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STABILISING AMINE UREA IN NITROGEN FERTILISER INCREASES LEAF CHLOROPHYLL CONTENT, TILLER BASE DIAMETER AND ROOT LENGTH OF WHEAT PLANTS

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Summary: Fertilisation of field crop plants with urea nitrogen is very inefficient because over half of this is degraded via hydrolysis and nitrification, releasing greenhouse gases and leaching nitrate into water systems. Technologies for stabilising urea N in fertiliser, and prolonging its availability for plants, have been developed. Here we investigate whether chemically stabilising urea amine N (in a product called 'Elona') in foliar fertiliser applied to pot-grown wheat, induces favourable physiological effects, compared to those of industry standard nitrogen fertilisers. All treatments contain identical amounts of nitrogen by weight, equivalent to a rate of 2.5 L/ha stabilised amine nitrogen (SAN) in 100L, and were applied every 3-4 weeks in March-June 2018, in a greenhouse in Preston, Lancashire, UK. The chlorophyll content of wheat leaves was significantly increased by SAN nutrition 3 and 10 days after the first treatment, initially at 4-5 tiller stage; and tillers were more upright. At 14-15 tiller stage tiller bases had an increased diameter. This gave rise to a higher tiller diameter – canopy height ratio. Three weeks later roots of SAN-treated plants were significantly longer, which gave rise to a larger root length – canopy height ratio. We discuss how these attributes relate to specific effects of ureic amine N on plant phenotype, and how they may affect yields in the longer term. We argue that genetic screening for high yield-linked phenotypic traits may be more effective when wheat is fertilised with stabilised urea.

INTRODUCTION

The effect of the form of nitrogen (N) within fertiliser on plant development and yielding has often been overlooked. One reason for this is that all N forms (ammonium, urea, nitrite, organic amines) are eventually degraded to nitrate (and gaseous pollutants) within hours to weeks of application (dependent on environmental conditions) unless they are stabilised (see Wilkinson et al. 2019a). When N form has, however, been studied on plant growth in the field, this mainly relates to differences between effects of nitrate and ammonium nutrition (e.g. Carlisle et al. 2012), which nevertheless show widely contrasting effects on many physiological characteristics which influence yield, including in wheat. As it was originally believed that non-leguminous plants could only take up these inorganic N forms from the soil, effects of urea in plants have not been investigated to the same extent. However this is a growing area of research, particularly as technologies for preventing urea degradation to nitrate and pollutants are becoming available, which can increase crop nutrient use efficiency and/or yield in some cases (e.g. Wang et al. 2015).

The effects of urea N, ammonium N and nitrate N on plants have frequently been compared in highly controlled experimental systems, such as in hydroponics or agar-filled pouches. Under these conditions plants grown in the presence of ammonium alone or urea alone can exhibit reduced growth, and generate symptoms of toxicity compared to nitrate nutrition (see Yang et al. 2015). When a detrimental effect of ammonium on hydroponic solution pH is corrected, however, ammonium nutrition improves biomass and tillering of wheat in comparison to nitrate (Chen et al 1998). Further increases in biomass and tillering occurred when both N forms were supplied together. When two N forms are supplied in ratios, for example 75-25 urea-nitrate compared to 25-75, nutritional effects on plants in experimental systems can still be attributed to the dominant N form (Pompeiano and Patton, 2017), whilst reflecting more closely conditions existing in the field. In the latter case both above and below ground biomass was greatest under a ratio of 75-25 urea-nitrate in greenhouse grown Zoysia grass. Different sets of genes are up-regulated when both N forms are present, increasing the efficiency of total N uptake, assimilation and use (Pinton et al. 2016).

New strands of research are emerging showing that plants have evolved to take up urea from soil, and possess highly conserved systems within root cells for doing so (Wang et al. 2016). We are gathering field and greenhouse data showing that the N form urea amine has unique and beneficial effects on plant form and function in comparison to nitrate and ammonium nutrition when it is stabilised. These effects can lead to greater, more uniform tuber yields in potato (Marks et al. 2018; Wilkinson et al. 2019b), and to increased flowering in ornamental species (Wilkinson et al 2019a). We have shown that, in the main, this is not related to the stabilisation-induced maintenance of nitrogen concentration per se (by preventing ammonification and nitrate leaching), although this positive effect may still additionally occur in the field. Instead, favourable effects of urea amine (Wilkinson et al. 2019a, b), in comparison to conventional ammonium nitrate and/or un-stabilised urea controls, can be some or all of the features of the specific phenotype generated by this N form: increased root-shoot ratio during early development, increased root development per se, reduced shoot extension rate, and increased chlorophyll content. At later developmental stages, aboveground biomass increases over and above that of controls, lateral shoot development is increased, and chlorophyll content remains high.

Here we describe the effects of foliar treatments of a range of N fertilisers, including chemically stabilised amine nitrogen (SAN), on wheat growth and physiology. We aimed to determine whether any of the above effects, several of which are known to contribute to and/or proxy for increased yields in the field (Bai et al. 2013), occur in pot-grown seedlings of this staple food crop. Experiments were conducted in compost in a greenhouse in Preston, UK, in 2017-2018.

MATERIALS AND METHODS

Triticum aestivum L. cv Anapolis was used in greenhouse trials. Seeds were drilled in modules in December 2017, in J. Arthur Bowers John Innes No. 2 compost (Westland Horticulture Ltd., Co. Tyrone, UK), at a rate of one per 2.5 x 2.5 cm module sub-compartment. Prior to tillering seedlings were transplanted singly to 5 L pots containing the same compost. The pH of this is 5.5-6.0, and it initially provides appropriate macro- and micro-nutrients to all plants. Foliar spray treatments with a range of nitrogenous compounds occurred every 3-4 weeks from the onset of tillering, in a heated and ventilated greenhouse under natural light (PPFD 200-1000 µmol m^2 s^{-1}), in Preston, northern England, UK. Night-time temperature was 12-16^{o}C, and day-time temperature was 16-32^{o}C. Plants were watered by hand to soil capacity as required. Each of the nitrogenous treatments comprised of five replicate wheat plants randomised within an area of 2.5 x 1.0 m^2.

Nitrogen (N) fertiliser treatments were applied as liquid formulations: a standard N-P-K control, stabilised amine nitrogen (SAN) in a formulation called ‘Elona’ (supplied by Levity Crop Science Ltd., Preston, UK), and a cereal-specific industry standard (IS). SAN was applied at a rate of 2.5 L ha^{-1} in 100 L water. It contains 15 % N (by weight), and the control and IS treatments were designed to provide the same amount of N to the plants (given that IS contains 24 % N). Both controls and commercial IS treatments contain a mixture of ureic and ammonium nitrate N. All plants were supplemented via the soil with standard N-P-K (control treatment) at 50% recommended strength every 3-4 weeks, approximately mid-way between main treatment dates, ensuring access to sufficient micronutrients and P-K. Main treatments with N fertiliser occurred approximately every 3-4 weeks, as specified in Table 1, at a rate of 20 cm^3 per m^2.

Leaf relative chlorophyll content, tiller angle, tiller basal diameter, canopy height and root lengths were measured once or on several occasions at different developmental stages over the course of the experiments (Table 1).

Relative chlorophyll content was measured in leaves as an index, with a FieldScout CM 1000 Chlorophyll Meter (Spectrum Technologies Inc., Illinois, USA). “Point-and-shoot” technology instantly measures the reflectance of ambient and reflected 700 nm and 840 nm light in a conical viewing area on the adaxial leaf surface 30-180 cm from the light receptor. Laser guides outline the edges of the sampling area, allowing replication of the position of this between plants. The light receptor comprises four photodiodes; two for ambient light and two for reflected light from the leaf. Measurement units are calculated as an index of relative chlorophyll content, 0-999 ± 5%. Leaf canopy height and root lengths were hand-measured with a ruler at the times detailed in Table 1. Tiller angle of the three largest tillers, with the soil surface as the horizontal plane, was measured using a protractor. Tiller basal diameter was measured at its widest point with a digital calliper.

Means and standard errors of each measurement type per treatment are displayed as bar charts. The significance of the differences between treatments was calculated using a one-tailed *t*-test for two independent means, and where treatments are significantly different from each other (at $p<0.1$), this is denoted by ‘a’, ‘b’, or ‘c’, above the appropriate column on the graphic representations of the data.

Table 1. Time course detailing foliar nitrogen (N) application occasions, and measurement activity, during experiments on greenhouse-grown *Triticum aestivum* L. cv Anapolis beginning in March 2018 (Preston, Lancashire, England, UK).

Days from start	Activity	Figure no.
0	N treatment 1	
3	Chlorophyll analysis	1A
5	Tiller angle measured	2
10	Chlorophyll analysis	1B
27	N treatment 2	
36	Tiller diameter measured	3A
36	Canopy height measured	3B
48	N treatment 3	
56	Root length measured	4A
56	Canopy height measured	4B

RESULTS

Figure 1A shows that the chlorophyll content of wheat leaves was significantly increased by SAN 3 days after the first foliar nitrogen treatment, at 4-5 tiller stage; by 11.6% in comparison to the controls, and by 19% compared to the industry standard (IS) treated plants. Figure 1B shows that the effect of SAN persists 7 days later. In between the chlorophyll measurements, tillers were more upright in SAN treated plants (Fig 2A), with an increased angle between the soil surface and the three largest tillers per plant (Fig 2B). The increase in angle was 51.5% in comparison to controls, and 50% compared to IS treated plants.

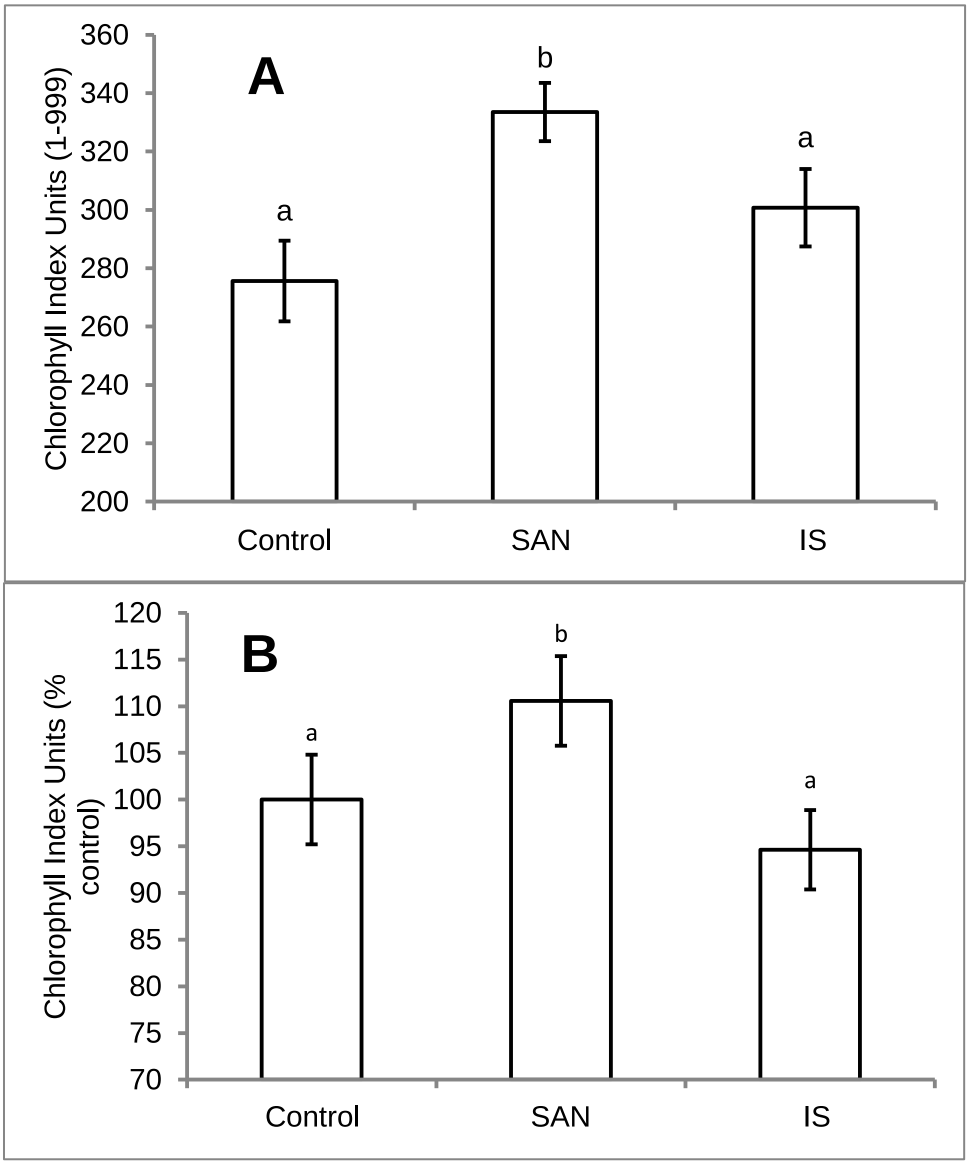

Figure 1. Effect of foliar SAN application on leaf chlorophyll content of wheat plants 3 days after treatment (A), compared to conventionally fertilized control and industry standard (IS) treated plants. Fig 1B shows the effects 7 days later.

At 14-16 tiller stage, tiller base diameter was significantly larger in SAN-treated plants (Fig 3A) than in both control and IS treatments. Thus there was a significantly higher tiller base diameter-canopy height ratio (3B), as canopy height was similar among treatments (not shown).

Four weeks later, after a total of 3 foliar N fertiliser treatments, root length below the pot was also the highest in SAN treated plants (Fig 4A), as was root length-canopy height ratio (4B), as again canopy height was similar among treatments.

DISCUSSION

Genes and/or agronomic practises relating to phenotypic variability in root architectural traits, nutrient uptake and metabolism, photosynthesis and canopy longevity, nitrogen remobilization and wheat grain N accumulation are being sought to improve field wheat nitrogen uptake efficiency (N taken up per unit N supplied) and/or nitrogen utilisation efficiency (grain yield per unit N taken up), and yield per se (Hawkesford 2014, 2017). Several of these characteristics are displayed during pre-anthesis growth of wheat seedlings, and have been linked to yielding of mature plants in the field. These have largely been determined by germplasm screening in a range of experimental and field systems, under a range of conditions including drought, heat, and low and high levels of N. However, these studies are rarely carried out on the basis of the N form(s) of the applied fertiliser. Here we demonstrate that some of these traits can be induced in greenhouse-grown wheat seedlings by a simple change in nutritional N form; these being increased relative leaf chlorophyll content (Figure 1) and increased root length (Figure 4). Furthermore increased wheat tiller basal internode diameter (Figure 3) is closely related to lodging resistance and grain yield (Tripathi et al. 2003, Khan et al 2019). Erect tillers (Figure 2) can enhance photosynthesis and dry matter production through greater sunlight capture (Abichou et al. 2019).

Improvements in root architecture (lateral root proliferation near the soil surface and at depth) and increases in root biomass have been viewed as promising targets for selection for NUE and yield amongst wheat genotypes (Hawkesford 2014, 2017). However, it has also been demonstrated that root development is largely dependent on genetic differences in above-ground shoot biomass and tillering processes (Allard et al. 2013), such that there is an argument that root traits may not be as important as selection targets for improved NUE and grain yield as originally believed. Allard et al. (2013) used ammonium nitrate as basal fertiliser in field trials, which can be assumed to have been converted to nitrate, which will have then been the dominant form of N in the soil. Given our research (e.g. Wilkinson et al. 2019b), we would maintain that the study by Allard et al (2013) was one based on genetic variation in nitrate use efficiency, rather than one based on wider nitrogen use efficiency per se. Nitrate from soil or foliar sources is preferentially allocated to shoots for above ground vegetative growth and tillering during early seedling development, at the expense of root biomass growth (Andrews et al. 2013, Wilkinson et al. 2019a, b). Compared to ammonium N and ureic amine N, this generates a phenotype with a reduced root-shoot ratio and relatively low internal nitrogen utilisation efficiency (nitrate assimilation is comparatively resource inefficient). Screening for root traits would thus have occurred within a narrowed phenotypic range, in which variations in vegetative traits would have provided a wider target. Had the authors used stabilised urea as basal or foliar fertiliser, which promotes the generation of the resource use efficient, stress resistant phenotype (characterised by high root-shoot ratio, initially reduced apical dominance, and increased leaf chlorophyll content), we propose that they would have found a wider variation in root traits within a more productive phenotypic range.

Given that increased photosynthesis (Figure 1), and increased rooting (Figure 4) do indeed show promising links to improved yields in other species (potato - Wilkinson et al 2019b, lettuce – Wilkinson et al 2020, manuscript in preparation), and that we show that these traits are easily altered by nutritional N form in wheat, we argue that wheat crop fertilisation in the field with stabilised urea will increase grain yield via the generation of a specific urea-amine phenotype.

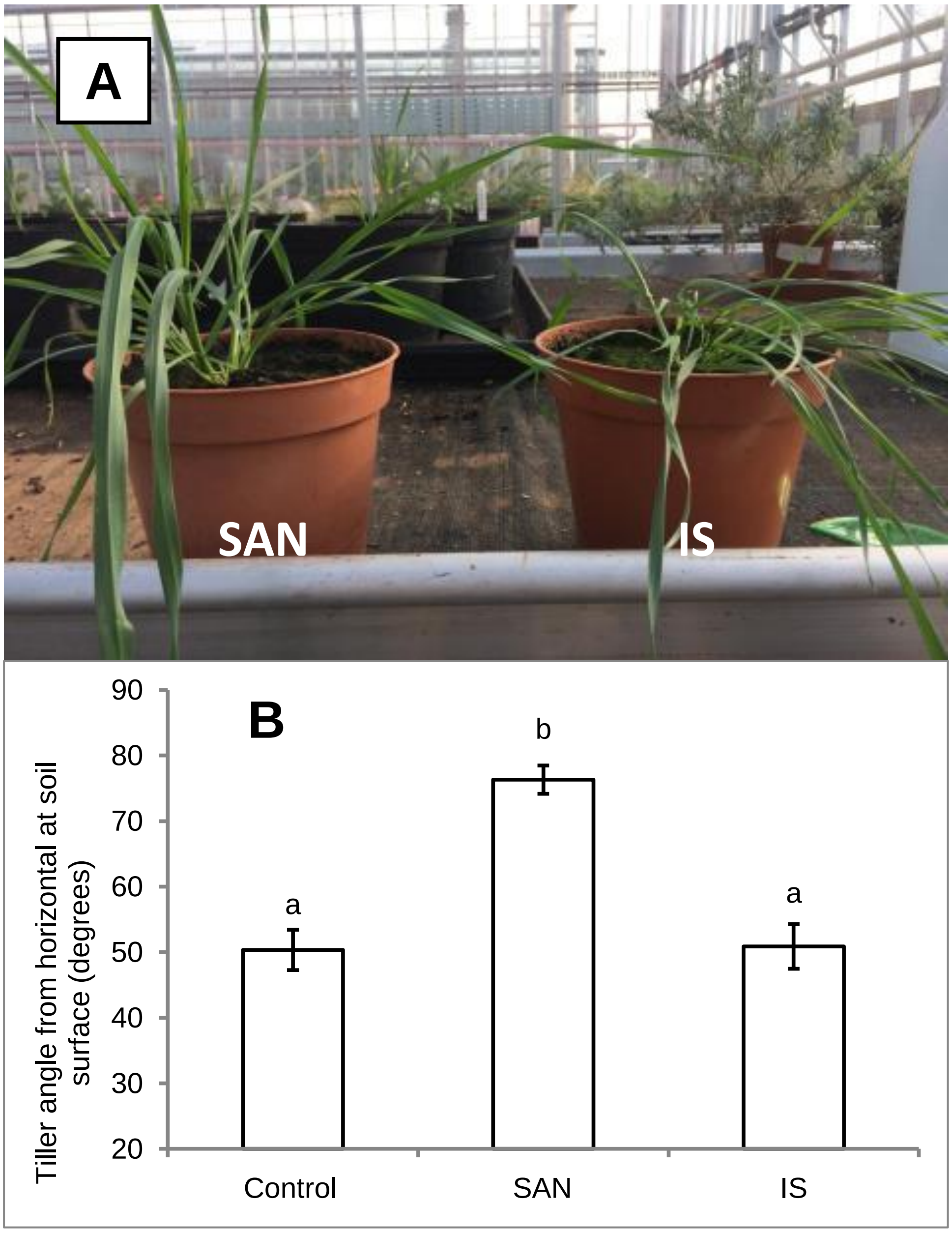

Figure 2. Comparison between the effects of SAN, control and industry standard (IS) foliar N fertilisation treatments on tiller angle.

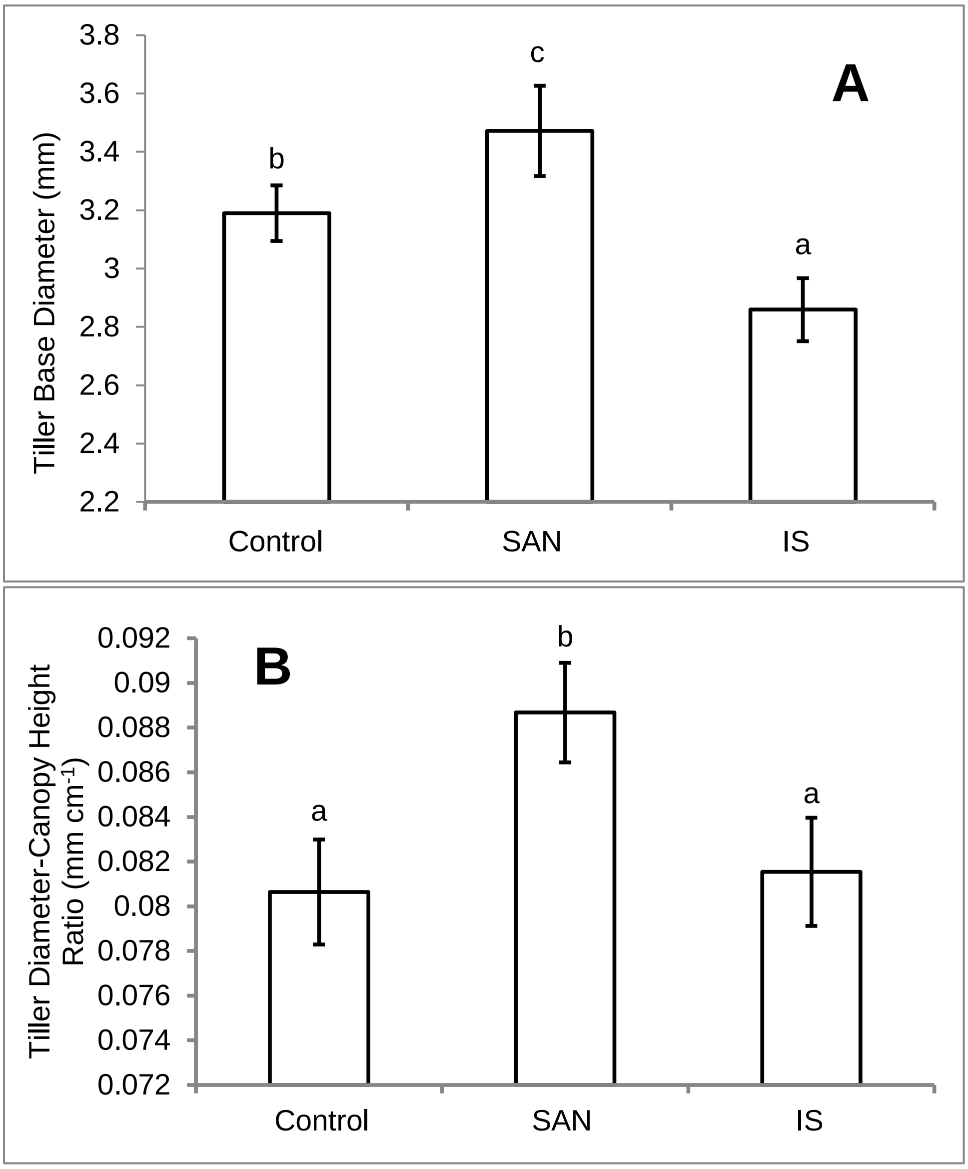

Figure 3. Comparison between the effects of SAN, control and industry standard (IS) foliar N fertilisation treatments on tiller diameter (A) and tiller diameter – canopy height ratio (B).

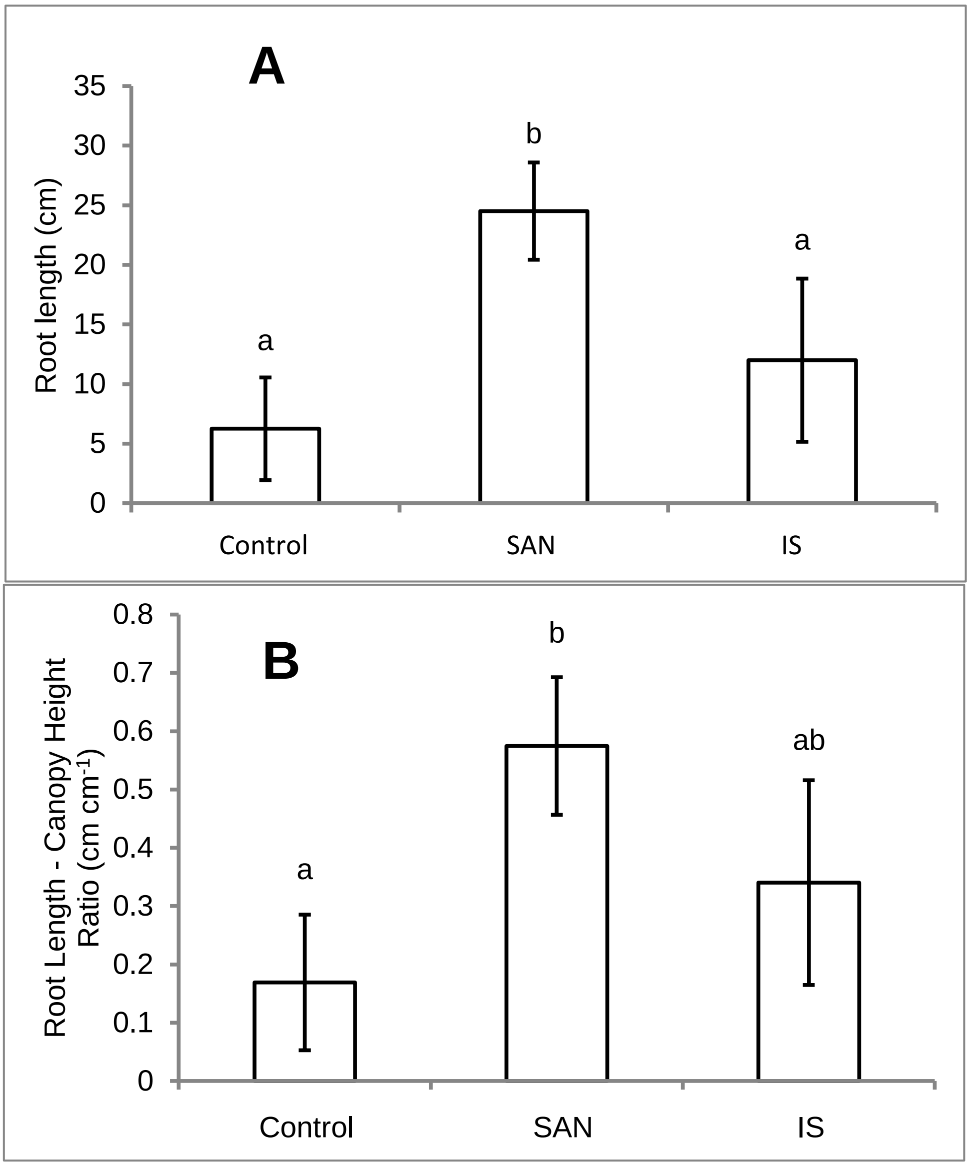

Figure 4. Comparison between the effects of SAN, control and industry standard (IS) foliar N fertilisation treatments on root length (4A) and root length – canopy height ratio (4B).

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