress induced also a significant reduction in both P700$_{ox}$ (oxidized PSI) and P700$_{m}$' (maximal fluorescence yield of illuminated sample with all PSI centres closed), compared to control plants, which means that the presence of salt induced a reduction of PSI oxidation (Fig. 6E,F). Interestingly, attenuation of this negative behaviour was recorded in the presence of silicon.

Lipid peroxidation: In the absence of silicon, our results showed that salt stress induced a significant increase in MDA contents of nearly 135% (Fig. 7). However, silicon supply alleviated this increase by lowering MDA contents by 35%.

Sodium and chloride contents: NaCl treatment increased sodium and chloride contents in both roots and shoots when compared to untreated plants (Table 1). Silicon

addition did not show any effects at the root level. Yet, it reduced shoot sodium and chloride contents by 27 and 28%, respectively.

Potassium content: Salt stress reduced potassium content in roots and shoots by 25 and 60%, respectively (Table 1). Silicon supply showed no beneficial effect, but rather a more pronounced decrease was recorded in shoot potassium contents.

Correlation analysis and Principal Component Analysis (PCA): To identify possible correlations between treatments and studied parameters, correlation analysis and Principal Component Analysis (PCA) were carried out (Fig. 1S, *supplement*; Table 1S, *supplement*). In this analysis, only results obtained under salt stress treatments (200 mM NaCl) were used to focus on the main purpose of this research (to investigate the effects of silicon under salt stress). At 200 mM NaCl and 0 mM Si, multiple significant negative correlations were obtained, for shoot FM, whole plant FM, shoot WC, whole plant WC, Chl *b* content, Chl (*a*+*b*) content, and F_m. These correlations indicated that the absence of silicon under saline conditions tended to favour the reduction of all these parameters. By contrast, this treatment was found to increase sodium and chloride contents in shoots and potassium content
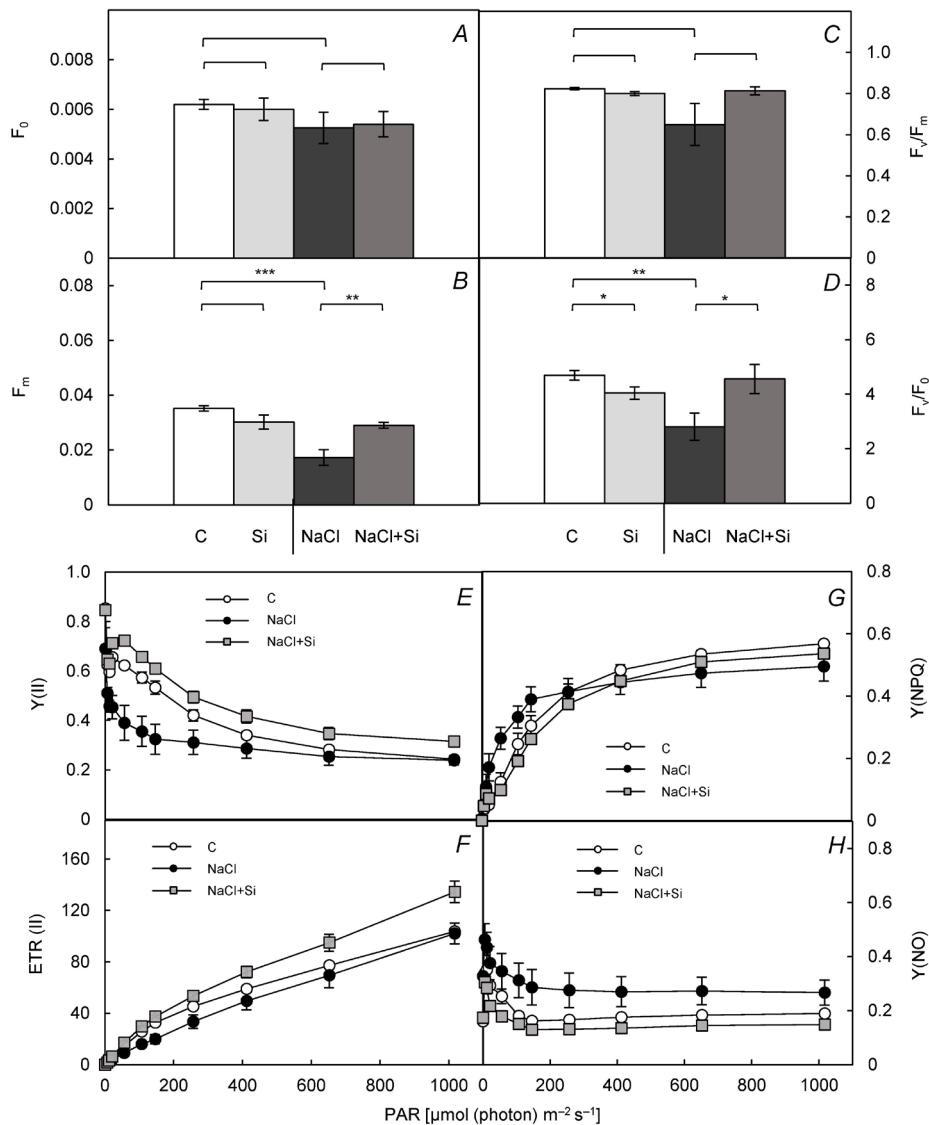
Fig. 5. Effect of silicon on fluorescence parameters of PSII for dark-adapted leaves (dark test) and light test in plants treated or not with salt. F_0 – minimum fluorescence (*A*); F_m – maximum fluorescence (*B*); F_v/F_m – maximum quantum yield of PSII primary photochemistry (*C*); F_v/F_0 – maximum quantum yield of PSII photochemistry (more sensitive than F_v/F_m) (*D*); $Y_{(II)}$ – quantum yield of photochemical energy conversion in PSII (*E*); ETR – electron transfer rate (*F*); $Y_{(NPQ)}$ – quantum yield of regulated nonphotochemical energy dissipation in PSII (*G*); $Y_{(NO)}$ – quantum yield of nonregulated nonphotochemical energy dissipation in PSII (*H*); PAR – photosynthetically active radiation. *Hordeum vulgare* plants were exposed to two concentrations of silicon (0 and 0.5 mM) in the absence or presence of NaCl (200 mM) during the last 7 d of culture. The values are averages of five replicates. Error bars correspond to standard errors. *Asterisks* mark the statistically significant differences according to *Student*'s *t*-test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

in roots and shoots. In the presence of Si under saline conditions, several significant positive correlations were obtained for all the above-mentioned parameters. In addition, a reduction of shoot sodium and chloride contents, as well as shoot and root potassium contents were observed.

Discussion

The purpose of this study was to investigate the effect of the interaction between silicon and salt (NaCl) on growth,

tissue hydration, mineral nutrition, and photosynthesis in *H. vulgare*. Our results showed that the application of 200 mM NaCl decreased the fresh mass of *H. vulgare* in roots, shoots, and whole plant (Figs. 1, 2). Similar results have been observed in several plant species, such as common wheat (*T. aestivum*), durum wheat (*Triticum turgidum*), barley (*H. vulgare*), and sea barley (*H. marinum*) (Munns *et al.* 1995, Chen *et al.* 2014, El-khawaga 2018, Laifa *et al.* 2021). Salt stress induces a reduction in plant growth mainly due to the osmotic pressure applied to the root area, which causes reduced water uptake and
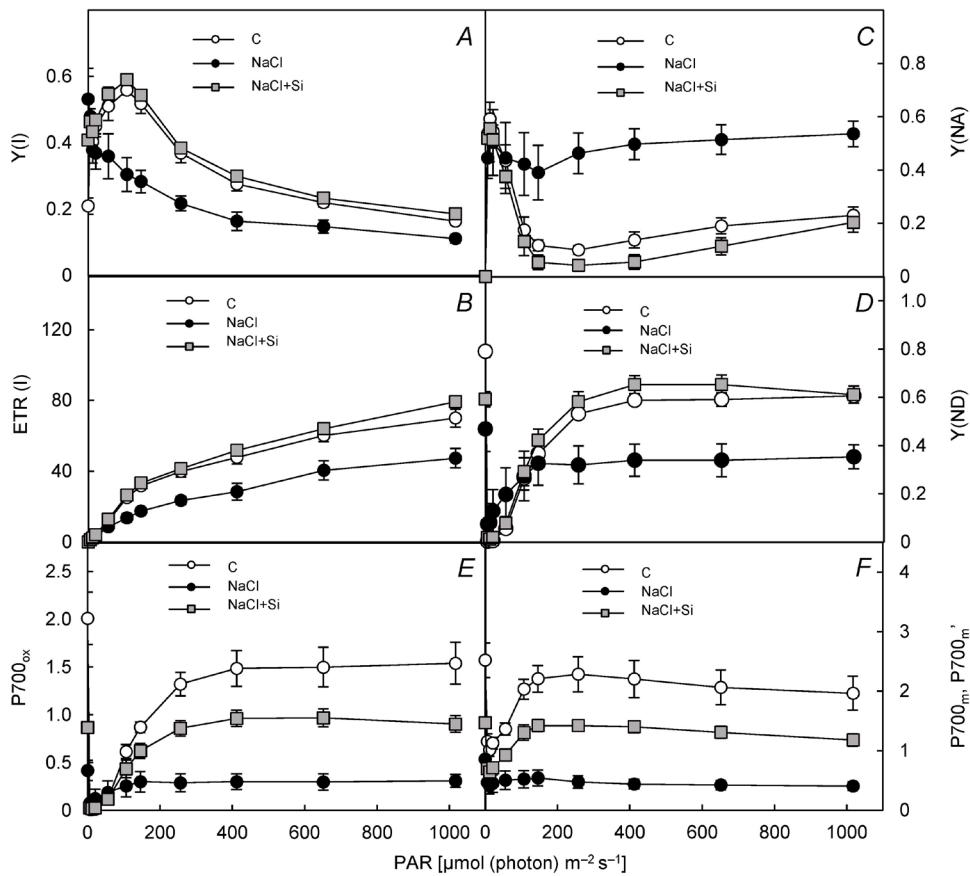
Fig. 6. Effect of silicon on fluorescence parameters of PSI for light-adapted leaves (light test) in plants treated or not with salt. $Y_{(I)}$ – quantum yield of photochemical energy conversion in PSI (A); ETR – electron transfer rate (B); $Y_{(NA)}$ – quantum yield of nonphotochemical energy dissipation in reaction centres limited by acceptor side (C); $Y_{(ND)}$ – quantum yield of nonphotochemical energy dissipation in reaction centres limited by donor side (D); $P700_{ox}$ – oxidized PSI (E); $P700_m$ – maximal fluorescence yield of dark-adapted sample with all PSI centres closed; $P700_m'$ – maximal fluorescence yield of illuminated sample with all PSI centres closed (F); PAR – photosynthetically active radiation. *Hordeum vulgare* plants were exposed to two concentrations of silicon (0 and 0.5 mM) in the absence or presence of NaCl (200 mM) during the last 7 days of the culture. Values are averages of five replicates. The error bars correspond to standard errors.
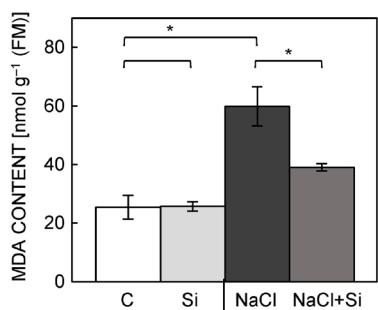
Fig. 7. Effect of silicon on malondialdehyde (MDA) contents in shoots of plants treated or not with salt. PAR – photosynthetically active radiation. *Hordeum vulgare* plants were exposed to two concentrations of silicon (0 and 0.5 mM) in the absence or presence of NaCl (200 mM) during the last 7 d of the culture. Values are averages of five replicates. The error bars correspond to standard errors. *Asterisks* mark the statistically significant differences according to *Student's* *t*-test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

turgor pressure, stomatal closure, reduced photosynthesis, and toxic mineral disturbances (Bandehagh *et al.* 2011, Adhikari *et al.* 2019, Hurtado *et al.* 2019, Attia *et al.* 2022) as well as a combination of them (Ashraf 2004).

According to many studies, the beneficial effects of silicon are generally expressed under abiotic stress conditions, especially under salt stress (Ma *et al.* 2001, Coskun *et al.* 2019). This is in agreement with our results, which showed that the beneficial effects of silicon were more expressed under salt stress. Silicon treatment showed a beneficial effect in terms of improving fresh mass which revealed strong positive correlations linking the application of silicon to the shoot fresh mass (correlation coefficient $R = 0.73$; Table 1S) and the whole plant ($R = 0.74$; Table 1S). These results are consistent with other studies on rice, sugarcane, tomatoes, cotton, and cucumber (Epstein 1994, 1999; Romero-Aranda *et al.* 2006, Yin *et al.* 2019).

Water uptake and tissue hydration are important aspects to assess salt stress tolerance in plants. In this study, the

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Table 1. Effect of silicon on sodium, chloride, and potassium contents in roots and shoots of plants treated or not with salt. *Hordeum vulgare* plants were exposed to two concentrations of silicon (0 and 0.5 mM) in the absence or presence of NaCl (200 mM) during the last 7 d of the culture. Values are means ± standard errors of seven individual plants. *Asterisks* mark the statistically significant differences according to *Student*'s *t*-test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

Ion	Treatment	Roots		Shoots	
Na$^+$ [mmol g^{-1}(DM)]	Control	0.081 ± 0.008		0.046 ± 0.002	
	Si	0.098 ± 0.009	]]***	0.095 ± 0.005	]***]***
	NaCl	1.147 ± 0.062	]	2.438 ± 0.085	]***
	NaCl + Si	1.357 ± 0.165		1.774 ± 0.101	
Cl$^-$ [mmol g^{-1}(DM)]	Control	0.170 ± 0.016		0.215 ± 0.012	
	Si	0.186 ± 0.020	]]***	0.255 ± 0.017	]]***
	NaCl	1.290 ± 0.040	]	3.995 ± 0.220	]**
	NaCl + Si	1.603 ± 0.176		2.853 ± 0.173	
K$^+$ [mmol g^{-1}(DM)]	Control	0.501 ± 0.014		1.063 ± 0.028	
	Si	0.528 ± 0.030	]]***	1.084 ± 0.038	]]***
	NaCl	0.376 ± 0.034	]**	0.429 ± 0.015	]***
	NaCl + Si	0.294 ± 0.004		0.319 ± 0.010	

application of NaCl induced a reduction in shoot water content (Fig. 3*B*), which is in agreement with the work of Gong *et al.* (2006). Interestingly, silicon supply partially restored shoot tissue hydration. Indeed, strong positive correlations associating silicon application to shoot ($R = 0.90$; Table 1S) and the whole plant water content ($R = 0.92$; Table 1S) were revealed. Zhu *et al.* (2015) and Romero-Aranda *et al.* (2006), who showed similar results in cucumber and tomato, respectively, extensively discussed this improving effect of silicon under salt stress conditions. Zhu *et al.* (2015) showed that silicon induced overexpression of the *PIP2* (plasma membrane intrinsic proteins 2) subfamily genes in roots. PIP are highly conserved aquaporins in plants, representing a major pathway for water exchange between cell membranes (Yaneff *et al.* 2015). Therefore, the restoring effect of silicon on water uptake and transport in barley may be related to the overexpression of the genes encoding these aquaporins. In addition to the overexpression of the aquaporin gene, Zhu *et al.* (2015) further explained that the adjustment of the osmotic potential in plants might also have contributed to better water uptake. Moreover, another aquaporin family (NIP – nodulin-like intrinsic protein), directly involved in the transport of silicon, was also reported to induce better water absorption (Deshmukh *et al.* 2020). However, Savant *et al.* (1999) suggested a different mechanism. According to these authors, silicon can prevent water loss by inhibiting transpiration through the formation of silica deposits around leaf epidermal cells. Vandegeer *et al.* (2021) suggested that silicon improves plant water status by forming a layer on the cuticle, which may act as a physical barrier that prevents water loss by reducing cuticular conductance.

Our results also showed that salt stress induced a decrease in Chl *a* and *b* and carotenoid contents (Fig. 4). A similar effect was indicated in barley and tomato (Al-aghabary *et al.* 2005, El-khawaga 2018). Srinieng *et al.*

(2015) linked this decrease to a nutritional deficiency caused by salt that inhibits the absorption of essential nutrients. In addition, Santos (2004) suggested that the drop in Chl contents could be due to a suppression of their synthesis as well as an increase in the activity of the enzymes involved in their degradation. Yasar *et al.* (2008) added that ROS, formed under salt stress, could potentially arouse Chl degradation. Regarding the combined effects of silicon application and salt stress, results showed no significant improvement in the contents of Chl *a* and carotenoids. However, the contents of Chl *b* ($R = 0.71$; Table 1S) and Chl (*a+b*) ($R = 0.67$; Table 1S) were found to be enhanced (Fig. 4*B,D*). Liang (1998) and El-khawaga (2018) found similar results in barley (*H. vulgare*) and Laifa *et al.* (2021) in sea barley (*H. marinum*).

Salt stress induced a reduction in maximal fluorescence (F$_m$) and F$_v$/F$_0$ ratio (a value that is proportional to the activity of the water-splitting complex on the donor side of the PSII), with no effect on minimum fluorescence (F$_0$) and F$_v$/F$_m$ ratio (Fig. 5*A–D*). These results are in agreement with those of Mahdieh *et al.* (2015) who showed no salt or Si effect on F$_v$/F$_m$ ratio in rice (*Oryza sativa*). The reduction of F$_m$ recorded under salt stress showed a decrease in the activity of the reaction centres in PSII. These results suggest that salt stress limits electron transfer from the light-harvesting complex to the reaction centre, which, in turn, limits the transfer of electrons between the acceptor and donor sides of PSII (Akhter *et al.* 2021). F$_v$/F$_0$ ratio is more sensitive to stresses than F$_v$/F$_m$ ratio. An increase in this ratio results from an improvement in photosynthetic electron transport (Ghassemi-Golezani and Lotfi 2015). Indeed, the presence of Si improved the yield of the F$_v$/F$_0$ ratio under salt stress and was comparable to the control level.

Concerning Y$_{(I)}$ and Y$_{(II)}$, salt stress led to a decrease in both yields, compared to those recorded in control plants (Figs. 5*E*, 6*A*). In general, high NaCl concentrations

decrease the efficiency of the photosystems (PSI and PSII) (Shin *et al.* 2020). This decrease is probably related to the reduction of Chl contents due to salt stress.

According to this study, $Y_{(I)}$ was more affected by salinity compared to $Y_{(II)}$. In fact, according to Zivcak *et al.* (2015), PSII is less affected than PSI under the altering effect of a saturating light pulse (SP). After this pulse, the PSII is partially recovered in a few minutes, then fully restored in a few days. However, no recovery is observable for PSI, even after 48 h of stopping treatment. This slow recovery of PSI has been observed by many authors who have studied PSI photoinhibition.

Plants grown in the presence of silicon showed a significant increase in $Y_{(I)}$ and $Y_{(II)}$ (Figs. 5*E*, 6*A*). The observed yields were even better than those of the control plants. We also found that the performance of $Y_{(I)}$ which was significantly affected by salt stress, showed the best improvement under the interactive effects of salinity and Si supply (Fig. 6).

The presence of NaCl showed a reduction in the electron transfer rates (ETR) in both photosystems. This decrease, together with the low Chl content caused by salt stress was responsible for the reduction in yields of PSI and PSII. Interestingly, the presence of silicon improved these rates, which is in agreement with the results obtained by Zivcak *et al.* (2015) and Zhang *et al.* (2018). Thus, these results suggest a better ability for the photosystems to convert light energy into chemical energy in the presence of silicon (Figs. 5*F*, 6*B*).

As for the energy dissipation (loss) in PSII, salt stress caused an alteration in this photosystem, increasing energy loss, which was more pronounced in its unregulated form [$Y_{(NO)}$] than in its regulated form [$Y_{(NPQ)}$]. The presence of silicon decreased both $Y_{(NPQ)}$ and $Y_{(NO)}$; the latter was even lower than the control level (Fig. 5*G,H*). Similar results were obtained by Zivcak *et al.* (2015). According to Zhou *et al.* (2019), $Y_{(II)}$, $Y_{(NPQ)}$, and $Y_{(NO)}$ are in 'competition', so an increase in one results in a decrease in the two others.

At a given set of environmental conditions, successful regulation generally tends toward maximum values of $Y_{(II)}$, with the remaining losses [$Y_{(loss)}$], tending towards a maximum ratio of $Y_{(NPQ)}/Y_{(NO)}$. Under stressful conditions, high $Y_{(NO)}$ values and low $Y_{(NPQ)}$ or $Y_{(NPQ)}/Y_{(NO)}$ values reflect an inefficient ability for photoprotective reactions, which will eventually lead to photodamage (Klughammer and Schreiber 2008a). Our results are consistent with these statements, as the addition of silicon improved $Y_{(II)}$ yields and maintained a higher $Y_{(NPQ)}/Y_{(NO)}$ ratio.

In PSI, salt treatment increased $Y_{(NA)}$ and decreased $Y_{(ND)}$ compared to the control (Fig. 6*C,D*). Similar results were reported by Zhou *et al.* (2019). The limitation of the acceptor side is observed when electrons are not transmitted from PSI to $NADP^+$, either by a reduction in the activity of the Calvin cycle or FNR (ferredoxin-$NADP^+$ reductase). In the absence of an electron acceptor, no oxidation of P700 can occur. The limitation of the donor side is observed when the electron transport between PSII and PSI decreases (Perreault *et al.* 2009, Schreiber and Klughammer 2016).

Silicon treatment substantially decreased $Y_{(NA)}$ and increased $Y_{(ND)}$ yields. According to Zivcak *et al.* (2015), the increase of $Y_{(NA)}$ causes the decrease of $Y_{(I)}$. Thus, silicon seems to decrease energy dissipation due to the limitation of the acceptor side [$Y_{(NA)}$] and improve, as a consequence, the nonphotochemical energy dissipation due to a limitation of the donor side [$Y_{(ND)}$]. Therefore, this response could induce an improvement in PSI.

Silicon also alleviated the depressive effect of salinity on P700$_m$' and P700$_{ox}$ (Fig. 6*E,F*). Therefore, it appears to enhance light absorption level when PSI is subjected to a saturating flash in the presence of actinic light (P700$_m$') as well as the oxidation state of PSI reaction centres (P700$_{ox}$). Some processes in plants are placed upstream and downstream of PSI to protect it from ROS, by increasing the oxidation of P700 (Takagi *et al.* 2017, Wada *et al.* 2019). It appears that silicon contributes to the enhancement of these protective mechanisms in the presence of salt.

In this study, it was also noticed that salt stress significantly increased membrane lipid peroxidation. Eraslan *et al.* (2007) and Alzahrani *et al.* (2018) reported similar effects. The most likely explanation for this negative result is an increase in ROS concentrations, which leads to a change in membrane integrity. Interestingly, results revealed that silicon supply lowered MDA contents (Fig. 7). Indeed, Liang *et al.* (2003) reported that silicon makes it possible to maintain better membrane integrity of cells, thus reducing their permeability.

Concerning sodium, chloride, and potassium concentrations, the obtained results showed an increase in sodium and chloride contents mainly in shoots of *H. vulgare* subjected to 200 mM of NaCl, which was concomitant with a decrease in K^+ content (Table 1). These results are in agreement with those of Ben-Abdallah *et al.* (2019) and Hmidi *et al.* (2019). Bosnic *et al.* (2018) suggested the reduction of the cytosolic pool of sodium, by increasing its vacuolar sequestration, as the main mechanism of plants to tolerate salt stress.

Sodium and chloride increases in shoots were alleviated by the addition of silicon. To elucidate this finding, silicon seems to prevent the translocation of sodium and chloride to shoots by storing these ions at the root level. This protective effect of silicon was also reported in tomatoes, barley, and sea barley (Liang *et al.* 1996, Romero-Aranda *et al.* 2006, Laifa *et al.* 2021). According to Liang *et al.* (2006), silicon enhanced plasma membrane H^+-ATPase activity under salt stress in barley (*H. vulgare*) plants. Higher H^+-ATPase activity partially contributed to the decrease of Na^+ concentration in the cytoplasm, which is very important for the photosynthesis machinery.

The decrease in potassium contents in *H. vulgare* plants subjected to 200 mM NaCl might be explained by either a decrease in the transpiration rate, since potassium transport is reliant on that of water, or a decrease in its absorption. The decrease in absorption could be due to the decline of the activity of potassium transporters in the presence of NaCl, or the competition between potassium and sodium at these transport sites (Hafsi *et al.* 2007).

Nevertheless, silicon supply did not appear to have any restoring effect on potassium concentration.

Overall, the analysis of all data and results obtained by Principal Component Analysis (PCA) and correlation analysis (Fig. 1S, Table 1S), confirmed the beneficial effects of silicon against the deleterious effects of salt stress (Fig. 2S, *supplement*). Therefore, using a silicon-rich fertilizer could be an effective method for dealing with salt stress.

Conclusion: Salinity stress negatively affected *Hordeum vulgare* growth, water status, mineral nutrition, chlorophyll pigment contents, MDA concentrations, and photosynthesis. Nonetheless, silicon addition partially or completely restored these studied parameters. Silicon presence enhanced both PS activities, by increasing their yields [$Y_{(II)}$ and $Y_{(I)}$], the ETR, and decreasing the different types of energy loss [$Y_{(NPQ)}$, $Y_{(NO)}$, $Y_{(NA)}$], which, in some cases, these results were even better than that of control plants. Interestingly, under severe salinity application, the performance of PSI, which was significantly affected, showed a better improvement, under the interactive effects of salinity and Si supply, compared to PSII.

These results provide further evidence that silicon is a very interesting product for the correction of the deleterious effects of salt stress. From the outcome of this study, it is possible to conclude that silicon application could be a promising approach to provide tolerance to salt-stressed plants and also improve salt tolerance in plants like barley mainly by improving and protecting the photosynthetic machinery. Therefore, silicon can be used as a fertilizer of prodigious importance due to its availability in large quantities and can compete with other fertilizers owing to its high efficiency and low price.

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