



## Mycorrhizal colonisation of cotton in soils differing in sodicity

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### ABSTRACT

Despite the reported importance of mycorrhizal symbioses for early growth and nutrient acquisition of cotton, little is known about how sodicity affects this relationship. Changes in mycorrhizal colonisation and nutrient uptake of cotton in a range of naturally non-sodic (exchangeable sodium percentages (ESP) < 6) and low-sodic soils (ESP 6–10), from cotton production areas in southern Queensland and northern New South Wales, with different ESP (ranged between 1.4 and 9.8) was investigated in a glasshouse experiment. The experiment was a complete factorial design with 11 recently-collected soils and two mycorrhizae treatments (either inoculated with fresh “live” mycorrhizal inoculum or without inoculum). Linear mixed model analysis showed minimal effects of sodicity, when ESP was less than 10, on mycorrhizal colonisation, associated plant growth and nutrient uptake. Principle component and regression analysis showed that other sources of variation including soil pH and soil P content, rather than sodicity, might drive cotton colonisation in Vertosols with low to moderate ESP. The colonisation percentage was positively linearly correlated with P, Mg, and Zn uptake of cotton plants. Further investigation into mycorrhizal spore density and species diversity under sodic soil conditions is warranted.

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### 1. Introduction

Of the world's 581 million hectares of sodic soils, more than half (340 million ha) are in Australia (Szabolcs, 1989; Murphy, 2005) where they make up more than 60% of Australian cropping soils (Rengasamy, 2002). In Australia, soils with exchangeable sodium (Na) constituting more than 6% (ESP) of the cation exchange capacity (CEC) are considered sodic (Northcote and Skene, 1972). These sodic soils are further characterised as moderately sodic with an ESP greater than 10, and highly-sodic with ESP > 15 (Smith, 2015). Large areas of the cotton growing regions in Australia are sodic (Rengasamy, 2002; Dodd, 2007), are often irrigated with saline groundwater (Rengasamy and Olsson, 1993; Dodd, 2007) and contain sodic soil conditions in the topsoil, subsoil or both (Northcote and Skene, 1972; Naidu et al., 1995). Sodic soil conditions include waterlogging, hard-setting, high bulk density, high pH, and high soil solution sodium concentrations [Na<sup>+</sup>] (Dodd et al., 2013).

Cotton is a mycorrhizally responsive plant (Rich and Bird, 1974; Thompson et al., 2012). Mycorrhization enhances growth and nutrient acquisition, especially P, of cotton plants (Rich and Bird, 1974; Smith and Roncadori, 1986; Bellgard and Williams, 2002). In controlled conditions, up to 90% of cotton root length can be colonised by arbuscular mycorrhizal fungi, whilst in field conditions, 50 to 70% is typically observed within 4 weeks of cotton germination in normal topsoils (McGee et al., 1998). Adverse physical and chemical conditions induced by high Na levels in sodic soils may affect the mycorrhizal symbioses of cotton plants.

Previous research (Eskandari et al., 2016, 2017) suggests cotton struggles to form mycorrhizal symbiosis in moderately (ESP 14) and highly-sodic soils (ESP 21) and the percentage of root length colonised by mycorrhizae and P uptake in cotton plants grown in highly-sodic soil is reduced significantly (by 16% and 20%, respectively) compared to plants in low-sodic soil. Dodd et al. (2013) believed that poor cotton performance in sodic soils is mostly related to soil physical constraints when ESP is less than 19. There is a possibility that adverse soil physical/chemical conditions, induced by high levels of Na in the soil, decrease colonisation and mycorrhizal hyphal exploration ability in the soil and consequently reduce P uptake and cotton growth (Eskandari

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et al., 2017). However, whilst sodic soils in those experiments were sourced from cotton growing regions, the ESP (14 and 21) was not indicative of the status of many of the sodic soils used by farmers within the Australian cotton growing regions. In fact, there are no reported research papers investigating the effect of low levels of sodicity (ESP 6–10) on mycorrhizal infection in Vertosols (Isbell, 2016).

Hence, the aim of this paper was to determine whether sodicity affects mycorrhizal colonisation of cotton over the range of sodicities commonly encountered in cotton soils in Australia. Earlier research into the effects of sodicity on cotton growth indicated that physical constraints to root extension, and water-logging associated with reduced hydraulic conductivity, reduce cotton growth at low soil ESP values. We hypothesized that these same constraints may also act through limiting mycorrhizal colonisation, exacerbating the reduced ability of cotton plants to acquire P, Zn and other nutrients.

## 2. Materials and methods

### 2.1. Soil collection & properties

Soils (0–20 cm) were collected from cotton fields within the upper Gwydir and McIntyre production regions in Australia. All soils were classified as Vertosols in the Australian soil classification (Isbell, 2016). Soils were air-dried after collection, crushed and passed through a 4 mm sieve to remove coarse organic matter and stones. Subsamples of each soil type were then ground to <2 mm and 2 g subsamples were shaken end over end for 1 h in 40 mL of 1 M NH<sub>4</sub>Cl (buffered to pH 8). The supernatant was filtered through a Whatman No. 42 paper and analysed for CEC (Ca, K, Mg, and Na) using inductively coupled plasma optical emission spectroscopy (ICP-OES) as described in Rayment and Lyons (2011). The ESP was calculated as the percentage of exchangeable Na on the cation exchange complex.

The pH and electrical conductivity (EC) of the soils were measured in a 1:5 soil water suspension and soil P concentrations were measured in 0.5 M NaHCO<sub>3</sub> extracts using the method outlined by Colwell (1963). All soils had an EC less than 4 ds m<sup>-1</sup>, which is the salinity threshold of a soil saturation extract at which soil salinity begins to decrease cotton crop yield (Silvertooth, 2001). Selected properties of the soils used in this study are presented in Table 1.

### 2.2. Experimental design

Eleven recently-collected soils (dried, crushed and 4 mm sieved) each with and without inoculum addition (designated as inoculated or uninoculated) were placed in plastic lined pots, 13.5 cm high and 12.5 cm in diameter containing 500 g of soil, with a few holes to allow, but limit, drainage. Pots were placed on a glasshouse bench with the diurnal temperature range controlled between 18 °C and 28 °C (night and day, respectively) and were watered every two days for 6 weeks to maintain a gravimetric water content of 0.6, assessed by constant weight, which was equivalent of field saturation.

A root-based mycorrhizal inoculum consisted of 20 g of a mixture of fresh roots and soil taken from a pot culture of highly colonised (80% of the root length, measured by method described in 2.4) 12-week-old maize (*Zea mays* L.), grown on a non-sodic pasture topsoil (Vertosol, ESP 0.7, pH = 6.5, Colwell P = 57 mg kg<sup>-1</sup>) collected from near the University of New England, Armidale, NSW (30°29' 12.28" S, and 151°38' 11.40" E), was used. The inoculum was placed 3 cm below the cotton seeds. Approximately 9.5 g (fresh weight) of colonised roots were present in each pot.

Before planting, basal nutrients, except P, were added in solution to the soil surface of each pot. Amounts were 200 mg kg<sup>-1</sup> of nitrogen (N) as urea, 2.5 mg kg<sup>-1</sup> of zinc as ZnSO<sub>4</sub>, and 100 mg kg<sup>-1</sup> of potassium (K) as K<sub>2</sub>SO<sub>4</sub>. Five cotton seeds (cv. Sicot 74BRF) were sown 2 cm deep into each pot, which was then covered with shade cloth to reduce evaporation and facilitate cotton germination and emergence. When the plants reached the two-leaf stage, the shade cloth was removed and the cotton plants were thinned to three per pot.

### 2.3. Plant and root measurements

At 6 weeks after sowing, shoots were removed, dried in a fan-forced oven at 60 °C for 48 h and their dry weights were recorded. They were then ground to <2 mm, and digested in 70% nitric acid using a microwave digestion technique (Milestone® UltraWAVE) and the nutrient composition of the samples was determined using ICP-OES. A standard plant sample was included in the analysis, to confirm the accuracy of the results. Total uptake of Zn (μg pot<sup>-1</sup>), K (mg pot<sup>-1</sup>), Ca (mg pot<sup>-1</sup>), Mg (mg pot<sup>-1</sup>), Na (mg pot<sup>-1</sup>), and Mn (μg pot<sup>-1</sup>) are reported in Table A. 1, Appendix A.

**Table 1**  
Chemical variables, Exchangeable Na Percentage (ESP), and management history of non- and low-sodic Vertosols, collected from southern Queensland and northern New South Wales, Australia, used to investigate the effect of ESP and inoculation on mycorrhizal colonisation and associated nutrient uptake of cotton. Values ± standard error (n = 3).

Soil	Exchangeable cation concentrations (cmol <sup>+</sup> kg <sup>-1</sup> ) <sup>a</sup>				(ESP) (%)	Colwell P (mg kg <sup>-1</sup> ) <sup>b</sup>	EC (ds m <sup>-1</sup> ) <sup>c</sup>	pH	Previous crop	Irrigated/ Dryland
	Ca	K	Mg	Na						
Soil 1	36.6 ± 0.2	0.8 ± 0.1	33.9 ± 0.4	1.0 ± 0.0	1.4 ± 0.0	34.7 ± 2.9	0.1 ± 0.0	7.6 ± 0.0	Cotton	Dryland
Soil 2	24.6 ± 0.1	1.1 ± 0.0	9.7 ± 0.0	1.0 ± 0.0	2.8 ± 0.0	28.7 ± 0.3	0.1 ± 0.0	8.5 ± 0.0	Cereal	Dryland
Soil 3	23.2 ± 0.1	1.2 ± 0.0	9.4 ± 0.1	1.0 ± 0.0	2.9 ± 0.0	39.0 ± 0.8	0.2 ± 0.0	8.2 ± 0.0	Fallow	Irrigated
Soil 4	21.8 ± 0.3	1.2 ± 0.0	10.9 ± 0.1	1.5 ± 0.0	4.1 ± 0.0	39.5 ± 1.6	0.2 ± 0.0	8.3 ± 0.0	Cotton	Irrigated
Soil 5	27.1 ± 0.1	0.9 ± 0.0	11.9 ± 0.1	2.1 ± 0.0	4.9 ± 0.0	6.6 ± 0.1	0.5 ± 0.0	7.9 ± 0.0	Cotton	Irrigated
Soil 6	24.4 ± 0.3	1.1 ± 0.0	15.3 ± 0.2	2.7 ± 0.0	6.2 ± 0.0	21.4 ± 1.4	0.2 ± 0.0	8.2 ± 0.0	Fallow	Irrigated
Soil 7	18.8 ± 0.4	1.0 ± 0.0	8.4 ± 0.1	2.2 ± 0.1	7.1 ± 0.1	37.9 ± 0.2	0.1 ± 0.0	8.3 ± 0.0	Cereal	Dryland
Soil 8	24.2 ± 0.2	1.0 ± 0.0	15.7 ± 0.2	3.5 ± 0.0	7.8 ± 0.0	21.3 ± 1.1	0.3 ± 0.0	7.7 ± 0.0	Fallow	Irrigated
Soil 9	21.5 ± 0.4	0.8 ± 0.0	14.6 ± 0.3	3.6 ± 0.1	8.9 ± 0.0	16.8 ± 1.2	0.4 ± 0.0	7.1 ± 0.0	Fallow	Irrigated
Soil 10	23.2 ± 0.5	0.9 ± 0.0	14.7 ± 0.3	4.1 ± 0.1	9.6 ± 0.0	18.9 ± 0.4	0.4 ± 0.0	8.0 ± 0.0	Fallow	Irrigated
Soil 11	22.6 ± 0.4	0.9 ± 0.0	15.1 ± 0.2	4.2 ± 0.1	9.8 ± 0.0	26.5 ± 0.8	0.4 ± 0.0	7.6 ± 0.0	Fallow	Irrigated

<sup>a</sup> 1.0 M NH<sub>4</sub>Cl (buffered to pH 8), Rayment and Lyons (2011).

<sup>b</sup> In 0.5 M NaHCO<sub>3</sub> extracts, Colwell (1963).

<sup>c</sup> 1:5 soil:solution ratio in H<sub>2</sub>O.

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