Nostoc, Microcoleus and Leptolyngbya inoculums are detrimental to the growth of wheat (*Triticum aestivum* L.) under salt stress

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Abstract

Background and aims This study investigated the effect of cyanobacterial inoculants on salt tolerance in wheat.
Methods Unicyanobacterial crusts of *Nostoc*, *Leptolyngbya* and *Microcoleus* were established in sand pots. Salt stress was targeted at 6 and 13 dS m^{-1}, corresponding to the wheat salt tolerance and 50 % yield reduction thresholds, respectively. Germinated wheat seeds were planted and grown for 14 (0 and 6 dS m^{-1}) and 21 (13 dS m^{-1}) days by which time seedlings had five emergent leaves. The effects of cyanobacterial inoculation and salinity on wheat growth were quantified using chlorophyll fluorescence, inductively coupled plasma-optical emission spectrometry and biomass measurements.
Results Chlorophyll fluorescence was negatively affected by soil salinity and no change was observed in inoculated wheat. Effective photochemical efficiency correlated with a large range of plant nutrient concentrations primarily in plant roots. Inoculation negatively affected wheat biomass and nutrient concentrations at all salinities, though the effects were fewer as salinity increased.
Conclusions The most likely explanation of these results is the sorption of nutrients to cyanobacterial extracellular polymeric substances, making them unavailable for plant uptake. These results suggest that cyanobacterial inoculation may not be appropriate for establishing wheat in saline soils but that cyanobacteria could be very useful for stabilising soils.

Keywords Cyanobacteria · *Nostoc* · *Microcoleus* · Biological soil crust · Wheat · Salinity

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Introduction

Approximately 7 % of the global soil surface (Mashali 1999), including 23 % of all cultivated land (Tanji 1990), is salt-affected. In Australia, 16 % of agricultural land is currently effected by salinity (Rengasamy 2006), while 67 % is highly susceptible to transient salinity, the seasonal accumulation of salts in the upper soil profile

during dry periods (Rengasamy 2002). Saline soils have an electrical conductivity of the soil-water extract (EC_e)>4 dS m^{-1} (Richards 1954) which correlates to an osmotic pressure of approximately 0.2 MPa (Munns and Tester 2008). A wide variety of metal salts are associated with salinised soils, with Na$^+$ and Cl$^-$ the most common (Charman and Wooldridge 2000).

Plant salt tolerance is described using the salt tolerance threshold (STT). STT is quantified using EC_e (Shaw 1999) which accounts for the effects of soil texture on the conductivity of the soil-water solution (Richards 1954). When all other factors are equal, salinities above the STT result in yield reductions (Maas 1986). Both salt tolerance and the degree of yield reduction above STT are highly variable between plant species and can range from 1 dS m^{-1} for salt-sensitive crops, to 10 dS m^{-1} for salt-tolerant crops (Maas 1986; Munns et al. 2006). The degree of reduced yield is a linear measure ranging from 2 % to 33 % yield decline per dS m^{-1} increase above the STT and generally has an inverse relationship with salt sensitivity, i.e. salt tolerant plants normally have a lower yield decline per dS m^{-1} above the STT (Maas 1986). Wheat (*Triticum aestivum* L.) is moderately tolerant to salinity with an STT of 6 and a yield decline of 7.1 % for each dS m^{-1} above the STT (Maas 1986). Salt sensitivity varies considerably over the growth cycle of wheat, with seedlings and early emergent plants more salt-sensitive than mature plants (Maas et al. 1977). Screening of wheat genotypes for salt tolerance is conducted at salinities above the STT to final salinities of between 12 and 25 dS m^{-1}, or the equivalent mM NaCl (El-Hendawy et al. 2005a, b; Sayed 1985; Sharma et al. 1992).

Increasing levels of salt in soils effects plant growth in two main ways. The primary effect is an increase in osmotic potential, making water removal from the soil increasingly difficult (Rengasamy 2006). Secondly, increasing tissue salt concentrations can have toxic effects (Munns and Tester 2008). Plant salt tolerance is regulated by osmotic stress tolerance, ion exclusion and ion tolerance in plant tissues (Munns 2005; Munns and Tester 2008). Rising osmotic potential induces turgor loss (Munns and Tester 2008) and reduces photosynthetic efficiency via induced stomatal closure (Parida and Das 2005). The ability to exclude sodium from wheat leaves is correlated to enhanced plant salt tolerance as is the maintenance of K$^+$ homeostasis (Tester and Davenport 2003; Zhu 2003). Ion tolerance in plant tissues is enhanced by antioxidant enzyme activity which protects the plant from salinity-induced reactive oxygen species (ROS) production (Benavides et al. 2000; Tester and Davenport 2003).

The observation of cyanobacteria–plant symbioses and their successful use as plant growth promoters (PGP) for rice cultivation (Kennedy et al. 2004; Kennedy and Islam 2001; Vaishamayan et al. 2001) has led to broader research into the use of cyanobacteria, and principally *Nostoc*, in cereal agronomy, including wheat cultivation. Cyanobacteria have been successfully isolated from wheat rhizospheres (Karthikeyan et al. 2009; Nain et al. 2010) and respond to hormogonia-inducing factors (HIFs) from wheat plants (Gantar et al. 1993). Cyanobacteria associate with wheat roots when exposed to light in liquid culture (Gantar et al. 1991b; Svircev et al. 1997) and penetrate through the epidermis to the root cortex (Gantar 2000; Gantar and Elhai 1999; Gantar et al. 1991a; Sood et al. 2011; Svircev et al. 1997). Diverse cyanobacteria have been assessed in laboratory and pot trial studies for plant growth promotion as well as specifically for ameliorating salt stress. This list includes *Nostoc*, *Anabaena*, *Anabaenopsis*, *Calothrix*, *Fischerella*, *Westiellopsis*, *Hapalosiphon*, *Aulosira* and *Phormidium* spp., as well as unclassified strains. Under laboratory and pot trial conditions cyanobacterial inoculants have colonised wheat roots, contributed to wheat N requirements (Gantar et al. 1995b; Karthikeyan et al. 2007; Nain et al. 2010; Obreht et al. 1993; Svircev et al. 1997) and produced phytostimulatory effects on root and shoot biomass (Mazhar and Hasnain 2011; Sood et al. 2011; Svircev et al. 1997). With regard to salt stress amelioration, cyanobacterial inoculation improved grain yield, straw yield and N uptake of rice, the dry weight of barley and antioxidant content of wheat grain when plants were exposed to salt (Abd El-Baky et al. 2008; Aziz and Hashem 2004; Hashem 2001; Issa et al. 1994; Rodriguez et al. 2006).

Cyanobacteria, including *Nostoc*, *Leptolyngbya*, and *Microcoleus*, are prolific producers of extracellular polymeric substances (EPS) (Mazor et al. 1996). EPS is correlated to cyanobacterial salt tolerance (Ozturk and Aslim 2010) and is an important factor in root colonisation studies (Gantar et al. 1995a). Cyanobacterial EPS binds soil particles (Mager and

Thomas 2011; Malam Issa et al. 2007) preventing their loss from wind erosion, thereby protecting the soil nutrients bound to soil particles (Belnap and Gillette 1998). Cyanobacterial EPS applied to the soil surface was also shown to enhance aggregate stability (Maqubela et al. 2009), organic C and the lipid content (Pardo et al. 2010) of soils. In saline soils cyanobacterial inoculation lowered soil EC_e (Hashem 2001; Subhashini and Kaushik 1981) and improved organic matter and N content (Aziz and Hashem 2003).

The aim of the current study was to explore the effects of cyanobacteria isolated from wheat fields on the chlorophyll fluorescence and nutrition of wheat grown under salt stress. Based upon previous studies that demonstrated salt stress amelioration for barley and rice (Aziz and Hashem 2004; Hashem 2001; Issa et al. 1994) we hypothesised that: (1) cyanobacterial inoculation would ameliorate salt stress of wheat, (2) *Nostoc* inoculants would have better growth outcomes for wheat than inoculants of *Microcoleus* or *Leptolyngbya*. We also proposed that chlorophyll fluorescence parameters obtained via Pulse Amplitude Modulation (PAM) fluorometry would provide valuable indicators of plant health. The information generated in this study provides important insights into the application of cyanobacterial inoculants as growth promoters of wheat and expands the role of chlorophyll fluorescence in plant nutrition studies.

Methods

Pot trial set up and growth conditions

Fine Sydney sand was oven dried at 70 °C overnight before 700 mL was added to 125 mm pots (RICO) along with 10 cm of 0.5" low density poly-pipe. The poly-pipe was placed in the centre of the pot and was used to apply all water and nutrient solutions. Pots were autoclaved at 121 °C for 15 min. Autoclaving was repeated after 24 h. Pots were thoroughly flushed with RO water to remove seed germination inhibitors produced by autoclaving, then 350 mL modified 1/2 strength Hoaglands solution was added to remove nutrient limitations on wheat growth. Hoaglands recipe 1 (Hoagland and Arnon 1938) was used with the following modifications: The molar equivalent of

0.5 % Ferric tartrate was added as 0.22 % Ferric citrate (Sigma) to provide a more stable Fe source; BG11 micronutrient solution (Rippka et al. 1979) was used to ensure sufficient Mo supply for N_2 fixation in heterocystous cyanobacteria. Pots were left to drain to field capacity and weighed.

Cyanobacterial inoculum preparation and watering regime

Three cyanobacterial isolates originally isolated from wheat trials in agricultural research stations at Yanco, New South Wales and Kerang, Victoria were selected due to their salt physiology, strong ability to associate with wheat roots in agar media and their robust growth. *Nostoc* PCC73102 was selected for use as a reference strain as it has a fully sequenced genome (Meeks et al. 2001) and was originally isolated as an endosymbiont from *Macrozamia* in Australia (Rippka et al. 1979). *Nostoc* sp. 'K1.3' and *Nostoc* PCC73102 cultures were maintained in liquid $BG11_0$ (Rippka et al. 1979) while *Leptolyngbya* sp. 'Y4.2' and *Microcoleus vaginatus* 'K2.2' were maintained in liquid BG11. Triplicate pots were inoculated at each salinity with 250 µg of wet weight stationary phase culture. *Nostoc* cultures were applied by diluting with 1/2 strength modified Hoaglands to a total volume of 1 mL which was applied across the soil surface. *Leptolyngbya* and *Microcoleus* were shredded and applied via forceps across the soil surface. The cyanobacteria were applied in these two manners to try to mimic natural cyanobacterial crust formation as opposed to the previous methods used by Gantar et al. (1995b) and Svircev et al. (1997) which used liquid inoculum in flooded pots to induce thick crust formation. A fifth set of pots were used as controls. Controls were not supplied with any inoculum but were otherwise treated the same as other pots. Pots were completely randomised and watered to field capacity every two days for 14 days by weighing plants on a balance. Soil electrical conductivity (EC_e) was adjusted over the 14 day growing period, commencing the day after inoculation. The target salinities were 6 and 13 dS m^{-1} which correspond, respectively, to the salt tolerance threshold (STT) and 50 % yield reduction of wheat (Maas 1986). Hereafter the non-salinised, 6 and 13 dS m^{-1} salinities are referred to as low, medium and high salinity, respectively. To raise the soil EC_e, volumes of 5 M NaCl were added via the irrigation pipe at watering.

A separate series of pots were used to monitor the EC_e of the medium and high salinity treatments. Salinity levels were determined using the soil-water extract method (Richards 1954). The experiment was conducted on three replicates in a Conviron PGR14 growth chamber with a 16-h light/8-h dark cycle at 25 °C (± 3 °C)/20 °C (± 2 °C) and 70 μmol photons m^{-2} s^{-1}.

Wheat source, germination, planting and culture maintenance

Wheat (*Triticum aestivum* L. 'EGA Gregory') seeds were surface sterilised by soaking in 70 % ethanol for one min, rinsed three times with sterile milliQ water and left to germinate for two days in sterile petri dishes. Five germinated wheat seeds were transferred to each pot 14 days after cyano-bacterial inoculation and planted 1 cm beneath the cyanobacterial crust. To supplement nutrient leaching prior to planting, 50 mL 1/2 strength modified Hoaglands solution was added to each pot. Pots were weighed and watered to field capacity daily. Soil EC_e was monitored intermittently and adjusted by adding 5 M NaCl at watering. On day 10, leaves on the uninoculated low salt wheat plants started turning yellow, indicating nutrient deficiency. Nutrient deficiency was relieved by adding 50 mL 1/2 strength modified Hoaglands solution to all pots. The trial was terminated on day 14 for the low and medium salinity treatments. For sufficient biomass production the high salinity pots were grown until day 21.

Pulse amplitude modulation (PAM) fluorometry

Pulse Amplitude Modulation (PAM) fluorometry was conducted using a PAM2500 (Walz) just prior to harvesting plant material. Dark leaf clips were applied to the midpoint of the newest fully-emergent leaves for 30 min to allow all photo-centres to open. The F_o and F_m values were measured in the dark with a red saturation pulse followed by 5 s far-red illumination. Red actinic light was applied at 1/2 the growth level of photosynthetically active radiation (PAR), growth PAR and 4× growth PAR, 35, 70 and 240 μmol photons m^{-2} s^{-1}, respectively, for five min intervals with red light saturation pulses applied every min to calculate F_t and F_m'. Each saturation pulse was followed

by 5 s of far-red illumination for the calculation of F_o'. Chlorophyll fluorescence parameters follow the nomenclature and calculations of van Kooten and Snel (1990) and Schreiber et al. (1986). Values of F_o, F_m, F_t, F_m' and F_o' were used to calculate the fluorescence parameters: maximum photochemical efficiency of photosystem II [$F_v/F_m=(F_m - F_o)/F_m$], non-photochemical quenching [$qN=1- [(F_m' - F_o')/(F_m - F_o)]$] and photochemical quenching [$qP=(F_m'-F_t)/(F_m'-F_o)$]. All qP and qN values are annotated relative to the proportion of growth PAR applied at measurement, i.e.: $qP_{0.5}$, qP_1 and qP_4 and $qN_{0.5}$, qN_1 and qN_4. Dark leaf clips were reapplied 3/4 of the way along the same wheat leaf and again left for 30 min. The F_o and F_m values were measured as above and red actinic light was applied at growth PAR, 70 μmol photons m^{-2} s^{-1}, for five min with red light saturation pulses applied every min to calculate F_t and F_m'. Each saturation pulse was followed by five s far-red illumination for the calculation of F_o'. The F_o and F_m values were used to calculate the maximum photochemical efficiency of photosystem II (F_v/F_m) to determine that there was no lag effect from the previous treatment to the leaf photosynthetic response. The F_m' and F_o' values were used to calculate the effective photochemical efficiency of photosystem II (Φ_{II}).

Plant growth and nutrient concentrations

The number of fully emergent wheat leaves was recorded. Plants were harvested and plant material from each pot was pooled. Sand was removed from the roots by gentle washing. Roots were visually examined for the presence of root colonisation by cyanobacteria and any persistent seeds were removed before root and shoot material was separated and dried overnight at 70 °C. Dry weights were measured before dried material was ground with a mortar and pestle. Plant material was digested using distilled 70 % nitric acid (AJAX Chemicals) for 24 h followed by hot digestion at 120 °C for four h. Once cool, 2 mL 30 % hydrogen peroxide (AJAX Chemicals) was added and gently heated at 120 °C for 30 min. After cooling milliQ water was added to give a total volume of 25 mL. A Perkin-Elmer Optima 7300 DV optical emission spectrometer was used with WinLab32 software to measure shoot and root concentrations of Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn.

Statistical analysis

Spearman's rank correlations were calculated between all fluorescence parameters and all plant nutrient concentrations (Table 1) using the cor and cor.test functions in R version 12.0.2 (R Development Core Team 2010). To determine the effects of salinity the results were pooled by salinity treatment, and the effect of salinity was assessed using one-way ANOVA. Pairwise comparisons were made using Tukey's Honest Significant Difference (HSD) when ANOVA demonstrated significant differences. Since we were most interested in the effects of the individual inoculants rather than cyanobacterial inoculation *per se*, one-way ANOVA was performed between each inoculant and control. One-way ANOVA and Tukey's HSD were performed using Minitab version 16.1.1. To clarify whether inoculation was confounding the effects of salinity on plant biomass and nutrient concentrations a two-way ANOVA was performed using R version 12.0.2 (R Development Core Team 2010). To reduce the heterogeneity of variance observed between inoculants, shoot dry weight and many shoot nutrient concentrations were $\log_{10}$ transformed.

Results

Electrical conductivity fluctuations and cyanobacterial crust formation in sand pots

During the course of seedling growth EC_e values varied considerably for both the medium and high salinity treatments. In the case of the medium salinity treatment the EC_e of the soil at planting was 5.17 dS m^{-1} and varied from 2.62 to 5.37 during the growth period with a final EC_e value of 5.63 dS m^{-1} at completion. The EC_e values for the high salinity treatment were more variable. At planting, the EC_e was 15 dS m^{-1} and varied from 5.57 to 12.54 during the growth period with a final EC_e of 18.1 dS m^{-1} at the conclusion of the experiment.

Cyanobacterial crusts formed in all inoculated pots, covering between 50 and 100 % of the soil surface. Before washing, some persistent crustal material from *Nostoc* sp.'K1.3′ and *Microcoleus vaginatus*'K2.2′ was observed on plant shoots at the soil surface. No root colonisation was observed on wheat seedlings growing in any of the inoculated pots.

Chlorophyll fluorescence parameters correlate well with shoot dry weight, shoot Na concentrations and all root nutrient concentrations

Significant Spearman's rank correlations between fluorescence parameters and biomass and nutrient concentrations were limited to shoot dry weight, shoot Na concentrations and all root nutrient concentrations measured (Table 1). Of the fluorescence parameters measured only qP_4 and F_v/F_m significantly correlated to all these variables. Overall, Φ_{II} was the best indicator of shoot biomass and plant nutrient concentrations. Φ_{II} was the strongest indicator of shoot biomass (rs= 0.63) and the root nutrient concentrations of Ca (rs= 0.7), Fe (rs=0.49), Na (rs=0.59), P (rs=0.72) and S (rs=0.44). Φ_{II} was significantly correlated with shoot Na concentrations and, with the exception of Zn (rs= 0.027, P=0.07), all root nutrient concentrations. However, Φ_{II} had a much poorer correlation with root concentrations of Cu (rs=0.36) and K (rs=0.57) relative to F_v/F_m (rs=0.48) and qN_4 (rs=−0.72) respectively.

Regardless of cyanobacterial inoculation, altering EC_e strongly effects fluorometry parameters and nutrient concentrations

The response of fluorescence parameters and nutrient concentrations to salt stress was highly variable (Table 2). Salt stress primarily effected fluorescence parameters and root nutrient concentrations though shoot dry weight and some shoot nutrient concentrations were also affected. A significant difference was found between the high salinity treatment and both the low and medium treatments with the high salinity treatment having significantly higher qN and shoot Na concentrations and lower F_v/F_m, Φ_{II}, qP and shoot dry weight, root Mg and root P concentrations. A significant difference was found between medium and high salinity nutrient concentrations with higher shoot P and S and lower root Mn at high salinity, however there was no difference compared to the controls. Root Ca, K and Na concentrations were lowest in the high salinity treatment and increased from lowest to highest concentration in the order high < low < medium salinity. Both root Cu and Zn concentrations were significantly higher under the low salinity treatment than in salinised treatments. No significant difference was found between low and high salt for root S concentrations, however,

Table 1 Spearman's rank correlation coefficients between photosynthetic parameters and wheat seedling characteristics. Nutrient concentrations are in mg kg^{-1}. Significant correlations are shown at $P=0.05$ (*), 0.01 (**), 0.001 (***)

	F_v/F_m	Φ_{II}	$qP_{0.5}$	qP_1	qP_4	$qN_{0.5}$	qN_1	qN_4
Dry weight (mg)								
Shoot	0.6***	0.63***	0.42**	0.52***	0.52***	−0.47***	−0.56***	−0.58***
Root	−0.15	−0.23	−0.12	−0.25	−0.28	0.003	0.02	0.15
Nutrient concentrations								
Shoot								
Ca	0.17	0.08	0.08	0.16	0.15	0.02	0.08	−0.16
Cu	0.02	0.05	0.04	0.07	0.02	0.0	0.08	−0.14
Fe	0.01	−0.15	−0.15	−0.09	−0.08	0.12	0.20	0.05
K	−0.08	−0.14	−0.11	−0.1	−0.14	0.12	0.23	0.05
Mg	0.17	0.09	0.09	0.18	0.16	−0.01	0.05	−0.19
Mn	−0.06	−0.07	−0.05	−0.02	−0.06	0.08	0.15	−0.02
Na	−0.44**	−0.47***	−0.42**	−0.5***	−0.46**	0.32*	0.33*	0.51***
P	−0.14	−0.15	−0.11	−0.09	−0.14	0.13	0.26	0.04
S	−0.16	−0.21	−0.2	−0.16	−0.2	0.2	0.33*	0.12
Zn	−0.11	−0.09	−0.03	−0.05	−0.05	0.13	0.19	0.04
Root								
Ca	0.67***	0.70***	0.57***	0.67***	0.63***	−0.56***	−0.67***	−0.69***
Cu	0.48***	0.36*	0.21	0.27	0.44**	0.03	−0.05	−0.19
Fe	0.44**	0.49***	0.46**	0.41**	0.42**	−0.33*	−0.39**	−0.37*
K	0.45**	0.57***	0.54***	0.59***	0.51***	−0.59***	−0.72***	−0.55***
Mg	0.81***	0.76***	0.50***	0.69***	0.78***	−0.35*	−0.49***	−0.61***
Mn	0.55***	0.60***	0.52***	0.58***	0.64***	−0.51***	−0.62***	−0.49***
Na	0.5***	0.59***	0.43**	0.56***	0.54***	−0.45**	−0.57***	−0.51***
P	0.63***	0.72***	0.48***	0.54***	0.61***	−0.35*	−0.47***	−0.47***
S	0.29*	0.44**	0.21	0.36*	0.35*	−0.19	−0.30*	−0.28
Zn	0.33*	0.27	0.18	0.12	0.35*	0.08	0.06	0.01

medium salinity samples had significantly higher concentrations.

Cyanobacterial inoculation detrimentally effects wheat nutrition but not biomass or chlorophyll fluorescence

Cyanobacterial inoculation did not significantly affect leaf number, root dry weight or shoot dry weight at any salinity (Tables 3, 4, 5, 6). Only one chlorophyll fluorescence parameter showed a significant response to cyanobacterial inoculation, and in only one instance, where wheat plants in the non-saline treatment had significantly lower qP_4 values ($P=0.049$) when inoculated with *Leptolyngbya* sp. 'Y4.2'. Cyanobacterial inoculation had few significant effects on root nutrient concentrations (Tables 3, 4, 5) with the exception of

root Fe concentrations, while salinity significantly effected all root nutrient concentrations (Table 6). Plants inoculated with *Nostoc* sp. 'K1.3' had lower root Na concentrations (8,726±153 mg kg^{-1}, $P=0.002$) relative to controls (10,317±177 mg kg^{-1}) in the high salinity treatment.

In all the other cases, plants inoculated with cyanobacteria had lower nutrient concentrations. Lower root Mg concentrations were observed in non-salinised treatments when inoculated with *Nostoc* sp. 'K1.3' ($P=0.012$), *Microcoleus vaginatus* 'K2.2' ($P=0.005$) and *Leptolyngbya* sp. 'Y4.2' ($P=0.003$) and also at medium salinity when inoculated with *Microcoleus vaginatus* 'K2.2' ($P=0.036$). Lower root Mn concentrations were observed at medium salinity when inoculated with *Nostoc* PCC73102 ($P=0.042$). All other significant root

Table 2 Pooled ANOVA results of all pots at EC_e of 0, 6 and 13 dS m^{-1}. Mean of 15 pots ± standard error. Different letters indicate significant differences between columns ($P=0.05$) using Tukey's HSD. Dry weights are in g, nutrient concentrations are in mg kg^{-1}

	0 dS m^{-1}	6 dS m^{-1}	13 dS m^{-1}
Φ_{II}	0.644±0.005[a]	0.631±0.009[a]	0.448±0.016[b]
F_v/F_m	0.767±0.001[a]	0.76±0.002[a]	0.726±0.005[b]
$qP_{0.5}$	0.955±0.003[a]	0.956±0.004[a]	0.924±0.003[b]
qP_1	0.938±0.002[a]	0.936±0.003[a]	0.848±0.008[b]
qP_4	0.669±0.011[a]	0.628±0.013[a]	0.364±0.023[b]
$qN_{0.5}$	0.205±0.006[a]	0.192±0.007[a]	0.264±0.013[b]
qN_1	0.241±0.008[a]	0.228±0.015[a]	0.490±0.019[b]
qN_4	0.669±0.012[a]	0.674±0.011[a]	0.785±0.008[b]
Shoot			
Dry weight	0.026±0.001[a]	0.027±0.001[a]	0.019±0.001[b]
No. leaves	4.6±0.2[a]	4.8±0.11[a]	4.8±0.22[a]
Ca	2,658±347[a]	2,096±372[a]	2,688±559[a]
Cu	4.53±0.76[a]	4.34±0.84[a]	5.28±0.87[a]
Fe	86.6±13.2[a]	107.6±50.8[a]	110.6±23.1[a]
K	28,423±5087[a]	17,229±3236[a]	32,823±6176[a]
Mg	2,962±349[a]	2,525±369[a]	3,135±568[a]
Mn	38.31±3.63[a]	36.48±4.13[a]	44.85±5.58[a]
Na	9,334±1763[a]	10,427±1979[a]	24,540±5695[b]
P	4,225±351[ab]	3,605±329[a]	5,070±473[b]
S	2,531±243[ab]	2,050±214[a]	3,125±297[b]
Zn	59.1±10.1[a]	44.2±6.1[a]	81.7±16.4[a]
Root			
Dry weight	0.011±0.001[a]	0.013±0.001[a]	0.013 ±0.001[a]
Ca	1,647±76.9[a]	1,967±104[b]	522±18[c]
Cu	18.1±1.8[a]	7.7±0.7[b]	7.7±0.5[b]
Fe	382±111[a]	256±28[a]	167±14[a]
K	5,521±284[a]	14,161±1174[b]	2,190±380[c]
Mg	1,662±123[a]	1,381±84[a]	353±34[b]
Mn	19.3±0.8[ab]	24.7±3.3[a]	12.0±1.8[b]
Na	17,444±833[a]	23,774±839[b]	12,123±1230[c]
P	2,299±101[a]	2,192±58[a]	1,480±53[b]
S	1,383±50[a]	1,572±46[b]	1,313±43[a]
Zn	348±69[a]	86±14[b]	116±15[b]

effects occurred in the non-salinised treatments. These included lower concentrations of K ($P=0.05$) when inoculated with *Nostoc* sp. 'K1.3', Ca ($P=0.042$) and Zn ($P=0.027$) when inoculated with *Microcoleus vaginatus* 'K2.2', and S ($P=0.047$) when inoculated with *Leptolyngbya* sp. 'Y4.2'.

The Oscillatorean isolates, *Microcoleus vaginatus* 'K2.2' and *Leptolyngbya* 'Y4.2', had a stronger effect on shoot nutrient concentrations than the Nostocalean inoculants (Tables 3, 4, 5). When inoculated with the Nostocalean cyanobacteria, shoot concentrations of Cu were significantly lower in the high salinity treatments. Shoot Zn concentrations were significantly lower when plants were treated with *Nostoc* PCC73102 at high salinity and when treated with *Nostoc* sp 'K1.3' at all salinities. Wheat plants inoculated with *Nostoc* sp. 'K1.3' also had significantly lower Mn and significantly higher Na concentrations in plant shoots at the low and medium salt treatments. When analysed in a two-way ANOVA, inoculation significantly effected most shoot nutrient concentrations (Table 6). In the case of shoot Mg, P, S and Zn salinity was also significant. An interaction was found between inoculation and salinity for shoot Ca, K and Na. The interactions resulted from differences in the effects of low, medium and high salinity depending on the inoculum used.

Inoculation effects from Oscillatorean cyanobacteria were more frequent than effects from inoculation with *Nostoc*. *Microcoleus vaginatus* 'K2.2' detrimentally affected most nutrient levels, with higher shoot Na and lower shoot concentrations of other nutrients relative to controls at all salinities. For the high salinity treatments all P values were below 0.1 for shoot Ca, Mn, P and S. *Leptolyngbya* sp. 'Y4.2' had fewer significant effects, especially at high salinity. Treatment with *Leptolynbgya* sp. 'Y4.2' followed the same pattern as *Microcoleus vaginatus* 'K2.2' with significantly higher shoot Na concentrations and significant reductions in other shoot nutrient concentrations. An exception to this trend was the higher shoot concentrations of K in *Leptolyngbya* sp. 'Y4.2' plants without added NaCl.

Discussion

Root colonisation absent

The successful colonisation of wheat roots by heterocystous and non-heterocystous cyanobacteria in liquid culture is well established (Ahmed et al. 2010; Gantar et al. 1991a, b, 1995a; Sood et al. 2011; Svircev et al. 1997) whereas colonisation in sand pots has been variable

Table 3 One-way ANOVA between individual isolates and the control at low salinity (0 dS m^{-1}). Mean of 3 pots ± standard error. Significant differences between treatment and control are shown by P=0.05 (*), 0.01 (**), 0.001 (***). Nutrient concentrations are in mg kg^{-1}

	Control	Nostoc PCC 73102	Nostoc sp. K1.3	Leptolyngbya sp. Y4.2	Microcoleus vaginatus K2.2
Chlorophyll fluorescencee					
Φ_{II}	0.651±0.014	0.646±0.0133	0.639±0.0118	0.636±0.0143	0.645±0.0125
F_v/F_m	0.766±0.005	0.77±0.004	0.769±0.002	0.764±0.003	0.767±0.003
$qP_{0.5}$	0.954±0.011	0.957±0.009	0.955±0.005	0.953±0.009	0.958±0.009
qP_1	0938±0.006	0.941±0.007	0.937±0.005	0.931±0.006	0.943±0.005
qP_4	0.673±0.012	0.682±0.014	0.648±0.029	0.632±0.009*	0.71±0.035
$qN_{0.5}$	0.226±0.024	0.198±0.01	0.198±0.004	0.203±0.011	0.2±0.01
qN_1	0.26±0.024	0.228±0.015	0.243±0.017	0.242±0.023	0.232±0.024
qN_4	0.679±0.018	0.624±0.049	0.686±0.01	0.682±0.015	0.673±0.017
Dry weight (mg)					
Shoot	27.03±0.76	28.69±3.45	25.23±3.06	25.48±1.41	23.99±1.06
Root	9.26±1.23	11.63±0.32	10.69±3.98	12.6±0.66	13.02±0.99
Nutrient concentrations (mg kg^{-1})					
Shoot					
Ca	4,237±174	3,160±639	3,098±391	2,124±198***	672±23***
Cu	8.77±1.49	4.93±1.28	4.26±0.96	2.79±1.08*	1.87±0.05**
Fe	115.7±16.1	126.73±52.4	83.26±18.7	72.33±10.2	35.11±0.38**
K	26,237±1,377	22,345±4,370	45,456±17,192	43,499±5,025*	4,579±384***
Mg	4,688±215	3,205±577	3,586±470	2,139±221***	1,193±55***
Mn	54.89±1.57	46.09±10.5	32.5±5.3*	33.87±5*	24.18±1.37***
Na	4,538±380	3,789±1,119	8,986±1,013*	7830.7±381**	21,526±1,959***
P	5,019±287	4,776±1,111	4,864±890	3,882±357	2,582±79.5***
S	2,941±72.4	2,313±468	3,421±720	2,655±285	1,326±13***
Zn	114.09±9.36	79.17±22.9	46.8±11.8*	31.42±3.96***	23.87±1.31***
Root					
Ca	1,904±175	1,659±110	1,774±136	1,609±163	1,288±115*
Cu	17.06±2.81	20.97±4.19	23.18±6.21	16.43±3.83	12.85±2.26
Fe	282±63	312±123	916±476	212±34	186±35
K	6,317±184	4,894±1,123	4,653±570*	6,077±274	5,665±264
Mg	2,345±155	1,751±379	1,511±109*	1,327±51	1,374±71**
Mn	19.58±1.23	20.62±2.28	20.24±3.22	16.54±0.42	19.39±0.45
Na	19,775±937	15,364±2,837	17,307±2,411	15,926±1,224	18,846±1,001
P	2,568±183	1,927±343	2,143±213	2,334±110	2,521±93
S	1,544±59	1,311±151	1,328±124	1,290±67*	1,440±130
Zn	343±1	250±86	654±309	261±44	228±33*

(Gantar et al. 1995b; Svircev et al. 1997). The effectiveness of hormogonia-inducing factors (HIFs) for both hormogonia induction and chemotaxis varies between plants (Nilsson et al. 2006; Svircev et al. 1997) as does the response by cyanobacteria (Gorelova 2006; Nilsson et al. 2005; Rasmussen et al. 1994; Svircev et al. 1997). From the available studies the differences between plants may also apply at the cultivar level, with some wheat cultivars producing more HIFs (Svircev et al. 1997). Most crustal biomass is concentrated in the top 3 mm of soil (Garcia-Pichel and Belnap 1996; Veluci et al. 2006) with cyanobacterial populations falling by approximately 90 % to approximately

Table 4 One-way ANOVA between individual isolates and the control at medium salinity (6 dS m^{-1}). Mean of 3 pots ± standard error. Significant differences between treatment and control are shown by P=0.05 (*), 0.01 (**), 0.001 (***). Nutrient concentrations are in mg kg^{-1}

	Control	*Nostoc* PCC 73102	*Nostoc* sp. K1.3	*Leptolyngbya* sp. Y4.2	*Microcoleus vaginatus* K2.2
Chlorophyll fluorescence					
Φ_{II}	0.624 ± 0.022	0.667 ± 0.005	0.637 ± 0.011	0.611 ± 0.022	0.617 ± 0.026
F_v/F_m	0.763 ± 0.003	0.766 ± 0.001	0.762 ± 0.005	0.76 ± 0.002	0.749 ± 0.007
$qP_{0.5}$	0.95 ± 0.008	0.968 ± 0.004	0.952 ± 0.012	0.948 ± 0.015	0.961 ± 0.003
qP_1	0.93 ± 0.01	0.947 ± 0.005	0.942 ± 0.003	0.925 ± 0.011	0.936 ± 0.002
qP_4	0.605 ± 0.025	0.673 ± 0.013	0.664 ± 0.017	0.582 ± 0.04	0.615 ± 0.009
$qN_{0.5}$	0.207 ± 0.024	0.185 ± 0.008	0.191 ± 0.012	0.2 ± 0.028	0.175 ± 0.008
qN_1	0.247 ± 0.036	0.207 ± 0.009	0.216 ± 0.007	0.266 ± 0.072	0.202 ± 0.009
qN_4	0.677 ± 0.019	0.649 ± 0.002	0.652 ± 0.013	0.719 ± 0.036	0.673 ± 0.024
Dry weight (mg)					
Shoot	28.39 ± 0.7	26.27 ± 0.96	26.07 ± 0.62	26.14 ± 1.86	28.46 ± 4.32
Root	11.99 ± 0.75	12.5 ± 2.24	11.83 ± 2.04	13.35 ± 1.21	14.98 ± 2.65
Nutrient concentrations (mg kg^{-1})					
Shoot					
Ca	3720 ± 391	3225 ± 308	2475 ± 334	520 ± 73[***]	541 ± 41[***]
Cu	8.051 ± 2.61	6.358 ± 1.05	3.578 ± 0.54	2.211 ± 0.75	1.502 ± 0.53
Fe	85.5 ± 13.8	64.61 ± 5.27	315.7 ± 249	38.52 ± 9.53[*]	33.76 ± 3.69[*]
K	26979 ± 5472	18886 ± 1083	31113 ± 4607	2864 ± 647[*]	6302 ± 2355[*]
Mg	4098 ± 638	3500 ± 382	2874 ± 500	1096 ± 189[*]	1058 ± 214[*]
Mn	58.48 ± 7.64	47.06 ± 2.1	31.42 ± 2.32[*]	22.13 ± 3.72[*]	23.27 ± 3.55[*]
Na	3534 ± 611	4373 ± 54	8008 ± 1362[**]	14872 ± 833[***]	21351 ± 4424[*]
P	4840 ± 658	4707 ± 249	3728 ± 172	2438 ± 495[*]	2314 ± 366[*]
S	2749 ± 383	2490 ± 252	2684 ± 92	1155 ± 202[*]	1168 ± 126[*]
Zn	79.97 ± 14.3	51.36 ± 3.48	31.84 ± 2.26[*]	33.85 ± 9.21	24 ± 1.86[*]
Root					
Ca	2044 ± 360	2116 ± 148	2102 ± 294	1877 ± 51	1695 ± 270
Cu	7.02 ± 1.9	7.37 ± 2.02	7.6 ± 1.37	7.26 ± 0.87	9.32 ± 1.92
Fe	180 ± 20	372 ± 80	296 ± 50	277 ± 52	154 ± 9
K	17305 ± 436	9997 ± 3122	10479 ± 3255	15877 ± 1062	17147 ± 538
Mg	1655 ± 165	1589 ± 161	1445 ± 189	1112 ± 131	1102 ± 69[*]
Mn	20.09 ± 1.15	16.4 ± 0.49[*]	26.6 ± 7.76	29.02 ± 8.5	31.53 ± 13.1
Na	25570 ± 2198	24191 ± 2597	24711 ± 1147	21133 ± 2301	23265 ± 825
P	2407 ± 122	2132 ± 230	2145 ± 70	2133 ± 104	2141 ± 76
S	1688 ± 44	1507 ± 211	1643 ± 66	1517 ± 54	1504 ± 69
Zn	62.05 ± 11.2	112.08 ± 67	101.05 ± 25.2	89.36 ± 16.7	63.44 ± 14.4

0.5×10^5 cells g soil^{-1} at 6–9 mm below the soil surface (Veluci et al. 2006). This suggests that the movement of cyanobacteria into the rhizosphere or bulk soil is driven primarily by the flushing of cyanobacterial filaments into soil pores. Once they are in darkness cyanobacteria may then become responsive to plant chemoattractants.

Inoculation confounds the effects of salinity on wheat seedling nutrition

Most studies agree that biomass of shoots and roots and shoot nutrient concentrations of wheat decrease incrementally with increasing salinity, whereas root nutrient concentrations increase under the same conditions. This

Table 5 One-way ANOVA between individual isolates and the control at high salinity (13 dS m^{-1}). Mean of 3 pots ± standard error. Significant differences between treatment and control are shown by $P=0.05$ (*), 0.01 (**), 0.001 (***). Nutrient concentrations are in mg kg^{-1}

	Control	Nostoc PCC 73102	Nostoc sp. K1.3	Leptolyngbya sp. Y4.2	Microcoleus vaginatus K2.2
Chlorophyll fluorescence					
Φ_{II}	0.46±0.019	0.48±0.013	0.407±0.04	0.477±0.065	0.415±0.011
F_v/F_m	0.737±0.006	0.734±0.003	0.724±0.008	0.721±0.019	0.715±0.01
$qP_{0.5}$	0.928±0.008	0.93±0.003	0.917±0.011	0.915±0.009	0.928±0.003
qP_1	0.854±0.014	0.857±0.013	0.837±0.023	0.847±0.037	0.846±0.005
qP_4	0.337±0.032	0.386±0.035	0.342±0.025	0.419±0.112	0.334±0.021
$qN_{0.5}$	0.257±0.049	0.253±0.036	0.261±0.022	0.266±0.016	0.282±0.04
qN_1	0.487±0.064	0.471±0.021	0.5±0.039	0.468±0.067	0.526±0.027
qN_4	0.777±0.033	0.783±0.006	0.792±0.023	0.776±0.022	0.80±0.008
Dry weight (mg)					
Shoot	17.95±0.62	23.15±2.27	19.25±1.6	16.12±0.84	20.53±0.98
Root	13.3±1.59	11.64±0.85	13.87±2.28	11.47±1.59	13.41±0.66
Nutrient concentrations (mg kg^{-1})					
Shoot					
Ca	5,723±1,785	2,805±476	2,785±384	1,262±305	865±26.2
Cu	10.67±1.24	4.64±0.79*	3.92±0.72**	4.68±2.04	2.48±0.43**
Fe	251.97±70.7	99.06±10.7	80.32±10.1	74.11±18.8	47.52±2.53*
K	33,019±5,503	59,377±9,631	53,964±6,877	10,740±3,380*	7,017±750**
Mg	6,516±1,752	2,915±430	2,612±387	2,142±410	1,490±67*
Mn	76.35±17.7	33.88±5.28	39.09±1.4	43.8±8.76	31.11±2.05
Na	6,842±1,333	11,535±1,952	12,033±3,006	56,103±15,069*	36,188±2,817***
P	7,249±1,483	5,023±908	4,771±457	4,847±918	3,460±113
S	3,916±868	3,646±608	3,421±408	2,818±635	1,823±27.9
Zn	1,871±23	83.8±21.7*	34.1±3.4**	70.4±22.2*	32.9±1.6**
Root					
Ca	462±52	540±20	523±17	573±65	512±18
Cu	6.33±1.26	6.89±0.78	7.23±1.21	9.25±1.67	8.66±0.73
Fe	165±9	146±5	164±4	152±19	207±74
K	1,623±139	2,357±677	1,632±91	3,425±1,849	1,915±73
Mg	303±5	390±55	280±14	446±164	345±23
Mn	8.65±0.71	12.19±1.37	10.11±0.71	17.96±9.05	11.14±0.79
Na	10,317±177	14,993±2,792	8,726±153**	14,672±5,283	11,907±1,557
P	1,521±80	1,510±132	1,348±102	1,558±219	1,461±64
S	1,267±10	1,436±90	1,218±107	1,357±170	1,287±54
Zn	84.97±5.75	174.27±59	89.15±14.5	108.49±28.9	120.59±18.9

includes shoot and root dry weights, leaf number, shoot Na, Ca, Mg, K, P and S, root Na, K, Ca and S (Atak et al. 2006; El-Hendawy et al. 2005a, b; Genc et al. 2007; Hu and Schmidhalter 1997; Husain et al. 2004; Islam et al. 2007; Rascio et al. 2001; Sharma et al. 1992; Yang et al. 2007; Zheng et al. 2008). Very few of the pooled data results, that is, when data was pooled by salinity rather than inoculant or control, matched the established trends of salt effects on wheat biomass and nutrient concentrations. Instead, most nutrient concentrations fluctuated in response to increasing salinity with maximal negative effects occurring with medium salt treatment (STT of 6 dS m^{-1}). However, shoot dry weights, root P and shoot Na concentrations only changed under high salinity. Two

Table 6 Two-way ANOVA results on the effects of inoculation verses salinity on plant biomass and nutrient concentrations. Unless otherwise indicated, all shoot variables were $\log_{10}$ transformed

	Treatment	Shoot		Root[a]	
		F	P	F	P
Dry weight	Inoculation	1.45	0.242	0.74	0.567
	Salinity	27.54	< 0.001	1.30	0.287
	Inoculation×Salinity	1.26	0.297	0.58	0.783
Ca[a]	Inoculation	79.07	< 0.001	1.75	0.165
	Salinity	9.66	< 0.001	102.21	< 0.001
	Inoculation×Salinity	4.36	0.001	0.67	0.718
Cu	Inoculation	13.99	< 0.001	0.30	0.868
	Salinity	1.46	0.248	28.26	< 0.001
	Inoculation×Salinity	0.61	0.761	0.92	0.511
Fe	Inoculation	8.01	< 0.001	3.03	0.033
	Salinity	1.79	0.184	7.36	0.003
	Inoculation×Salinity	1.47	0.211	2.17	0.059
K	Inoculation	46.34	< 0.001	2.25	0.087
	Salinity	15.69	< 0.001	112.97	< 0.001
	Inoculation × Salinity	9.91	< 0.001	0.94	0.497
Mg	Inoculation	35.22	< 0.001	2.52	0.06
	Salinity	3.57	0.041	205.46	< 0.001
	Inoculation×Salinity	1.76	0.124	1.87	0.102
Mn	Inoculation	15.66	< 0.001	0.56	0.691
	Salinity	2.90	0.070	17.64	< 0.001
	Inoculation×Salinity	1.84	0.108	0.84	0.578
Na	Inoculation	54.55	< 0.001	0.43	0.786
	Salinity	36.47	< 0.001	36.13	< 0.001
	Inoculation×Salinity	4.1	0.002	1.41	0.235
P	Inoculation	10.16	< 0.001	1.68	0.18
	Salinity	7.11	0.003	40.69	< 0.001
	Inoculation×Salinity	0.91	0.524	0.76	0.64
S[a]	Inoculation	15.90	< 0.001	0.52	0.720
	Salinity	11.65	< 0.001	7.80	0.002
	Inoculation × Salinity	1.77	0.122	0.89	0.54
Zn	Inoculation	27.27	< 0.001	0.30	0.873
	Salinity	7.42	< 0.001	34.37	< 0.001
	Inoculation × Salinity	0.32	0.318	1.07	0.452

[a] Analysis performed on untransformed data

important characteristics of inoculation are worth noting. Firstly, inoculation with all four cyanobacterial inoculants was generally detrimental to wheat seedling health, with higher shoot Na and lower concentrations of other nutrients (Tables 3, 4, 5). Overall, inoculation rather than salinity was the main effector of shoot nutrient concentrations (Table 6). Secondly, inoculation had greater negative effects on wheat seedling nutrition at lower salinities (Tables 3, 4, 5). As a result it seems that cyanobacterial inoculation is confounding the trends that should be observed by imposing salt stress on wheat seedlings. This would also explain why correlations between chlorophyll fluorescence parameters and root nutrient concentrations were so strong (Table 1), since

inoculation had fewer effects on root nutrient concentrations while salinity significantly affected them all (Table 6). In the absence of root colonisation, cyanobacterial exopolymeric substances in the cyanobacterial soil crust are the most likely mechanism that could explain how cyanobacterial inoculation is affecting wheat nutrition.

The role of exopolymeric substances (EPS)

In the absence of physical contact between cyanobacteria and wheat roots, the mechanism responsible for cyanobacterial effects on shoot nutrient concentrations is most likely cyanobacterial extracellular polymeric (exopolymeric) substances (EPS). Cyanobacterial EPS production is correlated with cyanobacterial salt tolerance (Ozturk and Aslim 2010) as well as being an effective sponge of metal ions (Blanco et al. 1998; Decho 2000) due to the presence of charged polysaccharides and proteins (Flemming 2011). Filamentous cyanobacteria are effective sorptive agents of Cu, Fe, Ni, Zn, Cd and Pb (Blanco et al. 1998, 1999; de Philippis et al. 2007; 2003; Singh et al. 1989; Yee et al. 2004). The sorption of Zn to cyanobacterial EPS is of particular concern for global nutrition since Zn-deficiency is the largest of the micronutrient deficiencies facing global agriculture (Alloway 2009). While all inoculants tested reduced shoot Zn concentrations, plants inoculated with either Nostoc sp. 'K1.3' or Microcoleus vaginatus 'K2.2' had lower shoot Zn at all salinities.

Both synergistic and competitive effects of metal ions on sorption in multimetal solutions have been reported (de Philippis et al. 2003, 2007; Pradhan and Rai 2001). In this regard, Na is less competitive for sorption sites than Ca (Khattak et al. 1989), however, increasing salinity by adding NaCl decreases the sorption of Mn (Khattak et al. 1989) and Cu (Bhaskar and Bhosle 2006), presumably by Na increasing competition for sorptive sites. The role of Na in competitive sorption in cyanobacterial EPS in the soil crust may explain why fewer detrimental effects on shoot nutrient concentrations occurred with cyanobacterial inoculation at high salinity (Tables 3, 4, 5).

In sand, cyanobacterial EPS encapsulates cyanobacteria within capsule, slime and sheath morphologies that bind sand particles (Mager and Thomas 2011; Malam Issa et al. 2007). Despite producing similar amounts of EPS in non-salinised conditions (Mazor

et al. 1996), crusts of Microcoleus have stronger sand binding properties, lower water penetration and higher runoff rates than Nostoc when established on sand (Hu et al. 2002, 2003). The different nature of EPS between the Oscillatorean cyanobacteria, such as Microcoleus, and Nostoc is the most likely explanation for the differences observed between Nostocalean and Oscillatorean inoculants. Leptolyngbya sp. 'Y4.2' and Microcoleus vaginatus 'K2.2' are also expected to have greater effects since they grew faster and covered almost 100 % of the sand surface before the end of the experiment (data not shown).

The data presented here suggest that the cyanobacterial inoculants used in this study are not suitable for establishing wheat crops in saline soils. It must be highlighted that plants in previous studies that demonstrated a reduction of salt stress on rice and barley by cyanobacterial inoculants were grown until harvest (Aziz and Hashem 2004; Hashem 2001; Issa et al. 1994). As well as plant growth promotion, cyanobacterial inoculation has been shown to have a range of benefits to saline soils including lower soil EC_e (Hashem 2001; Subhashini and Kaushik 1981), improved organic matter and N contents (Aziz and Hashem 2003). As a result, further exploration of cyanobacterial inoculants for use on salinised soils is justified, with particular focus on the use of cyanobacteria in stabilising and improving these degraded soils as has already been demonstrated on non-saline degraded soils (Maqubela et al. 2009; Pardo et al. 2010).

High salt stress, not inoculation, reduced the photochemical efficiency of wheat

Due to its non-invasive nature, chlorophyll fluorescence is a useful tool in assessing plant responses to environmental stress (Logan et al. 2007; Sayed 2003). Of the inoculants tested, the fluorescence parameters were only affected by inoculation with Leptolyngbya sp. 'Y4.2' that reduced qP_4 at low salinity (Tables 3, 4, 5). When the chlorophyll fluorescence data was pooled by salt there was no difference between the low and medium salinities whereas high salinity had strong effects on chlorophyll fluorescence parameters (Table 2). The reductions in Φ_{II}, F_v/F_m and qP and increases in qN measured at high salinity indicate that the shift above the STT to high salinity led to a reduction in photochemical efficiency (Φ_{II}) caused by a reduction in the number of open photocentres

for conducting photosynthesis (qP) and a decrease in non-photochemical quenching efficiency (F_v/F_m, qN) as previously described by Maxwell and Johnson (2000). The observed reduction of qP_4 values to below 0.6 and the reductions in F_v/F_m suggest reaction centre damage occurred while protective energy dissipation may or may not be occurring at the same time (Horton et al. 1996). Shoot Na was the only plant nutrient variable that followed the same pattern as the chlorophyll fluorescence parameters, suggesting that shoot Na concentrations are the primary mechanism of both reaction centre damage and the induction of protective energy dissipation.

When plant biomass and nutrient concentrations correlated with chlorophyll fluorescence parameters, they generally correlated with all chlorophyll fluorescence parameters (Table 1). It is noteworthy that, with the exception of shoot Na, cyanobacterial inoculation effects on shoot nutrient concentrations were not closely associated with chlorophyll fluorescence parameters (Tables 3, 4, 5). Significant correlations between pooled biomass and nutrient concentrations were often highest with Φ_{II}, suggesting it is the most useful parameter for indicating the effects of salt stress on a broad range of physiological parameters for wheat. Both F_v/F_m and Φ_{II} have previously been suggested as the easiest and most effective chlorophyll fluorescence parameters to measure (Maxwell and Johnson 2000; Woo et al. 2008) and both are useful indicators of crop stress (Sayed 2003). In agreement with this study, Φ_{II} has been recommended as an appropriate plant physiology indicator since it can be performed in the field under light conditions (Maxwell and Johnson 2000). F_v/F_m has previously been recommended to measure drought stress in *Arabidopsis thaliana* because it is faster to measure (Woo et al. 2008), however it was a poor indicator of salt stress above STT in 25–50 day old rice (Lutts et al. 1996) and flag leaves of barley (Belkhodja et al. 1999).

Conclusion

This study investigated the effects of cyanobacteria on the chlorophyll fluorescence and nutrition of wheat seedlings grown under salt stress and evaluated the potential of chlorophyll fluorescence parameters to indicate plant nutrition. Rather than improving wheat salt tolerance as hypothesised, cyanobacterial inoculation produced neutral or negative effects on plant nutrition when compared to non-inoculated wheat seedlings. The hypothesis that *Nostoc* would have greater ameliorative effects than *Leptolyngbya* or *Microcoleus* was partially correct, in that *Nostoc* inoculants induced fewer negative growth effects on wheat seedlings. Furthermore, the effects of cyanobacterial inoculation on wheat shoot nutrition were so strong they confounded the effects of salinity at 6 and 13 dS m^{-1}. EPS is the most likely cause of the observed effects of cyanobacterial inoculation on wheat seedling nutrition. The effects of cyanobacterial EPS and the absence of root colonisation would also explain the shift in focus of cyanobacterial inoculant research in recent years from plant growth promotion to using cyanobacteria to improve saline soil structure and as substrates for biosorption. Salinity rather than inoculation was principally responsible for the observed effects on the chlorophyll fluorescence of wheat, suggesting that chlorophyll fluorescence holds some promise for indicating shoot Na concentrations, although this requires further validation.

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