Nitrogen components, growth and gas exchange in spring wheat plants grown under interaction of silicon (Si) and nitrogen (N)

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Abstract

Some studies have reported a synergistic effect between N and Si in plant growth. The effect of interaction between Si and N on the nitrogen metabolism, growth and gas exchange in spring wheat plants genotype IAC 375 was therefore investigated in the present study. Two Si doses (0 and 1.8 mmol L^{-1}) and five N doses (1.4, 3.6, 7.1, 14.3 and 28.6 mmol L^{-1}) were used. The concentrations of leaf (R^2=0.66*) and root silicon (R^2=0.24*) were influenced by the dose of 1.8 mmol L^{-1} of Si while unaffected by N doses. On the other hand, N doses were more decisive than Si in nitrate reductase activity, and leaf and root nitrogen content. The stomatal conductance (R^2=0.91**), transpiration (R^2=0.93**) and intercellular CO_2 concentration (R^2=0.98**) were lower in the dose of 1.8 mmol L^{-1} of Si, however, net photosynthesis (R^2=0.98*) was higher in this Si dose. The instantaneous (R^2=0.71**) and intrinsic water use efficiencies (R^2=0.52**) were higher in the presence of 1.8 mmol L^{-1} of Si, but decreased with increasing N levels. Leaf area, shoot dry mass and root dry mass of plants grown in the presence or absence of Si were influenced by N and not by Si. Silicon was effective in improving water ratios in wheat plants, however the interaction between Si and N was not was sufficiently effective to increase N uptake and promote dry matter gains in wheat plants.

Keywords: Water use efficiency, stomatal conductance, transpiration, dry mass, *Triticum aestivum* L.
Abbreviations: Si_silicon, N_nitrogen, NRA_nitrate reductase activity, TN_ total nitrogen, Ci_intercellular CO_2 concentration, Pn/gs_intrinsic water use efficiency, Pn/E_instantaneous water use efficiency.

Introduction

Silicon (Si) is the second most abundant element in the earth's crust and is the mineral matrix of growth for many plants (Zhang et al., 2013). In soil, it is present in the form of SiO_2 in a fraction corresponding to 50-70% of the soil dry mass (Isa et al., 2010). However, it is not considered essential to plants because its participation in the plant metabolism is not clear (Ma, 2010). There is a body of evidence showing the importance of Si in growth and development of many plant species such as rice, sugarcane and sedges, considered accumulators of Si (Liang et al., 2005; Shi et al., 2010), although the Si concentration varies among plant species (Murozuka et al., 2014). Wheat plants are also considered accumulators of Si, with concentration between 1-2% of the plant dry mass (Kaparaju et al., 2009). After being absorbed by the root system, Si is transported via the transpiration stream to the shoot, polymerizing and forming amorphous silica bodies called phytoliths that are incorporated into the cell walls, giving them greater rigidity and roughness (Epstein and Bloom, 2006). Under these conditions Si induces specific structural and physiological responses by conferring more pronounced vertical growth of the stalk or stem, as well as horizontal growth in the form of plant leaves, in addition to inducing greater resistance to physical, chemical and biological stress factors existing in the growth medium of the plants (Marschner, 2004; Rizwan et al., 2012; Farshidi et al., 2012; Zhao et al., 2013; Rios et al., 2014). Under conditions of abiotic (*e.g* salts, heavy metals, water shortage and high temperatures) and biotic stress (*e.g* insects

and pathogens), Si can increase resistance or tolerance in a wide range of plants (Epstein, 2009; Keeping and Reinolds, 2009; Ye, et al., 2012; Vaculík et al., 2012; Ye et al., 2013). Studies have shown that leaf transpiration in some plants is considerably reduced by the use of Si (Agarie et al., 1998; Gao et al., 2004; Gao et al., 2006) and this effect on gas exchange may be associated with thickening of the leaf cuticle by deposition of Si (Savant et al., 1999) which results in water economy and increased plant growth. Moreover, under field conditions, fertilization with Si is widely adopted in order to increase rice production (Detmann et al., 2012). In this case, the effect of Si is attributed to its role in minimizing stresses of biotic and abiotic nature, as well as increased resistance to lodging and improved leaf stiffness which permits better light transmittance through the canopy, and therefore indirectly increases the photosynthetic process of plants (Tamai and Ma, 2008). Among the factors influencing plant growth nitrogen (N) is highlighted, the mineral nutrient essential to plant metabolism due to the countless functions it performs in plants. Nitrogen plays a role in the formation of organic molecules such as enzymes, nucleic acids, amino acids, proteins, formation of cell structures and vegetative growth of plants. For these reasons it is one of the nutrients absorbed in greatest quantities by plants. Therefore, it is necessary that the enzyme nitrate reductase (NR) (E.C 1.6.6.1) initially catalyzes the reduction of nitrate to nitrite using NADH/NAD(P)H/NADPH as an electron donor. This enzyme is the first step in the assimilation of nitrate into

organic compounds (Taiz and Zeiger, 2013; Nemie-Feyissa et al., 2013). Several factors contribute to its post-transcriptional regulation, highlighting the availability of nitrate, a primary factor that regulates *de novo* synthesis and enzyme activity (Ruiz et al., 2007). For example, in *Sorgum bicolor* subjected to aluminum (Al) toxicity, enzyme activity was reduced by 16% compared to the control treatment. In parallel there was a reduction of the leaf nitrate concentration, indicating the toxic role of Al in reducing the availability of nitrate, and hence the activity of NR (Cruz et al., 2014). Adequate nitrogen fertilization has promoted productivity gains for crops, but high nitrogen doses increase the possibility of self-shading and the incidence of lodging. Thus, to obtain positive responses regarding the application of N, appropriate management practices should be taken into consideration that allow for better use of nitrogen supplied to the plant, considering the fact that N is one of the main input factors in the production of cereals (Ávila et al., 2010; Nouriyani et al., 2012). In literature, several studies have shown better utilization of nutrients by plants due to the presence of Si in the growth medium (Savant et al., 1999; Mali and Aery, 2008; Shen et al., 2009). In this context, the interaction between Si and N seeking to promote growth and efficiency gains in the physiological responses of plants appears to be a simple and interesting strategy to maximize the use of environmental factors such as light, water and nutrients by plants. Based on the need to clarify the effect of interaction between Si and N on growth, the N metabolism and gas exchange in plants, the hypothesis was evaluated that the addition of Si in the nutrient solution can improve the response of wheat plants grown in the presence of increasing N concentrations. This study sought to evaluate the effects of interaction between Si and N on growth, N metabolism and gas exchange in spring wheat plants grown in nutrient solution.

Results

Leaf and root silicon concentration, nitrate reductase activity, leaf and root total nitrogen content

Table 1 presents the mean squares of the analysis of variance for the polynomial regression of leaf and root silicon concentrations, nitrate reductase activity, and leaf and root total nitrogen content for spring wheat plants, genotype IAC 375, submitted to increasing nitrogen doses in the presence or absence of silicon. There was an increasing linear polynomial response of leaf concentrations of silicon (Si) in the treatment with 1.8 mmol L^{-1} of Si (+Si) (Fig 1A) in relation to nitrogen (N) applied (R^2=0.66*). In the silicon control treatment (-Si), on the other hand, the effect of N levels on leaf Si concentration had no significant polynomial response. Within each dose of N, the concentration of Si in +Si was significantly higher in relation to -Si (Fig 1A). In the roots, similarly to the leaves, there was an increase in Si concentration in +Si (R^2=0.24*) although the polynomial response observed was of cubic order (Fig 1B). In -Si there was no significant polynomial response of N levels on the root Si concentration. The root concentration of Si in +Si, considering each N dose, was significantly higher than in -Si (Fig 1B). However, no significant differences in NRA between +Si and -Si were observed within each N dose, except at the dose of 28.6 mmol L^{-1} (Fig 2A). In leaves, the content of total nitrogen (TN) in +Si (R^2=0.83**) and -Si (R^2=0.85**) showed increasing linear polynomial responses as a function of N doses. Despite the absence of significant differences between +Si and -Si within each N dose, -Si

showed higher N content in all N levels (Fig 2B). In the roots, in +Si (R^2=0.90**) and -Si (R^2=0.72**) there was an increasing linear polynomial response of the TN content, with no significant differences observed between +Si and -Si with respect to the TN content within each N dose, except at the dose of 3.6 mmol L^{-1} (Fig 2C).

Gas exchange

Table 2 presents the mean squares of the analysis of variance for the regression coefficients of net photosynthesis, stomatal conductance, transpiration, intercellular CO_2 concentration, instantaneous and intrinsic efficiency for use of water in spring wheat plants, genotype 375 IAC, subjected to increasing nitrogen doses and the presence or absence of silicon. There was an increase in the net photosynthetic rate (Pn) in +Si as a function of N levels with quadratic polynomial response (R^2=0.98*), whereas a cubic order increase was observed in -Si (R^2=0.99*) (Figure 3A). Within each N dose, only in those of 7.1, 14.3 and 28.6 mmol L^{-1} was the Pn in +Si significantly higher compared to -Si (Fig 3A). Stomatal conductance (gs) in +Si presented a quadratic polynomial response (R^2=0.91**), with a slight decrease in the dose of 28.6 mmol L^{-1} of N (Fig 3B). On the other hand, in -Si the increase in gs had a cubic polynomial response (R^2=0.99**), with a decrease in the dose of 28.6 mmol L^{-1}. In -Si the gs was significantly higher in doses of 1.4, 3.6 and 7.1 mmol L^{-1} of N in relation to +Si (Fig 2B). The same response trend was observed in transpiration (E), where +Si showed a quadratic polynomial response (R^2=0.93**), while -Si presented a cubic polynomial response (R^2=0.99**) (Fig 3C). The E values were significantly higher in –Si in relation to +Si only in the doses of 1.4, 3.6, 7.1 and 14.3 mmol L^{-1} of N (Fig 3C). The intercellular CO_2 concentration (Ci) in +Si presented an increasing cubic polynomial response (R^2=0.98**), however, -Si showed no significant polynomial response (Fig 3D). Within each N dose, -Si presented significantly higher Ci values with respect to +Si (Fig 3D). Instantaneous water use efficiency (Pn/E) in +Si presented a decreasing linear polynomial response (R^2=0.71**) while for –Si the response was non-significant (Fig 4A). Within each N dose, only in the dose of 28.6 mmol L^{-1} of N did the treatment +Si not have a significantly greater Pn/E ratio in relation to -Si (Fig 4A). The intrinsic water use efficiency (Pn/gs) in +Si had a descending linear polynomial response (R^2=0.52**), however, -Si showed no significant polynomial response (Fig 4B). Within each N dose, the Pn/gs in +Si was significantly higher compared to -Si (Fig 4B).

Growth of the shoots and roots

Table 3 shows the mean squares of the analysis of variance for the polynomial regression of leaf area, and shoot and root dry mass of the of spring wheat plants, genotype 375 IAC, exposed to increasing doses of nitrogen and the presence or absence of silicon. The leaf area (LA) in +Si (R^2=0.82**) and -Si (R^2=0.73*) presented a positive quadratic response, with a declining trend in doses of 14.3 and 28.6 mmol L^{-1} of N, both in +Si and -Si (Fig 5A). No significant differences were observed in LA between +Si and -Si within each N dose (Fig 5A). Similar to the LA, the shoot dry matter (SDM) showed the same response trend, however, +Si presented a quadratic polynomial response (R2 = 0.74 **) and -Si a cubic response (R^2=0.89*) (Fig 5B). Furthermore, there were no significant differences between + Si and -Si within each N dose (Fig 5B). The root dry matter (RDM) in +Si presented a polynomial linear response (R^2=0.58**), however, in -Si the

Table 1. Mean squares of the analysis of variance for the polynomial regression of leaf and root Si, nitrate reductase (NR) and leaf and root total nitrogen (TN) of spring wheat plants, genotype IAC 375 exposed to increasing doses of nitrogen in the presence or absence of silicon.

Source of variation		Mean square +Si				
	DOF	Leaf Si	Root Si	NR	Leaf TN	Root TN
L.R.	1	0.2280**	0.0015ns	103.6152**	50.9767**	92.4300**
Q.R.	1	0.0312[ns]	0.0303[ns]	16.2461*	3.8878[ns]	4.8358[ns]
C.R.	1	0.0731[ns]	0.1719*	20.7189*	0.1471[ns]	5.2950[ns]
Treatment	4	0.0858	0.2103	35.1768	15.200	25.6484
Residue	15	0.0263	0.0301	2.9127	3.7474	3.9815
Source of variation		Mean square -Si				
	DOF	Leaf Si	Root Si	NR	Leaf TN	Root TN
L.R.	1	0.0180[ns]	0.0871[ns]	3.1794[ns]	93.3776**	42.8919**
Q.R.	1	0.0474[ns]	0.0125[ns]	37.1353**	0.7824[ns]	5.7051[ns]
C.R.	1	0.0171[ns]	0.0141[ns]	1.2292[ns]	11.7707[ns]	0.3002[ns]
Treatment	4	0.0218	0.0465	10.4915	27.3758	14.8511
Residue	15	0.0263	0.0301	2.9127	3.7474	3.9815

**,* Significant at 1 and 5% probability, respectively. ns, non-significant. Si, silicon. NR, nitrate reductase. TN, total nitrogen. +Si, presence of silicon. -Si, absence of silicon. L.R., linear. Q.R., quadratic. C.R., cubic. DOF, degrees of freedom.

polynomial response was not significant (Fig 5C). No significant differences were observed between +Si and -Si for each N dose on root dry mass (Fig 5C).

Discussion

Several studies have shown the ability of wheat plants to absorb silicon under stress conditions or otherwise (Rizwan et al., 2012; Gong and Chen 2012; Domiciano et al., 2013). In the present study, wheat plants showed considerable Si absorption, observing higher concentrations in plants of the +Si treatment compared to -Si, both in leaves and roots. However, the little influence of increasing N doses on increasing the absorption of Si was evident, since leaf Si concentrations in plants of the treatment +Si were very close to the average value of 2.01 g kg^{-1} in leaves and 2.13 g kg^{-1} in roots, independent of the N dose. Although nitrogen is a mineral nutrient demanded in large quantities by plants and contributes to plant growth (Marschner, 2004), in our study the results encountered indicate reduced interaction between N and Si. Similarly to that found in our study, Mauad et al. (2003) observed that increasing N doses did not cause increases in Si uptake by rice plants. Some studies have related a reduction in Si absorption to acidification of the soil-root interface at the NH_4^+-H exchange site when the main N source is ammonia. This pH reduction may decrease the absorption of Si by extruding H^+ from each NH_4^+ absorbed. On the other hand, when the N source is nitrogenated (NO_3^-) there may be greater availability of Si at the NO_3^--OH^- exchange site due to alkalinization of the soil-root interface. However this implies competition between Si and NO_3^- with probable reduction of Si absorption, as encountered in wheat plants subjected to interaction between N doses and sources (Wallace, 1989). The NRA is strongly modulated by the availability of nitrate. In this sense, NRA is considered a potential marker of nitrogen assimilation in plants (Singh et al., 2002), justifying its use in studies evaluating the interaction of N with environmental factors in plants. In both Si treatments, the NRA increased with increasing N doses. This evidence is reinforced by the fact that plants of both Si treatments showed increases in TN content for both the shoots and roots (Figure B and C, respectively), indicating that the increasing availability of N in the form of NO_3^- increased the NRA in wheat plants, although among Si treatments (-Si and +Si) within the N doses there were no significant differences in NRA. As noted in our study, Mauad et al. (2003) found no significant respo-
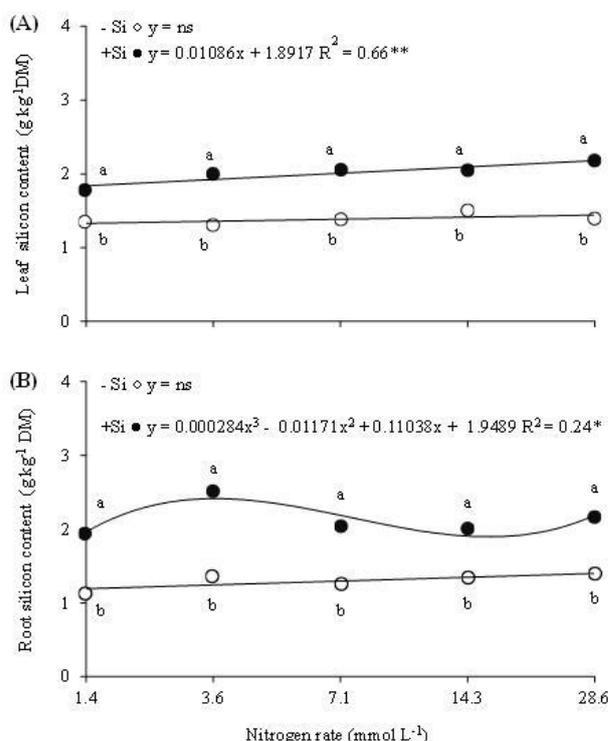
Fig 1. Effect of the interaction between silicon and nitrogen on the Si concentration in leaves (A) and roots (B) of spring wheat plants grown in nutrient solution. **, * Significant at 1 and 5% probability. ns, non-significant. Lowercase letters compare + Si and Si within each N dose. MSD, minimum significant difference. Leaf silicon content, MSD: 0.2346; Root silicon content, MSD: 0.2509.

nses to leaf TN content when assessing the interaction of N and Si in rice. Similarly, Viana and Kiehl (2010) observed that increasing N doses in association with K promoted increases in NRA in the leaves of wheat plants, however these authors found that N alone was effective in increasing the NRA. On the other hand, although Si promoted higher Pn in wheat plants of the +Si treatment (Fig 3A) and plant growth requires nitrogen, in our study the interaction between N and Si did not promote greater NRA, although there is

Table 2. Mean squares of the analysis of variance for the polynomial regression of net photosynthesis (Pn), stomatal conductance (gs), transpiration (E), intercellular CO_2 concentration (Ci), instantaneous efficiency (Pn/E) and intrinsic water use (Pn/gs) of spring wheat plants genotype IAC 375 exposed to increasing nitrogen levels in the presence or absence of silicon.

| Source of variation | DOF | Mean square + Si | | | | | |
		Pn	gs	E	Ci	Pn/E	Pn/gs
L.R.	1	89.5303**	0.0626**	36.4233**	16526.9348**	33.1319**	3943.2251**
Q.R.	1	215.4042**	0.0811**	21.7262**	2139.0632ns	5.7468*	1253.4387[ns]
C.R.	1	4.2110[ns]	0.0072[ns]	2.8010[ns]	6434.7666**	0.4322[ns]	128.1846[ns]
Treatment	4	77.5793	0.0392	15.6224	6287.1708	11.5597	1879.4340
Residue	15	2.6387	0.0025	0.7008	507.9663	0.8763	330.1296

| Source of variation | DOF | Mean square -Si | | | | | |
		Pn	gs	E	Ci	Pn/E	Pn/gs
L.R.	1	0.6193ns	0.0383**	7.1707**	71.0943[ns]	0.5853[ns]	419.9593[ns]
Q.R.	1	29.4210**	0.0116*	10.0681**	2.0279[ns]	0.0853[ns]	77.0309[ns]
C.R.	1	18.8016*	0.0167*	6.3329**	1.0088[ns]	0.0862[ns]	25.6200[ns]
Treatment	4	12.3215	0.0167	5.9343	29.8845	0.1922	132.3256
Residue	15	2.6398	0.0025	0.7008	597.9663	0.8763	330.1296

**,* Significant at 1 and 5% probability, respectively. ns, non-significant. Si, silicon. Pn, photosynthesis. gs, stomatal conductance. E, transpiration. Ci, intercellular CO_2 concentration. Pn/E, instantaneous water use efficiency. Pn/gs, intrinsic water use efficiency. +Si, presence of silicon. -Si, absence of silicon. L.R., linear. Q.R., quadratic. C.R., cubic. DOF, degrees of freedom.

evidence that high triose phosphate concentrations resulting from Pn contributed to NR activation by inhibiting kinases of NR (Weiner et al., 1993; Kaiser et al., 1999). In accordance with our results, Ebrahimian and Bybordi (2011) observed that in sunflower plants the NRA was not influenced by Si applied in nutrient solution. Gas exchanges in general were lower in the +Si treatment, except Pn which increased in comparison to plants of the -Si. The photosynthesis process corresponds to a basic and necessary input of energy and carbon in plants. It is essential to plants and is linked to leaf structure (Castro et al., 2009). Photosynthesis can vary in function of the plant environment and two major limiting factors in this process are CO_2 and light radiation (Nezami and Bybordi, 2011). The observed increase in Pn in plants of the +Si may have occurred due to possible morphological changes promoted by Si, as observed in banana seedlings nourished with Si in which an increase in stomatal density and thickness of the palisade parenchyma was observed, contributing to higher Pn (Asmar et al., 2013). This maximizes the influx of CO_2 and its incorporation into organic compounds via carboxylation by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39), although E, gs and C presented lower values in the +Si treatment compared to -Si, indicating greater carboxilative efficiency of plants in the +Si treatment. Furthermore, it is evident that the photosynthetic capacity is highly correlated with the nitrogen content, because most leaf N is contained in enzymes of the stroma and thylakoid proteins (Evans, 1989). On the other hand, in a study of the rice mutant genotype *lsi1*, defective in expressing Si transporters, it was observed that Pn elevation in wild plants was associated with increased mesophilic conductance of CO_2, which consists of CO_2 flux from the intercellular spaces to the fixation site in the stroma of chloroplasts (Detmann et al., 2012). Some studies have shown that water loss by transpiration can be reduced by Si (Epstein, 1994; Ma, 2004). This is due to deposition of silicon in epidermal cell walls. Its thickening therefore results in a reduction in stomatal transpiration as demonstrated in maize (Gao et al., 2006) and rice (Agarie et al., 1998). However, the reduction in gs, E and Ci observed in our study was compensated by an increase in Pn, indicating that Si promoted greater water use efficiency (WUE), as observed in Pn/E and Pn/gs (Figure 3A and B respectively) for plants of the treatment +Si. Ueno and Agarie (2005), in a classic study on deposition of silicon on the stomatal apparatus noted that Si is essential, for accumulating plants, in maintenance of physical and mechanical properties of the cell wall by preventing water loss through the stomatal pore by reduction of transpiration. However, water use efficiency promoted by Si in plants was not accompanied by significant gains in growth (leaf area, shoot and root dry mass), despite the increase in leaf area and slight gain of shoot dry mass in the doses of 3.6, 7.1 and 14.3 mmol L^{-1} of N in plants of the +Si treatment with respect to the -Si. This indicates that Si had a more important role in promoting water use efficiency in plants of treatment +Si in relation to plants of treatment -Si. In studies with wheat (Liang et al., 1994) and rice (Mauad et al., 2003) no increases were observed in shoot dry mass of plants cultivated in the presence of Si, reinforcing the evidence that growth of wheat plants in this study is attributed only to nitrogen nutrition, although Si plays the role of promoting protection of plants against biotic and abiotic stresses. For example, in cotton plants subjected to interaction between 1 µM of Cd and 1 mM of Si there was an increase in dry weight of plants on the order of 24% compared to plants treated with only 1 µM of Cd (Farooq et al., 2013). In another study, *Sorgum bicolor* seedlings subjected to 100 mM of NaCl in the presence of 0.83 mM of Si showed a 13% increase in fresh leaf weight in relation to the NaCl treatment, indicating the role of Si to stabilize or improve plant growth under stress conditions (Yin et al., 2013). Our results indicate that Si was effective in improving water ratios in wheat plants. It was observed that the interaction between Si and N was not effective to the point of increasing the N absorption and promoting dry matter gains. However, elucidation of the mechanisms that led to these results suggest a morpho-anatomical and molecular methodological approach for future studies involving this interaction, with the specific goal of indicating the role of Si and its relationship with N in the root system in order to understand the physiological response of wheat plants to the interaction of Si and N.

Materials and methods

Plant material, treatments and experimental conditions

This study was conducted at the Faculty of Agricultural and Veterinary Sciences, São Paulo State University, Campus in Jaboticabal, São Paulo, the period from 10/03 to 20/08/2012

(A)
$\diamond$ - Si $y = -0.01900x^2 + 0.54044x + 2.65172$ $R^2 = 0.96$ **
$\bullet$ +Si $y = 0.00311x^3 - 0.14716x^2 + 2.01976x + 0.167933$ $R^2 = 0.99$ *

Nitrate reductase (μmol NO_2^- g^{-1} FM h^{-1})

(B)
- Si $\diamond$ $y = 0.2198x + 47.0386$ $R^2 = 0.85$**
+ Si $\bullet$ $y = 0.1624x + 45.0452$ $R^2 = 0.83$**

Leaf nitrogen content (g kg-1 DM)

(C)
- Si $\diamond$ $y = 0.14903x + 41.6003$ $R^2 = 0.72$**
+ Si $\bullet$ $y = 0.21877x + 37.9309$ $R^2 = 0.90$**

Root nitrogen content (g kg-1)

Nitrogen rate (mmol L-1)
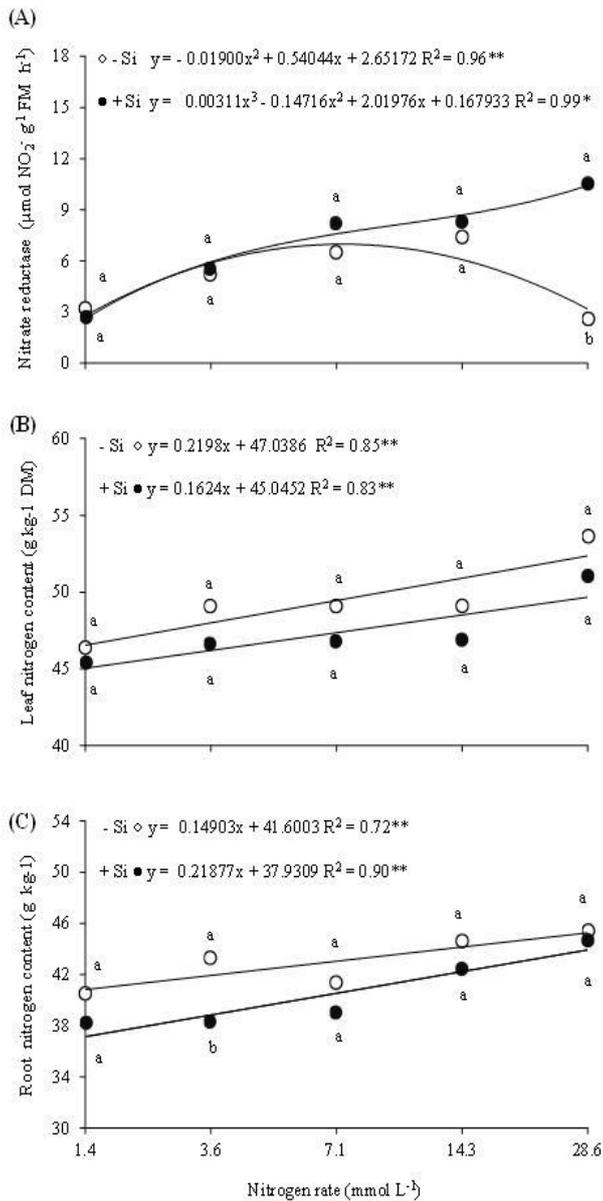
Fig 2. Effect of the interaction between silicon and nitrogen on nitrate reductase activity (A), total nitrogen content in leaves (B) and roots (C) of spring wheat plants grown in nutrient solution. **, * Significant at 1 and 5% probability. ns, non-significant. Lowercase letters compare + Si and Si within each N dose. MSD, minimum significant difference. Nitrate reductase, MSD: 2.4646; leaf nitrogen content, MSD: 2.7955; Root nitrogen content, MSD: 2.8815.

in controlled conditions of the plant grow room. For this, seeds of spring wheat (*Triticum aestivum* L.), genotype IAC 375, were sterilized with 5% NaClO for 5 minutes, rinsed with deionized water and surface moisture of the seeds was removed with paper towels. Next, the seeds were germinated in plastic trays containing sterile sand at 25°C in a germination chamber. After emergence of the second pair of leaves at four days after sowing, four uniform seedlings were transplanted to plastic pots containing 0.8 L of Hoagland and Arnon (1950) solution at ¼ ionic strength for three days and ¾ ionic strength for three more days for adaptation of the seedlings. After this adaptation period to the nutrient solution

it was maintained with complete ionic strength containing the treatments related to nitrogen and silicon doses. The nitrogen source consisted of a nitrate to ammonium nitrogen ratio (N-NO_3^-/N-NH_4^+) of ⅘ while the silicon source used was the H_2SiO_3 (Sigma-Aldrich). At 31 days after sowing, the evaluations related to gas exchange, shoot and root growth, collecting plant samples for the determination of silicon, total nitrogen and leaf nitrate reductase activity were conducted. Preparation of the nutrient solution included the use of deionized water with renovation of the solution at three day intervals, with continuous aeration throughout the experiment. The pH of the solution was monitored daily and maintained at 5.8±0.1, using a NaOH or HCl solution of 0.5 mol L^{-1} when necessary. Plants were maintained in a growth chamber under controlled conditions of temperature (25±1 °C), humidity (50±5%), light intensity (450 μmol m^{-2} s^{-1}) and photoperiod of 12 h (day/night) throughout the entire experimental period.

Determination of leaf and root silicon

Samples of leaf or root dry mass (0.1g) were digested in polyethylene tubes using 50% NaOH. The samples were digested with NH_4F before the Si determination by molybdenum blue colorimetry as described by Kraska and Breitenbeck (2010).

Determination of nitrate reductase activity

Nitrate reductase activity (NRA) (NR; EC 1.6.6.1) was measured according to Jaworski (1971), with some modifications. Samples of fresh leaf tissue (1.0 g) were incubated in test tubes containing 400 mM potassium phosphate buffer, pH 7.2, 200 mM KNO_3, 4% (v/v) *n*-propanol and subjected to a vacuum (60 cm Hg min^{-1}) for two minutes. Next, the samples were incubated in a water bath at 30°C for 90 minutes and the nitrite formed was quantified by colorimetry at 540 nm.

Determination of the total nitrogen content

Leaf and root total nitrogen levels were determined using 0.3 g dry mass by digestion with concentrated hydrochloric acid and the samples were quantified by titration with 0.1M HCl as proposed by the Kjeldahl method, and described by Bataglia et al. (1983).

Measurement of gas exchange

Photosynthesis (Pn), stomatal conductance (gs), transpiration (E) and intercellular CO_2 concentration (Ci) were measured in the second fully expanded leaf from the apex of the plant by means of an IRGA (Infrared gas analyzer, model LI-6400XT, LI-COR, Lincoln, NE, USA). Measurement of gas exchange was conducted under artificial light of 1000 μmol m^{-2} s^{-1}, CO_2 concentration of 380 μmol mol^{-1} and leaf chamber temperature of 25°C. Also determined were instantaneous water use efficiency (Pn/E) and intrinsic water use efficiency (Pn/gs).

Determination of shoot and root growth

The leaf area was determined using an image analysis system model Delta-T Devices LTD which uses the Delta-T Image Analysis System software. Shoot and rood dry mass were determined on an analytical balance, after drying in an oven at 65 °C to constant weight.

Table 3. Mean squares of the analysis of variance for the polynomial regression of leaf area (LA), shoot dry matter (SDM) and root dry matter (RDM) of spring wheat plants, genotype IAC 375' submitted to increasing nitrogen doses in the presence or absence of silicon.

Source of variation	Mean square +Si			
	DOF	LA	SDM	RDM
L.R.	1	54.656191^{ns}	0.000364^{ns}	$0.002445**$
Q.R.	1	$669.928138**$	$0.002045**$	0.000375^{ns}
C.R.	1	151.714594^{ns}	0.000558^{ns}	0.000756^{ns}
Treatment	4	219.514667	0.000805	0.001037
Residue	15	44.135749	0.000225	0.000305
Source of variation	Mean square - Si			
	DOF	LA	SDM	RDM
L.R.	1	74.970984^{ns}	$0.001028*$	0.000705^{ns}
Q.R.	1	$186.152227*$	$0.004094**$	0.000848^{ns}
C.R.	1	52.295277^{ns}	$0.001072*$	0.000002^{ns}
Treatment	4	88.798137	0.001732	0.000412
Residue	15	44.135749	0.000225	0.000305

**,* Significant at 1 and 5% probability, respectively. ns, non-significant. Si, silicon. LA, leaf area. SDM, shoot dry matter. RDM, root dry matter. +Si, presence of silicon. -Si, absence of silicon. L.R., linear. Q.R., quadratic. C.R., cubic. DOF, degrees of freedom.
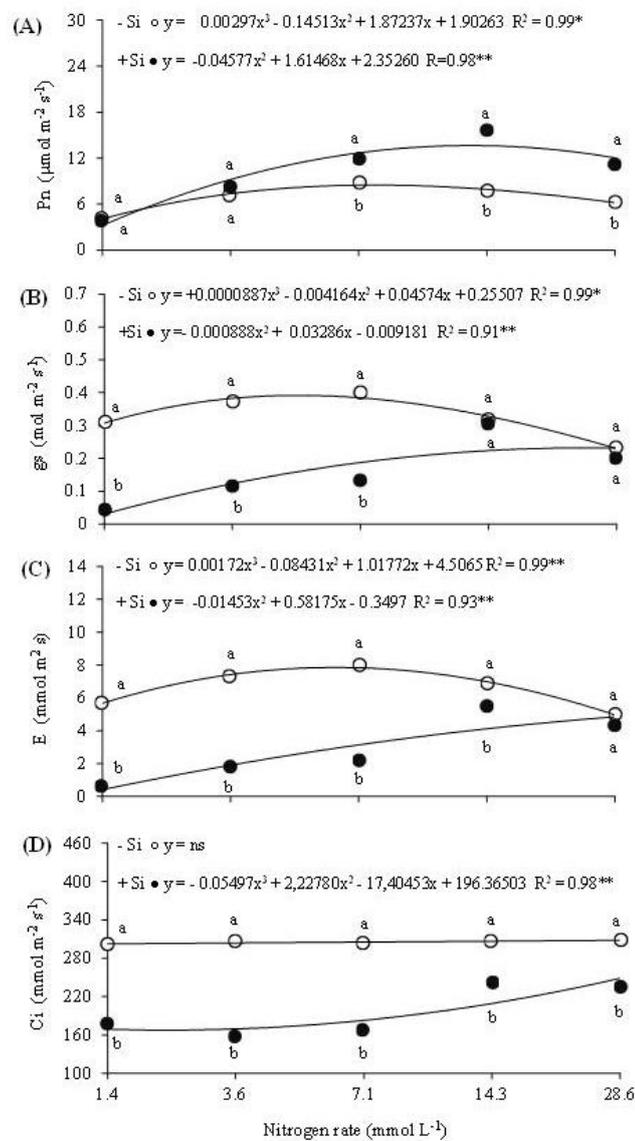
Fig 3. Effect of the interaction between silicon and nitrogen on the net CO_2 assimilation (Pn) (A), stomatal conductance (gs) (B), transpiration (E) (C) and intercellular CO_2 concentration (Ci) (D) in spring wheat plants grown in nutrient solution. **, * Significant at 1 and 5% probability. ns, non-significant. Lowercase letters compare + Si and Si within each N dose. MSD, minimum significant difference. Pn MSD: 2.3463; gs, MSD: 0.0733; E, MSD: 1.2090; Ci MSD: 35.3132.
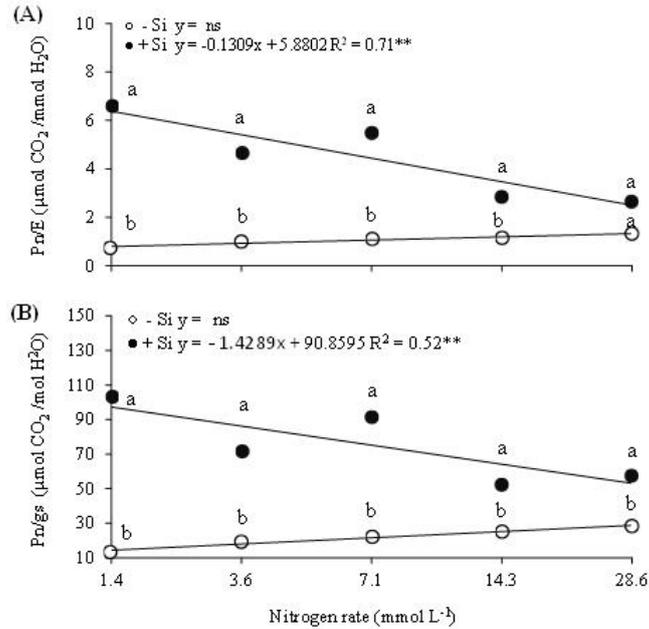
Fig 4. Effect of the interaction between silicon and nitrogen on the instantaneous (Pn/E) (A) and intrinsic water use efficiency (Pn/gs) (B) in spring wheat plants grown in nutrient solution. **, * Significant at 1 and 5% probability. ns, non-significant. Lowercase letters compare + Si and Si within each N dose. MSD, minimum significant difference. Pn/E, MSD: 1.3519; Pn/gs, MSD: 26.2386.
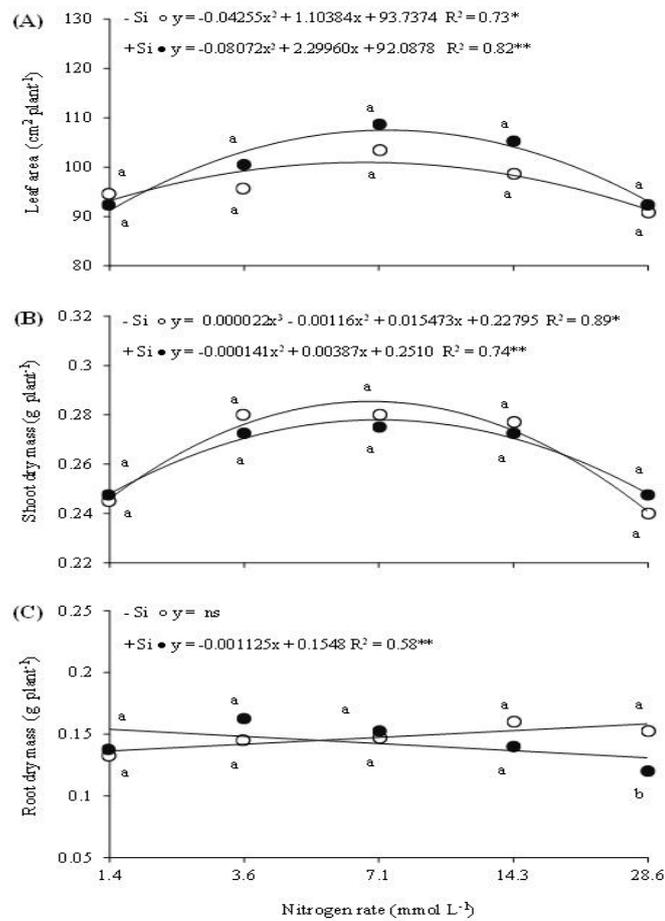
Fig 5. Effect of interaction between silicon and nitrogen on leaf area (A), leaf dry matter (B) and root dry matter (C) in spring wheat plants grown in nutrient solution. **, * Significant at 1 and 5% probability. ns, non-significant. Lowercase letters compare + Si and Si within each N dose. MSD, minimum significant difference. Leaf area, MSD: 9.5939; Shoot dry matter, MSD: 0.0217; Root dry matter, MSD: 0.0252.

Experimental design and statistical analysis

The experimental design was completely randomized in a 5 x 2 factorial (nitrogen doses x silicon doses) with each treatment consisting of 4 repetitions. Data was subjected to analysis of variance by the F-test at 5% probability. For unfolding of the degrees of freedom of the quantitative factor, represented by the nitrogen doses, a polynomial regression analysis was performed and selection of mathematical models was determined by the significance of the regression parameters. For the qualitative factor represented by the silicon levels, the Tukey test at 5% probability was applied.

Conclusion

In this article it was demonstrated that Si improves water use efficiency by wheat plants at the expense of N participation in this process. Furthermore, the N shows its greater importance in increasing the leaf area and dry mass of the shoots in relation to silicon, confirming its role in plant growth. In non-stress conditions, as observed in this study, the interaction between Si and N was not sufficiently effective to promote gains in plant growth, suggesting a morpho-anatomical and molecular methodological approach for future studies of this interaction, with the objective to specifically present the role of Si and its relation with N in the root system so as to understand the physiological response of wheat plants to interaction of Si and N.

Acknowledgements

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