

# Silicon application and plant growth promoting rhizobacteria consisting of six pure *Bacillus* species alleviate salinity stress in cucumber (*Cucumis sativus* L)

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## ABSTRACT

As water quality and availability decreases in many parts of the world, salinity is becoming a major challenge that reduces crop yield, even in soilless cultivation systems. Therefore, novel strategies are needed to promote plant salt tolerance in these systems. We hypothesized that the non-essential element silicon (Si) and plant-growth promoting *Bacillus* spp. can alleviate salt stress of cucumber (*Cucumis sativus* L.) grown in hydroponics. We tested this hypothesis by growing cucumber seedlings with and without salt stress (75 mM NaCl) and with and without 1.5 mM Si and an inoculum of six rhizosphere *Bacillus* species in a full-factorial design. Seedlings were grown in a climate room for two weeks in independent deep-water culture containers. The applied salt stress strongly reduced plant biomass, whereas Si application under salt stress resulted in a substantial increase in cucumber shoot and root biomass. This beneficial impact of Si was also observed in increased plant height, leaf area, specific leaf area, root length, specific root length, root surface area and root volume. The *Bacillus* species increased root dry weight, specific leaf area as well as specific root length. In seedlings grown under salt stress, Si application increased shoot and root Si concentration, whereas  $\text{Cl}^-$  concentration was reduced in the plant shoots. A reduction in  $\text{Cl}^-$  concentration of the shoots was also apparent in the *Bacillus* treatment. Under non-stress conditions, neither Si nor *Bacillus* species affected plant growth parameters. However, shoot mineral content was affected as Si application reduced shoot  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  concentrations, and inoculation with *Bacillus* species decreased K concentration. We conclude that Si does promote salt stress alleviation during the early growth stage of cucumber grown in deep water culture and this has implications for soilless crop production. Seed inoculation with *Bacillus* species showed a beneficial trend for some plant growth characteristics and nutrient status under high salinity, although not as pronounced as for Si.

## 1. Introduction

Global crop production is challenged by numerous abiotic stresses (Calanca, 2017). Salinification of soils and irrigation water is a major environmental threat, currently affecting more than 20% (approximately 240 million ha) of the total irrigated land (Munns and Tester, 2008). Projections for 2050 show a further increase in the scale and

impact of this environmental threat (Shrivastava and Kumar, 2015; Hassani et al., 2020).

Saline soils are defined as those with an electrical conductivity (EC) higher than  $4 \text{ dS m}^{-1}$  (approximately 40 mM NaCl) (Shrivastava and Kumar, 2015). However, the effects of salinity stress on plant growth depend on species susceptibility and their growth conditions as well as the ionic composition of salinity stress (Munns and Tester, 2008). Silicon

**Abbreviations:** Rdia, root diameter; RLT, total root length; Rsurf, Root surface area; Rvol, root volume; SRL, specific root length; LA, leaf area; SLA, specific leaf area.

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**Table A1**

Salts recipe used for the preparation of the nutrient solution with and without silicon addition.

	- silicon mmol L <sup>-1</sup>	+ silicon mmol L <sup>-1</sup>
Macro salts solid		
KNO <sub>3</sub>	6.75	3.75
MgSO <sub>4</sub> * 7 H <sub>2</sub> O	1.38	1.38
Ca(NO <sub>3</sub> ) <sub>2</sub> * 4 H <sub>2</sub> O	4.00	4.00
KH <sub>2</sub> PO <sub>4</sub>	1.25	1.25
NH <sub>4</sub> NO <sub>3</sub>	1.25	1.25
NaCl	0.25	0.25
	mmol L <sup>-1</sup>	mmol L <sup>-1</sup>
Macro salts solution		
50% K <sub>2</sub> SiO <sub>3</sub>	0.00	1.50
38% HNO <sub>3</sub>	0.00	3.00
	μmol L <sup>-1</sup>	μmol L <sup>-1</sup>
Micro salts solution		
Na-EDTA-Fe	30.00	30.00
H <sub>3</sub> BO <sub>3</sub>	25.00	25.00
ZnSO <sub>4</sub> * 7 H <sub>2</sub> O	10.00	10.00
CuSO <sub>4</sub> * 5 H <sub>2</sub> O	0.75	0.75
MnSO <sub>4</sub> * H <sub>2</sub> O	10.00	10.00
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub>	0.50	0.50

(Si) has been appraised for its beneficial properties in alleviating abiotic stresses such as drought and salinity (Thorne et al., 2020), and it increases the yields of a diverse range of crops under stress conditions, including: rice, wheat, sugarcane, soybean, apples (Ma and Takahashi, 2002; Liang et al., 1994; Korndörfer and Lepsch, 2001; Wijaya, 2016; Pati et al., 2016; Artyszak, 2018). Si is taken up from the soil and moved into the transpiration stream in the form of silicic acid. The silicic acid is translocated to the leaves where it is deposited as amorphous silica, in solid bodies known as phytoliths, or in other structures on the leaf surface or within cells (Hartley et al., 2015). This transport, distribution and deposition within plants involves both passive and active mechanisms (Kumar et al., 2017; McLarnon et al., 2017; Thorne et al., 2020). Si uptake can reduce the impact of abiotic stress through a range of

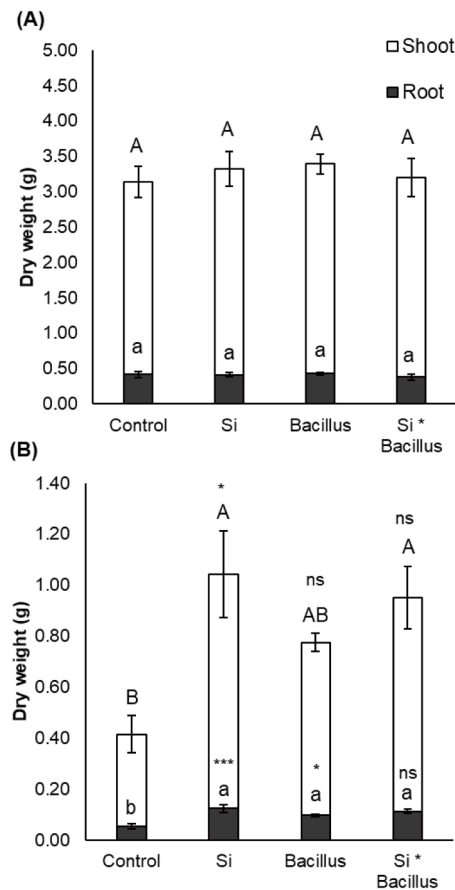
mechanisms e.g., reduction of oxidative stress (e.g. increased antioxidant enzyme activity), reduction of sodium ion accumulation in the shoots, reduction of stomatal conductance and subsequent transpiration losses, and by increasing osmolyte concentration and photosynthetic efficiency (Thorne et al., 2020). Si addition thus enhances mechanisms to reduce the osmotic stress associated with salinity which occur in the absence of Si (Khalid et al., 2020), but the magnitude of the additional benefits provided by Si varies between species and studies (Thorne et al., 2020).

Plant growth-promoting rhizobacteria (PGPR) are known to increase the tolerance of plants to abiotic stresses, while they affect the physiological and biochemical plant characteristics to a lesser extent under optimal conditions (Etesami and Maheshwari, 2018; Asghari et al., 2020). The PGPR can alleviate water deficit stress in plants by forming biofilms around the roots, increasing the relative water content in the leaves, and retaining a water layer around the root cells (Sandhya et al., 2010; Kasim et al., 2016). Under sub optimal conditions PGPR also facilitate shoot and root growth by increasing root traits such as root surface area, lateral root development, primary root elongation, root hair formation, root branching and root exudation; thereby enhancing nutrient access, nutrient uptake and plant water status (Vardharajula et al., 2011; Vacheron et al., 2013; Brazelton et al., 2008). Alongside these changes, PGPR can stimulate enzymatic activity, and production of antioxidants and osmoprotectants (e.g. proline) (Wang et al., 2018). Lastly, both PGPR (Groppa et al., 2012) and Si application (Rios et al., 2017) upregulate aquaporin gene expression under salt stress thereby increasing or maintaining root water uptake (Thorne et al., 2020). Yet, the stimulation of these salt tolerance traits by PGPR often results in a growth trade-off under optimal conditions (Rosier et al., 2018). Furthermore, most effects were demonstrated by application of individual PGPR strains, the beneficial effect of a multi-species inoculum has received far less attention. PGPR inoculum may be larger than individual application where synergies and mutualism are prevalent in the inoculum (Vacheron et al., 2013). Thus, quantifying the physio-morphological effects of a multi-species inoculum on the growth

**Table B1**

Test results of the effect of Bacillus, silicon (Si) and salt and their combinations on cucumber seedlings response variables. Effects were tested using Linear Mixed effects Models (LMM) with the treatments as fixed effects and block with growth cabinet as random effects at  $P \leq 0.05$ . DWS= shoot dry weight, DWR= root dry weight, DWT= total dry weight, LRWC= leaf relative water content, LA= leaf area, SLA= specific leaf area, SRL= specific root length, RLT= total root length, Rsurf= root surface area, Rdia= root diameter, Rvol= root volume. Bold values are indicated in bold. Non-significant trends are underlined.

	Plant height (cm)			DWS(g)			DWR(g)			DWT(g)		
	d.f.	F	p-value	d.f.	F	p-value	d.f.	F	p-value	d.f.	F	p-value
Si	1	4.787	0.039	1	2.174	0.154	1	0.092	0.764	1	1.79	0.195
Bacillus	1	0.003	0.956	1	0.496	0.504	1	0.012	0.915	1	0.388	0.553
Salt	1	121.045	0.000	1	294.419	0.000	1	301.608	0.000	1	301.221	0.000
Si * Bacillus	1	0.482	0.495	1	1.924	0.179	1	1.506	0.233	1	1.899	0.182
Si * Salt	1	3.182	0.088	1	1.614	0.217	1	3.432	0.078	1	1.833	0.189
Bacillus * Salt	1	0.007	0.933	1	0.030	0.864	1	0.731	0.402	1	0.069	0.795
Si * Bacillus * Salt	1	1.490	0.235	1	0.007	0.934	1	0.000	0.992	1	0.005	0.942
	LRWC (%)			LA (cm <sup>2</sup> )			SLA (cm <sup>2</sup> g <sup>-1</sup> )			SRL (cm g <sup>-1</sup> )		
	d.f.	F	p-value	d.f.	F	p-value	d.f.	F	p-value	d.f.	F	p-value
Si	1	6.701	0.015	1	0.341	0.565	1	3.214	0.086	1	10.474	0.003
Bacillus	1	2.002	0.167	1	0.337	0.578	1	1.345	0.279	1	4.048	<u>0.053</u>
Salt	1	0.441	0.512	1	284.319	0.000	1	328.008	0.000	1	45.942	0.000
Si * Bacillus	1	0.29	0.594	1	1.104	0.305	1	4.197	<u>0.052</u>	1	2.517	0.123
Si * Salt	1	0.615	0.439	1	1.605	0.218	1	12.974	0.001	1	11.844	0.002
Bacillus * Salt	1	0.528	0.473	1	0.068	0.797	1	0.43	0.519	1	1.458	0.237
Si * Bacillus * Salt	1	0.023	0.880	1	0.008	0.931	1	3.852	0.062	1	6.418	0.017
	RLT (cm)			Rsurf (cm <sup>2</sup> )			Rdia (mm)			Rvol (cm <sup>3</sup> )		
	d.f.	F	p-value	d.f.	F	p-value	d.f.	F	p-value	d.f.	F	p-value
Si	1	0.002	0.966	1	0.005	0.946	1	0.000	0.983	1	0.045	0.833
Bacillus	1	0.161	0.699	1	0.28	0.613	1	0.056	0.818	1	0.366	0.564
Salt	1	117.874	0.000	1	145.376	0.000	1	20.101	0.000	1	149.708	0.000
Si * Bacillus	1	2.121	0.159	1	1.686	0.208	1	0.126	0.726	1	0.935	0.344
Si * Salt	1	0.778	0.387	1	1.401	0.249	1	1.396	0.249	1	1.944	0.177
Bacillus * Salt	1	1.151	0.295	1	1.022	0.323	1	0.075	0.787	1	0.794	0.383
Si * Bacillus * Salt	1	0.546	0.468	1	0.331	0.571	1	0.248	0.623	1	0.084	0.775



**Fig. 1.** Effect of silicon, *Bacillus* spp. and their combination on shoot (open bars) and root (close bars) dry weight of cucumber seedlings under (A) non-stress and (B) salt stress conditions. Bar size is mean  $\pm$  S.E. ( $n = 5$ ). Different letters (lower case for root; upper case for shoot) indicate significant differences between treatments at  $P \leq 0.05$  level using Fisher's LSD test. ANOVA analysis main effects of Si, *Bacillus* and their interaction on total dry weight is reported with ns= not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . Under non-stress there were no significant (ns) effects of Si, *Bacillus*, nor Si\* *Bacillus*.

and nutrient uptake of cucumber seedlings would provide experimental insights in an understudied field. In addition to this, research on the combined application of Si and PGPR has been scarce, yet their co-application is expected to exert great benefits for plant biotic and abiotic stress alleviation (Etesami, 2018; Al-Garni et al., 2019; Vishwakarma et al., 2020).

The use of hydroponic systems, such as nutrient film techniques (NFT) and deep water-culture are becoming more widespread. This is due the higher control over rhizosphere conditions compared to soil

grown crops, which results in yield quantity, quality, and security gains. Closed-loop hydroponic system recycle the nutrient solution and are mandatory in e.g. the Dutch horticultural sector; this to reduce water use and environmental impact of fertilizer and pesticide runoff compared to freely draining systems. Closed-loop hydroponic systems are, however, prone to salinity stress as NaCl can accumulate because of polluted fertilizers and low water quality (high salinity) (Katsoulas and Voogt, 2014). High-quality water sources are scarce in many regions and are likely to become even scarcer in the near future (Assouline et al., 2015). It is therefore not uncommon for plants grown in hydroponics systems to be affected by salt stress (Niu et al., 2018). The need to screen new techniques to alleviate salinity constraints on plant growth in recirculating nutrient solution has been recognized (Neocleous et al., 2017). Application of biostimulants, such as Si and PGPR is one way forward but research on their potential for stress alleviation has been limited for hydroponic systems (du Jardin, 2015; Sambo et al., 2019; Singh et al., 2020). Cucumber (*Cucumis sativus* L.) is an important horticultural crop that is globally predominantly grown in hydroponics to mitigate the adverse biotic and abiotic factors which reduce its production in soil-based systems (Engindeniz, 2004; Walters et al., 2020). Cucumber is considered a moderate Si accumulator (Ma and Yamaji, 2006) and a moderately salinity-sensitive plant ( $<2.5$  dS  $m^{-1}$ ) (Stepien and Klobus 2006). Most knowledge on salinity stress alleviation by Si originates from experimental work on plant species of the Poaceae family, reflecting the fact that plants in this family are high accumulators of Si and their significance for crop production as both staple foods and pastures (Guntzer et al., 2012). In contrast, there is limited research on the benefits of Si in hydroponic systems, particularly on salinity stress alleviation in vegetable crops such as cucumber.

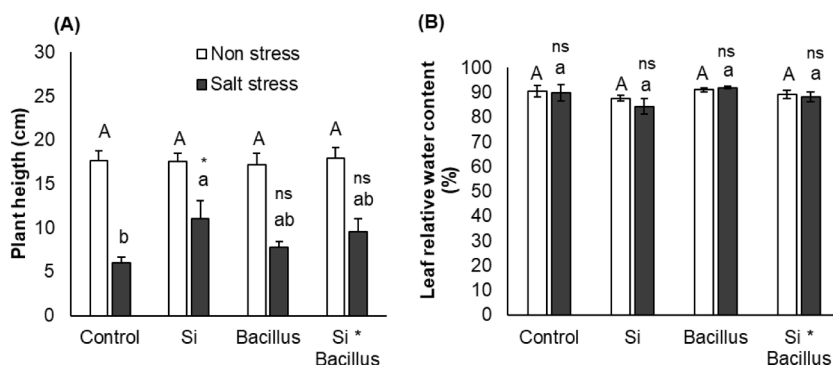
Thus, the aim of this study is to test the physio-morphological effects of PGPR, solubilized silicon, and their interaction on cucumber (*Cucumis sativus* L.) seedlings under salt stressed and non-stressed conditions in deep water culture under fully controlled environmental conditions.

## 2. Materials and methods

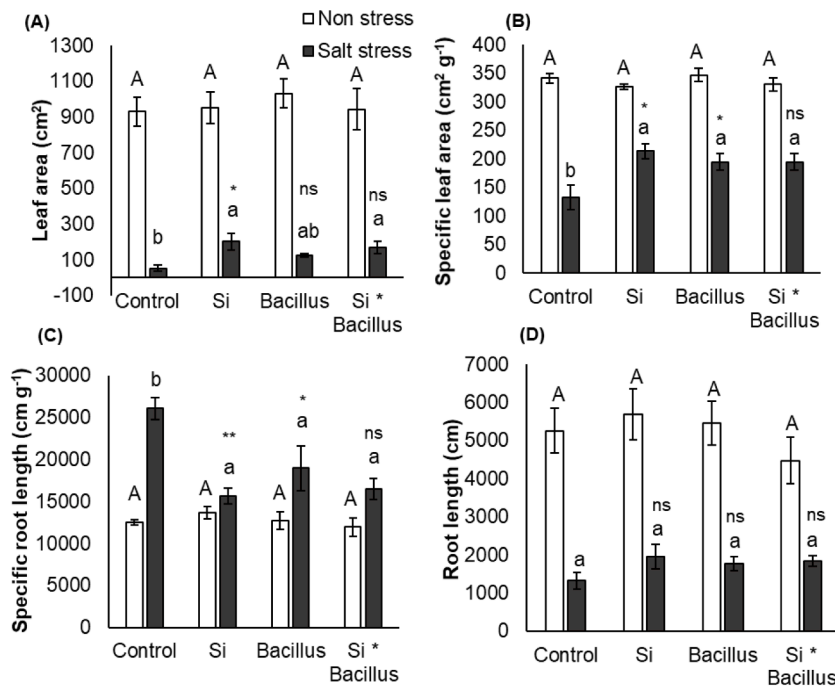
### 2.1. Experimental design

The deep-water culture system of Tocquin et al. (2003) was redesigned to facilitate highly controlled cucumber seedling growth (Fig. D1). Clean food grade polypropylene (PP) containers were used to minimize NaCl and Si contamination in the control treatment, rule out Si-substrate interactions, and facilitate PGPR growth by maintaining sufficient oxygen supply, adequate temperature and pH in the rhizosphere (Tyson et al., 2008; Song et al., 2017; Liang et al., 2005).

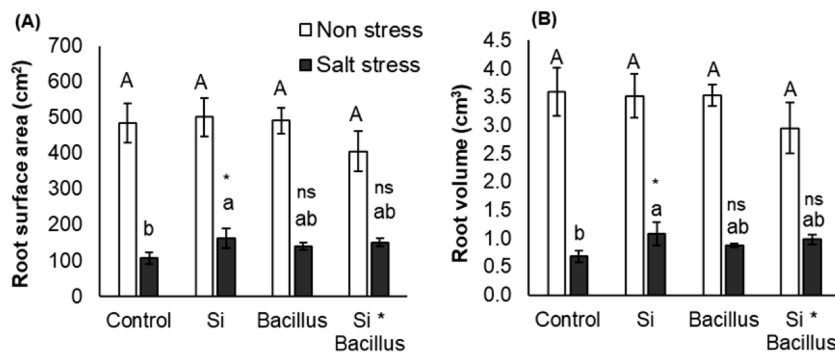
The experimental design contained three factors i.e., presence or absence of an inoculum of six pure *Bacillus* spp., 1.5 mM silicon and 75 mM of NaCl and their full factorial combination. This led to 8 treatments each replicated 5 times resulting in a total of 40 containers with one plant. To minimize the risk for bacterial contamination in plants without



**Fig. 2.** Effect of silicon, *Bacillus* spp. and their combination on (A) plant height and (B) LRWC of cucumber seedlings under non (open bars) and salt (close bars) stress (75 mM) conditions. Bar size is mean  $\pm$  S.E. ( $n = 5$ ). Different letters (lower case for salt stress conditions and upper case for non-stress) indicate significant differences between treatments at  $P \leq 0.05$  level using Fisher's LSD test. ANOVA analysis main effects of Si, *Bacillus* and their interaction is reported with ns= not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . Under non-stress there were no significant (ns) effects of Si, *Bacillus*, nor Si\* *Bacillus*.



**Fig. 3.** Effect of silicon, *Bacillus* spp. and their combination on leaf area (LA) (A), specific leaf area (SLA) (B), specific root length (SRL) (C) and total root length (RLT) (D) of cucumber seedlings under non (open bars) and salt (close bars) stress (75 mM) conditions. Bar size is mean  $\pm$  S.E. ( $n = 5$ ). Different letters (lower case for salt stress conditions, upper case for non-stress) indicate significant differences between treatments at  $P \leq 0.05$  level using Fisher's LSD test. ANOVA analysis main effects of Si, *Bacillus* and their interaction is reported with ns= not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . Under non-stress there were no significant (ns) effects of Si, *Bacillus*, nor Si \* *Bacillus*.



**Fig. 4.** Effect of silicon, *Bacillus* spp. and their combination on root surface area (A) and root volume (B) of cucumber seedlings under non (open bars) and salt (close bars) stress (75 mM) conditions. Bar size is mean  $\pm$  S.E. ( $n = 5$ ). Different letters (lower case for salt stress conditions, upper case for non-stress) indicate significant differences between treatments at  $P \leq 0.05$  level using Fisher's LSD test. ANOVA analysis main effects of Si, *Bacillus* and their interaction is reported with ns= not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . Under non-stress there were no significant (ns) effects of Si, *Bacillus*, nor Si \* *Bacillus*.

PGPR addition, cucumber seedlings were grown in two identical growth cabinets (Weiss Technik Ltd) each containing 20 containers. In each cabinet, the five replicates of each treatment (plants with and without NaCl, and with and without Si addition) were arranged in a randomized complete block design to account for the within-cabinet light and temperature variations (Fig. A2). Prior to the onset of the experiment, we recorded heat distribution (handheld infrared camera; FLIR E6, FLIR Systems, Inc., Oregon, USA) and light intensity distribution (quantum light-meter; LI-250A, Li-cor, Nebraska, USA) to ensure the growing conditions of the seedlings were similar.

## 2.2. Preparation of the bacterial inoculum

The PGPR inoculum was composed of six pure *Bacillus* spp. (TM1: *Bacillus megaterium*, TM2: *Bacillus licheniformis*, TM3: *Bacillus subtilis*, TM4: *Bacillus amyloliquefaciens*, TM6: *Bacillus mycoides*, TM7: *Bacillus methylotrophicus*). These species were originally isolated from the rhizosphere of tomato plants by Dr. P. Garbeva (NIOO-KNAW) and had confirmed plant-growth promoting properties as root endophytes exhibited in tomato plants (Garbeva, personal communication). The stock culture of each pure *Bacillus* spp. was stored at  $-80^{\circ}\text{C}$  and was cultured on Tryptic Soy Broth Agar (TSBA). PGPR were propagated by inoculating a loopful of each stock culture to freshly prepared TSBA

plates in triplicate. Subsequently, the cultures were placed in an incubator at  $25 \pm 1^{\circ}\text{C}$  for 5 days. The growth curve for each of the six *Bacillus* species was measured on a spectrophotometer (AquaMate Plus UV-Visible Spectrophotometer, Thermo Fisher Scientific, Inc., Waltham, MA, USA) by determining the optical density at 600 nm (OD600) and then counting colony forming units (CFU)  $\text{ml}^{-1}$ . Serial dilutions were done using phosphate buffer to obtain aliquots of cells with a concentration of  $10^{-7}$  CFU  $\text{ml}^{-1}$ .

## 2.3. Plant material and bacterial inoculation

Cucumber seeds of the cultivar PROLOOG RZ F1 (24-148) coated with fungicide tetramethylthiuram disulphide, were provided by Rijk Zwaan, the Netherlands. The seeds were surface sterilized with 0.5% sodium hypochlorite (NaOCl) and 80% ethanol, thoroughly rinsed with sterilized water, and then placed on double filter paper to germinate in the dark at  $25^{\circ}\text{C}$ .

One day after sowing (DAS) the bacterial inoculum was added on the emerging root. The inoculum was prepared by mixing equal volumes from the individual *Bacillus* species that were kept in the dark at  $4^{\circ}\text{C}$ ; 200  $\mu\text{l}$  of bacterial suspension was added on every seed. To account for the innate effect of inoculum application, e.g. a possible effect of the phosphate buffer on seed germination and plant growth, 200  $\mu\text{l}$  of sterile

**Table B2**

Test results of the effect of Bacillus, silicon (Si) and salt and their combinations on cucumber seedlings nutrient status. Effects were tested using Linear Mixed effects Models (LMM) with the treatments as fixed effects and block with growth cabinet as random effects at  $P \leq 0.05$ . Bold values are indicated in bold. Non-significant trends are underlined.

		Leaf Si (%)			Leaf P (%)			Leaf Cl (%)			Leaf Ca (%)			Leaf K (%)	
	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value
Si	1	3100.782	0.000	1	2.577	0.122	1	21.538	0.000	1	0.626	0.437	1	0.736	0.400
Bacillus	1	2.808	0.104	1	0.496	0.502	1	0.391	0.553	1	0.110	0.749	1	11.710	0.011
Salt	1	525.049	0.000	1	162.960	0.000	1	18,303.17	0.000	1	1429.688	0.000	1	9869.650	0.000
Si * Bacillus	1	0.515	0.478	1	0.020	0.888	1	0.261	0.615	1	0.855	0.365	1	0.280	0.602
Salt * Si	1	219.258	0.000	1	0.016	0.900	1	0.456	0.507	1	5.556	0.027	1	0.031	0.862
Salt * Bacillus	1	0.655	0.425	1	5.268	0.032	1	3.690	0.068	1	2.125	0.159	1	5.320	0.031
Salt * Si * Bacillus	1	1.346	0.255	1	0.191	0.666	1	2.366	0.139	1	0.440	0.514	1	1.224	0.281
		Leaf S (%)			Leaf C (%)			Leaf N (%)			Root Si (%)			Root P (%)	
	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value
Si	1	1.933	0.178	1	0.001	0.975	1	1.304	0.265	1	90.162	0.000	1	0.006	0.937
Bacillus	1	0.727	0.419	1	0.001	0.975	1	0.966	0.356	1	0.338	0.565	1	0.892	0.375
Salt	1	762.755	0.000	1	0.057	0.813	1	7.522	0.012	1	529.023	0.000	1	1341.176	0.000
Si * Bacillus	1	0.032	0.860	1	3.769	0.062	1	2.698	0.114	1	1.317	0.260	1	0.311	0.582
Salt * Si	1	7.016	0.014	1	0.420	0.522	1	0.034	0.854	1	0.773	0.386	1	0.593	0.449
Salt * Bacillus	1	0.030	0.865	1	1.464	0.236	1	1.058	0.315	1	2.357	0.135	1	0.157	0.695
Salt * Si * Bacillus	1	0.973	0.334	1	0.001	0.974	1	0.033	0.858	1	6.049	0.020	1	0.254	0.619
		Root Cl (%)			Root Ca (%)			Root K (%)			Root S (%)				
	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value
Si	1	1.923	0.176	1	0.007	0.936	1	0.003	0.957	1	0.595	0.446			
Bacillus	1	4.937	0.034	1	2.429	0.130	1	0.033	0.856	1	2.023	0.165			
Salt	1	3551.840	0.000	1	0.154	0.697	1	1556.474	0.000	1	70.798	0.000			
Si * Bacillus	1	1.408	0.245	1	0.060	0.809	1	0.682	0.416	1	1.339	0.256			
Salt * Si	1	1.616	0.213	1	0.089	0.768	1	0.004	0.948	1	2.093	0.158			
Salt * Bacillus	1	0.120	0.732	1	0.478	0.495	1	0.143	0.708	1	4.004	<u>0.055</u>			
Salt * Si * Bacillus	1	0.000	0.999	1	0.212	0.648	1	0.398	0.533	1	4.101	<u>0.052</u>			

buffer was also added to each seed of the non-inoculated treatment. All treatments were applied under sterile conditions in a laminar flow cabinet.

Seeds were sown in two containers (37.5 (l) x 24 (w) x 4 (h) cm) containing rinsed and sterilized bioplastics (Polypropylene (PP) granules obtained from <https://shop.breiwinkel.nl>) and 500 ml half-strength Hoagland solution (1 DAS) and incubated at 22 °C, 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity and 60% relative humidity.

#### 2.4. Seedlings' growth conditions

Nine DAS, forty uniform seedlings were transplanted in the containers. Abiotic parameters in the growth cabinets were set to 16 h photoperiod, coinciding with a 25 °C day / 22 °C night temperature, 70% relative humidity and 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity (LED, Hettich Benelux B.V., Geldermalsen, the Netherlands). The nutrient solution was modified from [de Kreijl et al. \(1999\)](#) (in  $\text{mmol L}^{-1}$ : 1.25  $\text{NH}_4^+$ , 16.00  $\text{NO}_3^-$ , 8.00  $\text{K}^+$ , 4.00  $\text{Ca}^{2+}$ , 1.38  $\text{Mg}^{2+}$ , 1.38  $\text{SO}_4^{2-}$  and in  $\mu\text{mol L}^{-1}$ : 30.00 Fe, 25.00 B, 0.75 Cu, 10.00 Zn, 10.00 Mn, 0.50 Mo.) with EC of  $2.3 \pm 0.3 \text{ dS m}^{-1}$  and pH of  $5.2 \pm 0.1$ , adjusted using citric acid (0.1 M) and  $\text{K}_2\text{HCO}_3$  (0.1 M). As the fertilizers (Table A1) used for the nutrient solution were purified salts of laboratory quality and the water was deionized, we added 0.25 mM NaCl to all solutions. This to account for the potential "NaCl growth stimuli" observed for many species at low NaCl dose ([Marschner, 2012](#)). The solution was formulated such that pH and ion composition were the same for all treatments (Table A1); they only differed in the intended 1.5 mM Si (from potassium silicate ( $\text{K}_2\text{SiO}_3$ ) and 75 mM NaCl (EC:  $10.7 \pm 1 \text{ dS m}^{-1}$ ). Dissolved oxygen (DO) was measured using a pH/ISE/Conductivity/RDO/DO meter (Thermo Scientific™ Orion™ Star A329 pH/ISE/Conductivity/RDO/DO Portable Meter, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and always exceeded 90%.

#### 2.5. Plant measurements

All physio-morphological measurements were done during or post-harvest, i.e. 23 DAS. Plant height was defined as the distance between root-shoot interface and from the upper boundary of the top leaf. All fully expanded leaf blades including 1 cm of their petiole were collected and their fresh weight was recorded. The leaf area (LA) of the samples was measured using a leaf area meter (LI-3100C, Li-Cor Inc., Lincoln, NE, USA), then one fully expanded leaf per plant was placed in a zipper bag with its petiole submerged in deionized water and stored at 4 °C. After 12 h the turgid weight (TW) of the leaves was measured as described in [Wilson et al. \(1999\)](#). All dry weights (leave, stem, roots) were obtained by oven-drying at 70 °C until constant weight (max. 4 days). Leaf relative water content (LRWC) was calculated using the equation as per [Abd El-Mageed and Semida \(2015\)](#):

$$\text{LRWC} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

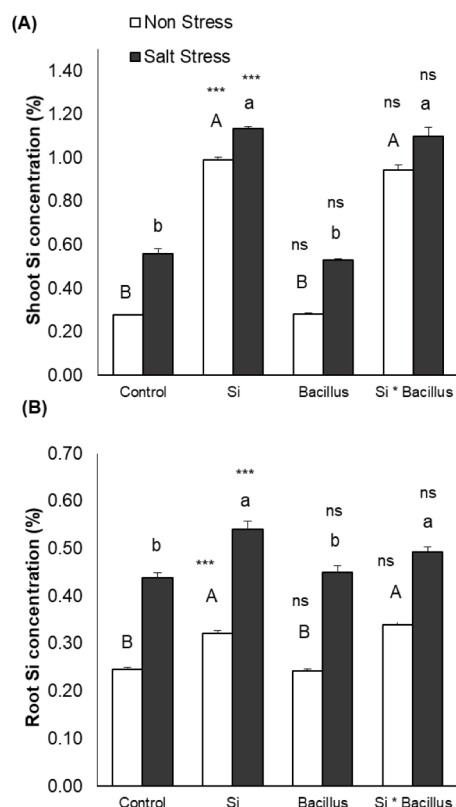
Specific leaf area (SLA) was calculated by using the equation as per [Wilson et al. \(1999\)](#).

$$\text{SLA} = \frac{\text{Leaf area}}{\text{Dry Weight}}$$

After, the fresh weight of the roots was recorded, the roots were placed in zipper bags in the fridge at 4 °C wrapped in water-moistened tissue paper. Two days later, they were divided in two samples. The first sample was directly oven-dried at 70 °C to constant weight (DWrs). The second sample was stored in a 100 ml tube with deionized water until root scan measurements on the same day. Following the root scans, these samples were also oven-dried (DWscan). The total dry weight of the root (DWR) was calculated as:  $\text{DWR} = \text{DWrs} + \text{DWscan}$ .

Root images were acquired using a high-resolution flatbed scanner (Epson Perfection V700 PHOTO scanner, Epson, Nagano, Japan). WinRHIZO software (Regent Instruments Ltd, Ontario, Canada) was used to obtain the following root parameters from these images: total





**Fig. 5.** Effect of silicon, *Bacillus* spp. and their combination on Si concentration of cucumber shoot (A) and root (B) under non (open bars) and salt (close bars) stress (75 mM) conditions. Bar size is mean  $\pm$  S.E. ( $n = 5$ ). Different letters (lower case for salt stress conditions, upper case for non-salt) indicate significant differences between treatments at  $P \leq 0.05$  level using Fisher's LSD test. ANOVA analysis main effects of Si, *Bacillus* and their interaction is reported with ns= not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

length (RLT), surface area (Rsurf), volume (RVol), average diameter (Rdia). All root measurements were expressed as a fraction of DWR. Specific root length (SRL) was calculated as the RLT to DWR ratio.

To determine plant mineral content, dried aboveground and belowground plant samples were separately ball-milled to a fine powder and pressed into pellets. These pellets were analysed for Si, phosphorus (P), chloride ( $\text{Cl}^-$ ), calcium ( $\text{Ca}^{2+}$ ), potassium ( $\text{K}^+$ ) and sulfur (S) using a portable P-XRF spectrometer (Niton XL3t900 GOLDD Analyzer; Thermo Scientific, Winchester, UK) (Reidinger et al., 2012). Carbon (C) and nitrogen (N) content were measured on subsamples of the fine powder using a CN elemental analyser (TruSpec Micro Elemental Analyzer, LECO, Germany).

## 2.6. Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA), using a significance threshold of  $\alpha = 0.05$ . To test for difference between means of response variables, linear mixed effects regression models (LMM) were used. Initially, LMM were built for all response variables with treatments as main effect and nested blocks within growth cabinets as random intercept effect. The residuals of the LMM were tested for normality (Shapiro-Wilkinson test and histogram inspection) and homogeneity (QQ-plots). The NaCl treatment was found to have a clear and highly significant effect on all response variables creating two distinct data types in terms of variance, i.e. non-stress and salinity stress. To avoid the inherent heteroscedasticity, the non-stress and salinity-stress treatments were analyzed separately in order to identify the effect and interaction of the Si and *Bacillus* treatments within 'salt stress' or 'non-stress'

**Table C1**

Effect of plant growth-promoting *Bacillus* species and silicon (Si) on the nutrient concentration of cucumber seedlings under salt stress. Data are mean  $\pm$  S.E. ( $n = 5$  for every treatment). Different letters in a column indicate significant differences between the treatments at  $P \leq 0.05$  using Fisher's LSD test. Significance: ns= not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . "Control": 0 mM Si and 75 mM NaCl.

Treatment	Leaf P (%)	Leaf Cl (%)	Leaf Ca (%)	Leaf K (%)	Leaf S (%)	Leaf C (%)	Leaf N (%)
Control	1.59 $\pm$ 0.10a	5.87 $\pm$ 0.15a	1.42 $\pm$ 0.05a	1.65 $\pm$ 0.06a	0.340 $\pm$ 0.02a	33.15 $\pm$ 3.87a	3.71 $\pm$ 0.53a
<i>Bacillus</i>	1.47 $\pm$ 0.03a	5.52 $\pm$ 0.14b	1.36 $\pm$ 0.03a	1.58 $\pm$ 0.03a	0.336 $\pm$ 0.01a	30.48 $\pm$ 1.75a	3.59 $\pm$ 0.27a
Si	1.52 $\pm$ 0.11a	5.22 $\pm$ 0.02b	1.48 $\pm$ 0.09a	1.68 $\pm$ 0.06a	0.340 $\pm$ 0.01a	31.77 $\pm$ 1.24a	3.33 $\pm$ 0.10a
Si * <i>Bacillus</i>	1.40 $\pm$ 0.05a	4.56 $\pm$ 0.17b	1.41 $\pm$ 0.05a	1.58 $\pm$ 0.04a	0.360 $\pm$ 0.02a	36.99 $\pm$ 1.59a	3.98 $\pm$ 0.25a
Significance							
<i>Bacillus</i>	ns	*	ns	ns	Ns	ns	ns
Si	ns	**	ns	ns	ns	ns	ns
Si * <i>Bacillus</i>	ns	ns	ns	ns	ns	ns	ns
Treatment	Root P (%)	Root Cl (%)	Root Ca (%)	Root K (%)	Root S (%)	Root C (%)	Root N (%)
Control	1.52 $\pm$ 0.05a	3.66 $\pm$ 0.15a	1.11 $\pm$ 0.04a	1.93 $\pm$ 0.12a	0.428 $\pm$ 0.02a		
<i>Bacillus</i>	1.49 $\pm$ 0.02a	3.54 $\pm$ 0.12a	1.06 $\pm$ 0.02a	1.93 $\pm$ 0.10a	0.496 $\pm$ 0.01a		
Si	1.50 $\pm$ 0.05a	3.88 $\pm$ 0.26a	1.09 $\pm$ 0.06a	1.96 $\pm$ 0.05a	0.438 $\pm$ 0.03a		
Si * <i>Bacillus</i>	1.47 $\pm$ 0.02a	3.32 $\pm$ 0.15a	1.06 $\pm$ 0.02a	1.91 $\pm$ 0.03a	0.440 $\pm$ 0.02a		
Significance							
<i>Bacillus</i>	ns	ns	ns	ns	ns		
Si	ns	ns	ns	ns	ns		
Si * <i>Bacillus</i>	ns	ns	ns	ns	ns		

treatments. Least-squares means of all response variables were compared for identifying differences between the treatments using Fisher's least significant difference (LSD).

## 3. Results

### 3.1. Plant growth parameters

Seedling growth was significantly reduced by the salt stress treatment and this was evident for all plant parameters (Table B1). In non-stress conditions, neither shoot nor root dry weights were affected by the application of Si, *Bacillus* spp. or their combination (Fig. 1A). In contrast, salt-treated seedlings with Si application exhibited a 2.5-fold increase in biomass compared to plants without Si application (Fig. 1B). Seedlings with salt stress showed higher root but not shoot dry weight following *Bacillus* spp. application (Fig. 1B). Yet, no interaction between Si and *Bacillus* treatments was observed.

In non-stress conditions, seedlings did not differ significantly in height with Si or *Bacillus* application or with both treatments. A decrease in plant height was observed following salt stress for all treatments (Fig. 2A and Table B1). Plant height of salt-treated seedlings without Si application was 54.1% of that of plants with applied Si. Salinity did not lead to significant changes in leaf relative water content (LRWC) (Fig. 2B).

**Table C2**

Effect of plant growth-promoting *Bacillus* species and silicon (Si) on the nutrient concentration of cucumber seedlings without salt stress. Data are mean  $\pm$  S.E. ( $n = 5$  for every treatment). Different letters in a column indicate significant differences between the treatments at  $P \leq 0.05$  using Fisher's LSD test. Significance: ns= not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . "Control": 0 mM Si and 0 mM NaCl.

Treatment	Leaf P (%)	Leaf Cl (%)	Leaf Ca (%)	Leaf K (%)	Leaf Ca/Leaf S (%)	Leaf C (%)	Leaf N (%)
Shoot							
Control	1.07 $\pm$ 0.04a	0.034 $\pm$ 0.002b	2.76 $\pm$ 0.03a	5.56 $\pm$ 0.08a	0.628 $\pm$ 0.01a	36.56 $\pm$ 3.41a	4.74 $\pm$ 0.34a
Bacillus	1.09 $\pm$ 0.02a	0.033 $\pm$ 0.002b	2.85 $\pm$ 0.07a	5.23 $\pm$ 0.03b	0.596 $\pm$ 0.01a	31.08 $\pm$ 2.10a	4.61 $\pm$ 0.25a
Si	1.00 $\pm$ 0.02a	0.026 $\pm$ 0.002a	2.70 $\pm$ 0.06b	5.53 $\pm$ 0.05a	0.647 $\pm$ 0.01b	30.05 $\pm$ 5.29a	3.69 $\pm$ 0.74a
Si * Bacillus	1.06 $\pm$ 0.04a	0.029 $\pm$ 0.001ab	2.68 $\pm$ 0.03ab	5.33 $\pm$ 0.09ab	0.599 $\pm$ 0.02a	32.72 $\pm$ 1.06a	4.54 $\pm$ 0.23a
Significance							
Bacillus	ns	ns	ns	*	Ns	ns	ns
Si	ns	**	*	ns	**	ns	ns
Si * Bacillus	ns	ns	ns	ns	Ns	ns	ns
Treatment	Root P (%)	Root Cl (%)	Root Ca (%)	Root K (%)	Root S (%)	Root C (%)	Root N (%)
Control	0.82 $\pm$ 0.03a	0.20 $\pm$ 0.02a	1.09 $\pm$ 0.01a	6.57 $\pm$ 0.26a	0.37 $\pm$ 0.01a		
Bacillus	0.78 $\pm$ 0.01a	0.18 $\pm$ 0.01a	1.08 $\pm$ 0.01a	6.81 $\pm$ 0.14a	0.35 $\pm$ 0.01a		
Si	0.81 $\pm$ 0.02a	0.19 $\pm$ 0.03a	1.10 $\pm$ 0.03a	6.73 $\pm$ 0.29a	0.37 $\pm$ 0.01a		
Si * Bacillus	0.82 $\pm$ 0.02a	0.15 $\pm$ 0.01a	1.08 $\pm$ 0.02a	6.62 $\pm$ 0.11a	0.37 $\pm$ 0.00a		
Significance							
Bacillus	ns	ns	ns	ns	Ns		
Si	ns	ns	ns	ns	Ns		
Si * Bacillus	ns	ns	ns	ns	Ns		



**Fig. A1.** Plant growing system inside one of the two growth cabinets. Each 2 L non-transparent containers (180  $\times$  130  $\times$  130 mm) with a transparent lid, covered with black plastic membrane to avoid algae development, was used to support the growth of one plant. A wooden stick was adjusted in the containers for mechanical support of plant growth. The plant was grown by floating on the nutrient solution onto foam discs. An aeration tube was adjusted in the containers providing sufficient amounts of oxygen. All the individual aeration tubes were connected to a large diameter PVC tube with plug valves for air flow adjustment. The PVC tube was connected to an air pump outside the cabinet.

Salt stress markedly reduced leaf area (LA) and specific leaf area (SLA) (Table B1), yet following either Si or *Bacillus* spp. application SLA showed a 1.5-fold increase (Fig. 3B). Si application also increased LA under stress and the effect of *Bacillus* spp. were similar to Si but not significantly different from the control treatment. Under non-stress conditions, neither Si nor *Bacillus* spp. application had a significant effect on LA or SLA (Fig. 3A and B).

Salt stress increased SRL (Fig. 3C) and reduced RLT (Fig. 3D) (Table B1). There was a doubling of SRL in control seedlings (without Si and/or PGPR) in such conditions. In the absence of salt stress, there were no effects of the Si and *Bacillus* treatments on RLT and SRL (Fig. 3D and C). In seedlings subjected to salt stress, there was a major decrease in SRL following Si or *Bacillus* application (Fig. 3C). The same trend was not observed for RLT in plants in the non-stressed conditions where Si and *Bacillus* treatments did not differ significantly from the plants without these treatments (Fig. 3D).

Salt stress significantly reduced Rsurf and Rvol (Table B1). Without salt stress there was no significant change in the response of Rsurf and

Rvol to Si or *Bacillus* treatments. However, in salt stress conditions a significant increase in Rvol and Rsurf was observed following Si application, but not for *Bacillus* treatments or the two treatments combined (Fig. 4A and B). No interaction was observed for the different treatments under low and high NaCl concentrations.

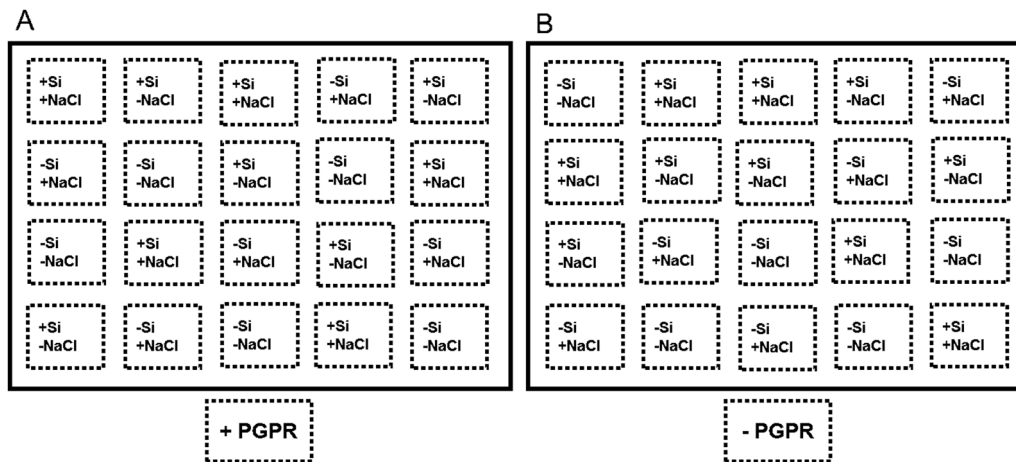
Some key response parameters were highly correlated. To get a better appreciation of the correlations between the response parameters we added Pearson correlations plots as supplementary material (Fig. D1).

### 3.2. Plant mineral concentration

Salinity significantly altered plant mineral concentration in both the aerial and root parts of cucumber seedlings, with the exception of root  $\text{Ca}^{2+}$  concentration and carbon concentration (Tables C1 and B2). In seedlings grown under salt stress, Si application increased both shoot and root Si concentration (by 29% and 50% respectively). There was no effect of the *Bacillus* treatment on the Si levels in the seedlings (Fig. 5). In absence of salt stress, Si application led to increased Si concentration in both shoots and roots, whereas the *Bacillus* treatment had no effect. Si concentration was higher for the aboveground than for belowground plant parts (Fig. 5A and B).

Salinity increased P, and  $\text{Cl}^-$  concentration of shoots by 35% and 198% respectively as compared to non-stress conditions (Table C1). Similar responses were observed in the roots, with a 60% increase in P and 181% increase in  $\text{Cl}^-$ . In seedlings grown under salt stress, the shoots had a lower concentration of  $\text{Ca}^{2+}$ ,  $\text{K}^+$  and S (reduction of 64%, 108% and 57% respectively compared to plants under non-stress conditions). Roots of seedlings under salt stress also had lower  $\text{K}^+$  and S concentration (110% and 22% respectively). N concentration was decreased by 19% following salt stress, whereas C concentration remained similar in both stress and non-stress conditions. Both individual Si and PGPR treatments reduced  $\text{Cl}^-$  concentration in the shoots with no interaction observed. No differences in nutrient status of the roots were documented for either treatments under salinity (Table C1).

In seedlings grown without salt stress and with Si, the shoots had significantly lower concentrations of  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  and significantly higher concentrations of S. In seedlings grown without salt stress and inoculated with *Bacillus* the shoot  $\text{K}^+$  concentration was reduced (Table C2).



**Fig. A2.** Diagram of the experimental design with the two growth cabinets (A, B) in solid lines and the individual containers with their respective treatments within each cabinet in dotted lines. To avoid bacterial contamination, PGPR-treated seedlings were placed in cabinet A while non PGPR-treated seedlings were placed in cabinet B. The abiotic conditions of the growth cabinet were the same.

#### 4. Discussion

For cucumber seedlings grown under optimal conditions in deep water culture there was no effect of silicon (Si) and *Bacillus* spp. (PGPR) application on plant growth parameters. Yet, under salt stress, which significantly impaired growth with an 8-fold reduction in dry weight (Fig. 1 and Table B1), Si application was beneficial and partly alleviated the impact of salinity. The growth reducing effect of salinity on cumpers has been demonstrated in greenhouse studies (Chartzoulakis, 1992). The beneficial role for Si alleviating this stress has also been documented (Zhu et al., 2004; Yin et al., 2019). A number of possible mechanisms could underpin this beneficial effect of Si, including an amelioration of salt-induced oxidative stress through increased accumulation of polyamine levels (Zhu et al., 2004), and an increase in root water uptake through upregulation of aquaporin activity (Yin et al., 2019). In addition, Si deposition in the transpiration stream at the epidermis of the leaf can act as a mechanical barrier preventing water loss and thus maintaining high leaf relative water content (LRWC, Chen et al., 2018), as well as enhancing photosynthetic rate Ma et al. (2004). The increased Si concentration in the roots as well as in the shoots of the seedlings (Fig. 5) could be significant: according to the apoplastic obstruction hypothesis, the deposition of Si in the Casparian strip in the root could reduce the uptake of sodium ions ( $\text{Na}^+$ ) (Coskun et al., 2019; Thorne et al., 2020). This mechanism, i.e. Si deposition in the root and the associated obstruction of bypass flow resulting in reduced  $\text{Na}^+$  uptake has also been observed in rice (Yan et al., 2021). Here, we did not quantify  $\text{Na}^+$  levels but we did observe reduced chloride ion ( $\text{Cl}^-$ ) concentration in plant roots and leaves under NaCl stress combined with Si application.

Salinity can decrease the specific leaf area (SLA) of plants, partly due to investment in the production of osmoprotectants essential for adaptation to water limitation (Gong et al., 2003), and this reduction also improves water use efficiency by increasing leaf thickness (Omamt et al., 2006). We found that Si significantly increased SLA of salt-stressed seedlings, as did PGPR. The beneficial effect of PGPR on salt stress alleviation in cucumber has been previously linked to reduced activity of antioxidants (Kang et al., 2014). Si, but not bacteria, had a significant effect on the plant height of seedlings in salt stress conditions (Fig. 2A). Overall, PGPR addition had a less pronounced effect on salt stress alleviation than Si.

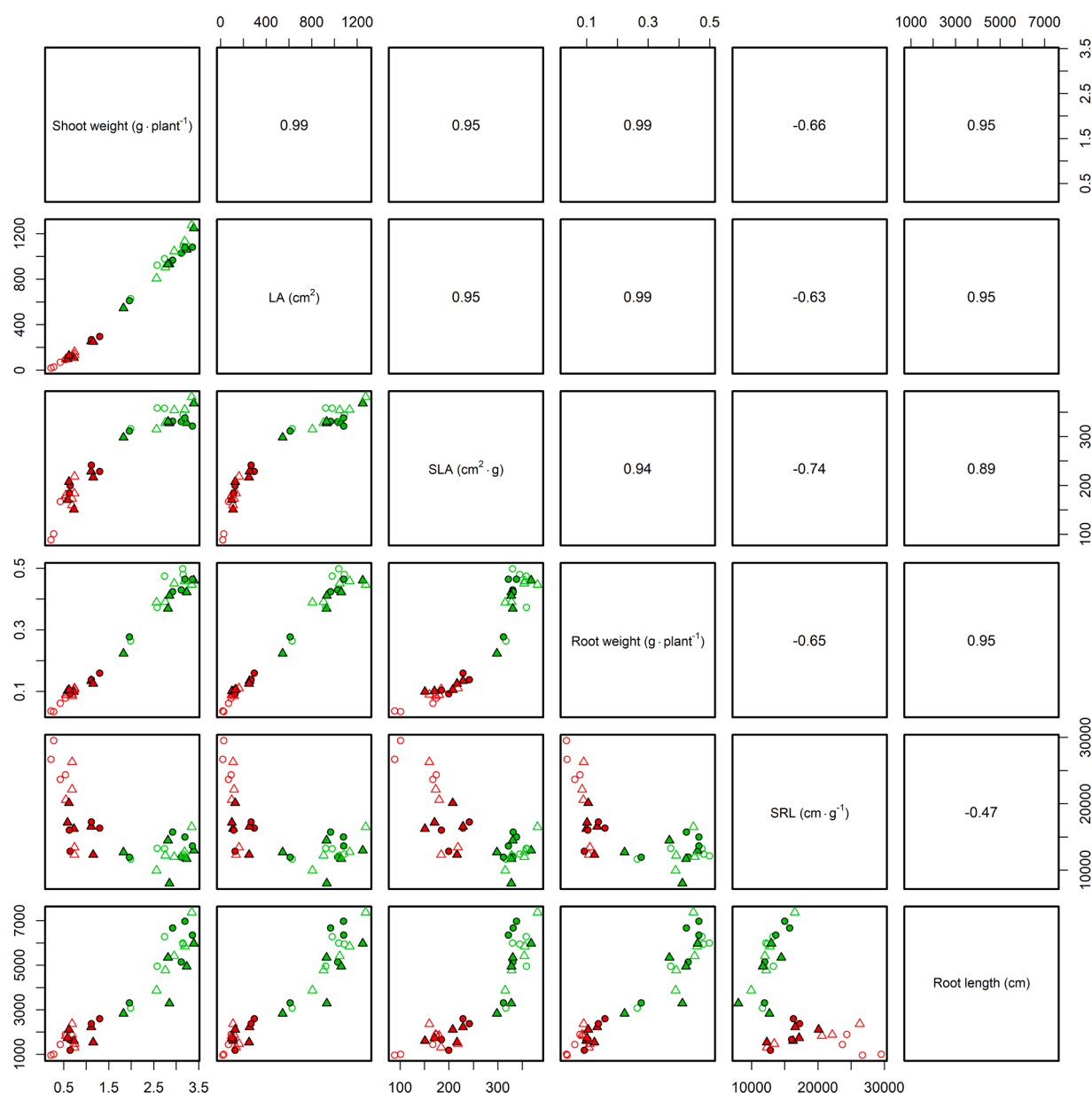
Compared to aboveground traits, salt stress had smaller impact on root biomass, but again this impact was reduced when Si was added in the nutrient solution. This effect is consistent with Wang et al. (2015) who attributed stress alleviation due to Si to enhanced plant water balance through a higher LRWC together with an increase in potassium

( $\text{K}^+$ ): sodium ( $\text{Na}^+$ ) ion ratio and polyamine accumulation. In our study, LRWC was unchanged in Si treated seedlings, but we did observe increased specific root length (SRL) in seedlings with salinity stress, indicating changes in the resource allocation strategy of the plants. Higher SRL values reflect reduced investment in biomass for the production of certain root length, potentially supporting faster resource acquisition (Cheng et al., 2016). Silicon and PGPR application under salt stress reduced the SRL values, indicating higher biomass investment for root length production. Neither Si nor PGPR application increased root diameter under salt stress (data not shown) but Si alone increased root surface (Rsurf) and root volume (Rvol) of the seedlings implying an important structural adaptation of the roots. Taken together, these results imply that Si application induced changes in the rooting system (SRL, Rsurf, Rvol) in salt-treated seedling enabling them to increase their shoot biomass in the face of salinity stress. Although Si application resulted in a significantly larger aboveground biomass, SLA, and total length area (LA), the root length was unaffected by Si, again pointing to improved root functioning due to Si.

Si and PGPR did not alter plant growth parameters under non-stress conditions, as found by Yin et al. (2016), although some studies have observed that Si improved plant performance even under non stress growth conditions (Zhu et al., 2004; Flam-Shepherd et al., 2018). In our study, PGPR application did not affect the growth of seedlings, though it did reduce their  $\text{K}^+$  concentration under non-stress conditions. This is similar to Gómez-Bellot et al. (2018), who also reported a decrease in  $\text{K}^+$  content but no effect on plant height following PGPR (*Acetobacter fabarum*, *Acinetobacter johnsonii*, *Candida boidinii*, *Nocardiaopsis alba*, *Penicillium chrysogenum* and *Azospirillum brasilense*) addition to laurustinus (*Viburnum tinus* L.) growing in soil, under non-stress conditions. In our study, the bacterial inoculum applied in salt treated seedlings was expected to colonize the root system and provide additive benefits to the salt stress alleviation effects of Si. Moreover, due to their plant growth-promoting qualities, i.e. stimulation of growth hormone production by seedlings, they were expected to increase root traits that are associated with salt stress resilience (Numan et al., 2018). Even though PGPR establishment in the roots of the seedling was confirmed, by isolating them from root tissues, their effect was not pronounced in our study. Neither synergy nor competition between bacteria and Si was observed, indicating no interaction effect between these parameters in this experimental setup.

In addition to growth, plant mineral status was also affected following, salinity, Si and PGPR addition. Shoot nitrogen (N) concentration was significantly decreased under salt stress conditions, which indicates reduced functioning of plant roots to acquire N from the





**Fig. D1.** Pearson's correlations coefficients and plots between shoot weight, leaf area (LA), specific leaf area (SLA), root weight, specific root length (SRL) and root length for cucumber seedlings growing under non (green color) and salt (red color) stress. Open symbols refer to seedlings without silicon (Si) addition, while closed symbols refer to Si-treated seedlings. Round symbols refer to seedlings without *Bacillus* spp. addition, while triangle symbols refer to *Bacillus* spp.-treated seedlings. This figure was plotted using R version 4.0.4.

nutrient solution. An increased concentration of  $\text{Cl}^-$  was observed for both shoot and root. Increased  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations have been observed under salt stress in cucumber, together with reduced  $\text{K}^+$  concentration and lower biomass production (Alpaslan and Gunes, 2001). We did not see a significant decrease in  $\text{K}^+$  concentration, but our results do support a relationship between increased  $\text{Cl}^-$  concentration and biomass reduction under salt stress.

Our seedlings responded to salt-stress by increasing Si concentration both belowground and aboveground. However, in a meta-analysis, Cooke and Leishman (2016) found a negative or neutral relationship between Si supply and Si accumulation in plant shoots and roots respectively when combining various types of abiotic stresses. Despite this, the same analysis found that Si consistently increased productivity and reduced oxidative damage in plants under abiotic stress. Here we found similar beneficial stress-alleviating effects but with an increase in Si uptake. Although salt-stressed plants had higher concentrations of

$\text{Cl}^-$  than non-stressed ones, in both cases, Si addition reduced those concentrations. This finding is in accordance with literature where a decrease in the apoplastic transportation of both  $\text{Na}^+$  and  $\text{Cl}^-$  in plants with higher Si uptake is highlighted as a potential salt stress alleviating mechanism (Liu et al., 2019; Thorne et al., 2020). Inoculation of seedlings with the *Bacillus* spp. inoculum also led to a reduction  $\text{Cl}^-$  concentration in seedlings' shoots, but only in stressed conditions.

Salinity induces higher efflux of electrolytes (such as  $\text{K}^+$ ) due to changes in cell membrane permeability resulting in lower ionic concentrations (Volkov, 2015). In the absence of Si or PGPR,  $\text{K}^+$  and sulfur (S) concentration of both shoots and roots were significantly reduced in high NaCl concentration. Other studies have found a reduction in  $\text{K}^+$  and in  $\text{K}^+:\text{Na}^+$  ratio after applying a NaCl treatment in cucumber (Tiwari et al., 2010; Wang et al., 2015). Si can increase this ratio, alleviating the detrimental effects of salinity, though some studies fail to demonstrate this effect (Thorne et al., 2020). Shoot calcium ion ( $\text{Ca}^{2+}$ ) concentration

was lower following salinity stress and it was not increased by adding Si in the nutrient solution, possibly explaining our observed lack of Si-effect on  $K^+$  concentration as low  $Ca^{2+}$  concentration upregulates  $K^+$  efflux channels (Shabala et al., 2006). Under both control and salt stress conditions, we found  $Ca^{2+}$  uptake was reduced in the presence of Si. This is consistent with results from previous studies on grasses (Hammond et al., 1995; Brackhage et al., 2013). The observed decrease in S in both the roots and the shoots of our seedlings under salt stress may be caused by the competition of the faster diffusing  $Cl^-$  with sulfate ( $SO_4^{2-}$ ) for anion adsorption sites in the root (Aghajanzadeh et al., 2019).

No effect of PGPR inoculation or its interaction with Si under salt stress was observed for the majority of nutrients measured in plant shoots and roots, except for a reduced  $Cl^-$  concentration in shoots. A reduction in Na has previously been demonstrated for cucumber plants following PGPR inoculation, namely *Burkholderia*, *Promicromonospora* and *Acinetobacter* species (Kumar et al., 2020).

Although the addition of Si to seedlings grown in the absence of salinity stress did not affect growth parameters, they did contain a higher concentration of Si, as found by Adatia and Besford (1986). This could provide a benefit for the crops when the quality of the recirculating solution is not constant throughout the growing period and accumulation of NaCl occurs. At the same time, Si is known to be effective for alleviating biotic stresses. It can be reasonable to assume that Si-rich plants growing in optimum conditions can be better prepared in terms of protection against pests and diseases that are common problem in cucumber cultivation (Reynolds et al., 2009; Massey and Hartley 2009).

Although the seedlings were grown in optimum conditions, there are always low amounts of NaCl present in the solution and therefore, Si exhibited the same effect on  $Cl^-$  concentration as in salt stress conditions. Si treated seedlings under low salinity conditions, had lower  $Ca^{2+}$  and increased S concentration. Thus, the Si: $Ca^{2+}$  availability ratio has been proposed to be important for certain plant groups although this was not evident in our study as no effect on crop growth was observed under non-stress conditions (Schaller et al., 2017).

## 5. Conclusions

The addition of soluble silicon (Si) to the nutrient solution in hydroponic systems has the potential to increase several growth-associated parameters of cucumber seedlings and so alleviate the adverse effects of salinity. The enhancement of aboveground growth under salt stress by Si application was coupled with a reduction in chloridex ( $Cl^-$ ) shoot concentration and an alteration in root growth traits. Under salt stress conditions, a root system of larger volume and biomass was observed in response to Si application, providing significant benefit to seedlings.

A multi strain inoculum of plant-growth promoting *Bacillus* spp. (PGPR) showed similar but smaller effects than Si addition, affecting fewer growth parameters significantly. The positive effect of the combined application of Si and PGPR was due to Si alone and therefore, no synergy was evident in our study. Thus, Si, and to a lesser extent PGPR application, confers changes in many morpho-physiological plant parameters that are important for the understanding of salt stress alleviation. Based on these conclusions we recommend that in cases where Si concentration in the fertigation water is low or the substrate is not a source of soluble Si, growers using deep-water culture systems for horticulture should consider the addition of Si to their nutrient solution as a strategy to mitigate the effects of salt stress.

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## CRedit authorship contribution statement

**Nikolaos Kaloterakis:** Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Sander H. van Delden:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. **Sue Hartley:** Methodology, Writing – review & editing. **Gerlinde B. De Deyn:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that there are no conflicts of interests.

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## Appendix A

Figs. A1 and A2.

## Appendix B

Tables B1 and B2

## Appendix C

Tables C1 and C2

## Appendix D

Fig. D1

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