SUMMER SCHOLARSHIP
Final Report

Part 1 - Summary Details

Cotton Catchment Communities CRC Project Number:

Project Title:

Improving cotton yields on sodic soils – a new role for plant growth regulators?

Project Commencement Date: 12/12/2011
Project Completion Date: 10/2/2012
Research Program:

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Part 3 – Scholarship Report

1. Background:

Sodic soils present a major management constraint for large parts of Australia's cotton producing districts. A combination of structural, chemical and water constraints in these soils leads to reduced plant growth and yields (Yadav *et al.* 2011). One primary area of concern is the effect of sodicity in reducing the drought tolerance of cotton plants, particularly seedlings. Because much of the soil moisture stored in the top 10cm of soil is lost to evaporation, it is vital that seedlings can quickly access moisture at greater depths. Sodic soils compound this problem in multiple ways. The chemical environment reduces the osmotic potential, and thus the ability of the young plants to draw sufficient water. Also, the dispersive nature of these soils creates crusting and blocks macropores, reducing infiltration and overall water supply under rain-fed conditions (Ghosh *et al.* 2010). Along with these factors, sodic soils, particularly Vertosols, can demonstrate high soil strength which makes it difficult for seedling roots to penetrate the soil (Odeh and Onus 2008). Cotton plants are well adapted to water stress and the chemical environment of sodic soils (Dodd *et al.* 2010). Most growth constraints, therefore, are likely to be attributable to structural limitations in physically preventing root access to water.

Mepiquat chloride is a synthetic plant growth regulator widely used in cotton production to maintain a regular crop and prevent excessive vegetative growth under high temperature conditions (Yeates, Constable and McCumstie 2005). It reportedly interacts with natural plant hormones, increasing the levels of auxin and cytokinin hormones in the middle region of the primary root (Duan *et al.* 2007), and suppressing gibberellin biosynthesis (Yeates, Constable and McCumstie 2005). The suppression of gibberellin production is believed to slow internode lengthening by limiting cell enlargement (Reddy, Reddy and Hodges 1996), which is the agronomic effect intended. Duan *et al.* (2007) found that mepiquat chloride also increased lateral root primordia development and lateral root growth, apparently in connection with the increased levels of auxins and cytokinins. Other reports have also found increases in root growth from treatment with mepiquat chloride (Xu and Taylor 1992; Iqbal *et al.* 2005), which can have beneficial effects in terms of drought tolerance, particularly for seedlings (Xu and Taylor 1992).

2. Aims and Objectives:

The potential for using mepiquat chloride (MC) to improve the tolerance of cotton seedlings to drought stress conditions is significant, particularly for the many Australian cotton growing regions constrained by sodic soils. Therefore this study sought to determine whether mepiquat chloride can increase root growth of cotton seedlings in a sodic Vertosol soil.

3. Methodology:

In order to determine if mepiquat chloride can increase cotton seedling root growth in a sodic Vertosol soil, two glasshouse pot trials were established simultaneously in order to improve the ability to detect responses that may vary over time. 36 pots were prepared for each of the two harvest groups, consisting of three rates of Pix (mepiquat chloride) by three rates of Polyacrylamide (PAM) with four replications. PAM was used as a control to ameliorate any physical constraints (but not chemical) in the sodic soil.

Initially, three lots of 48 kg of an air-dry sodic Vertosol soil were treated with the three rates of PAM: either 0, 0.005 or 0.1% weight/weight. The ESP (exchangeable sodium percentage) of the soil was 9 %. The PAM was mixed with 8L of water before being thoroughly incorporated into the soil, which was allowed to dry.

Cotton seeds were soaked overnight in a growth cabinet set at 30°C in Reign® solutions of either 0, 500 or 1000 mg/L.

White PVC pots 30 cm tall and 9 cm in diameter were lined with plastic bags and filled with 1.6 kg of the treated soil in two stages, with 200 mL of water applied at each stage to wet the soil to 80% field capacity. Soil bulk density in the pots was approximately 0.9 g/cm^3. Two germinated seeds were placed in each pot, and the soil surface covered with sand to weight. Pots were randomly arranged within replication groups.

Seedlings were maintained at field capacity, and 10 mL of 21.76 g/L urea solution was applied to all treatments after 15 days of growth. Seedlings were thinned to 1 plant per pot after 11 days of growth.

The first harvest was conducted 19 days after sowing and the second at 28 days. Roots were washed out over a 1 mm sieve and stored in 70% ethanol, while shoot height and fresh mass were recorded.

Roots were scanned on a flatbed scanner at a resolution of 600 dots per inch. The digital images were analysed using the software program WinRHIZO^{TM} (Regent Instruments), which was used to categorise the roots in the images into a series of diameter classes, and to provide measurements of root features according to these root classes. The program was configured to identify 60 root classes, the interval width being 0.1 mm. The parameters of interest were total root length, average root diameter, lateral and tap root lengths and lateral and tap root volumes.

Root fresh and dry mass was determined on a four decimal-point balance, as was shoot dry mass.

All data was collated into Windows Excel, and the statistical package GenStat 13th Edition was used for statistical analyses. Analysis of Variance tests were used with $\alpha=0.05$.

4. Results:

Plant height and shoot dry matter

For both harvest 1 and 2, plants treated with MC were significantly shorter than un-treated plants ($P<0.05$; Figure 1 and Figure 2). By Harvest 2, shoot dry mass of MC-treated plants was also significantly lower than that of the un-treated plants (Table 1 and Table 2). No consistent effect of the PAM was found on plant height or shoot dry mass.

Root length and root mass

At harvest 1, there was no effect ($P>0.05$) of either MC or PAM on total root length (Figure 3). At Harvest 2 total root length of MC-treated plants was significantly lower than that of untreated plants (Figure 4) but no effect on root dry mass was detected (Table 2). As with shoot mass and plant height, no consistent effect of PAM was found on total root length or root mass.

Root: shoot

Root to shoot ratio increased in the 500 and 1000 MC treatments over the un-treated plants for both harvests ($P<0.05$; Figure 5 and Figure 6). Only the 0.1% PAM treatment showed an increase in root:shoot over the control ($P<0.05$; Figure 6 and Figure 7).

Root morphology

Some small changes were observed in root morphology in response to MC treatment, including a decrease in average root diameter with MC treatment ($P<0.05$; data not shown). However, this was not consistent across harvests or with other root morphological traits, such as specific root mass (data not shown).

Overall, plant biomass (both root and shoot) increased from harvest 1 to harvest 2.

Table 1: Effect of mepiquat chloride (MC) and PAM on the shoot and root dry masses of cotton grown in a sodic Vertosol for 19 days (data ±1 standard deviation; n=4).

	Shoot dry mass (g)			Root dry mass (g)		
	MC 0	500	1000	0	500	1000
PAM 0	0.38±0.03	0.32±0.07	0.30±0.06	0.12±0.00	0.12±0.02	0.11±0.03
0.005 %w/w	0.33±0.08	0.29±0.11	0.28±0.08	0.10±0.02	0.11±0.03	0.12±0.04
0.1 %w/w	0.36±0.05	0.34±0.04	0.28±0.05	0.14±0.01	0.15±0.01	0.12±0.02
MC x PAM $P<0.05$	Interaction not significant			Interaction not significant		

Table 2: Effect of mepiquat chloride (MC) and PAM on the shoot and root dry masses of cotton grown in a sodic Vertosol for 28 days (data ±1 standard deviation; n=4).

	Shoot dry mass (g)			Root dry mass (g)		
	MC 0	500	1000	0	500	1000
PAM 0	1.14±0.11	0.75±0.12	0.67±0.15	0.36±0.06	0.29±0.04	0.25±0.05
0.005 %w/w	0.89±0.23	0.74±0.15	0.79±0.09	0.30±0.06	0.29±0.07	0.32±0.03
0.1 %w/w	0.99±0.21	0.70±0.16	0.56±0.23	0.35±0.08	0.32±0.11	0.29±0.05
MC x PAM $P<0.05$	Interaction not significant			Interaction not significant		

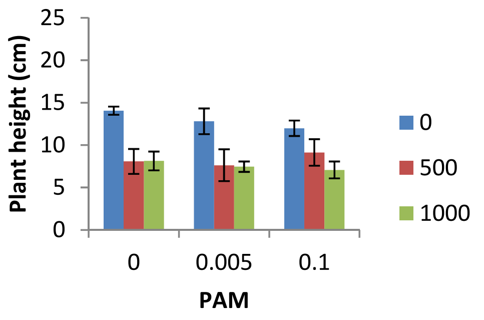

Figure 1: Harvest 1 plant heights according to MC rate (mg/kg) and PAM treatment (% weight)

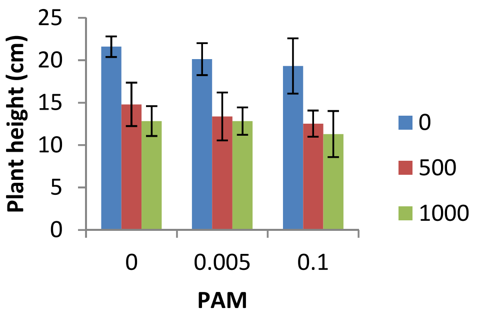

Figure 2: Harvest 2 plant heights according to MC rate (mg/kg) and PAM treatment (% weight)

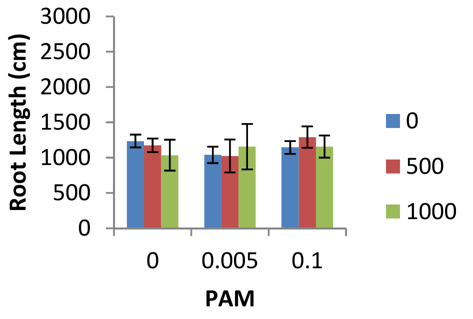

Figure 3: Harvest 1 total root length according to MC rate (mg/kg) and PAM treatment (% weight)

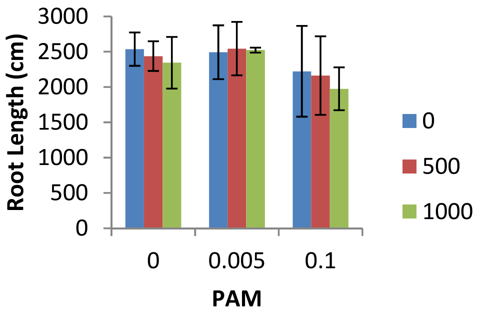

Figure 4: Harvest 2 total root length according to MC rate (mg/kg) and PAM treatment (% weight)

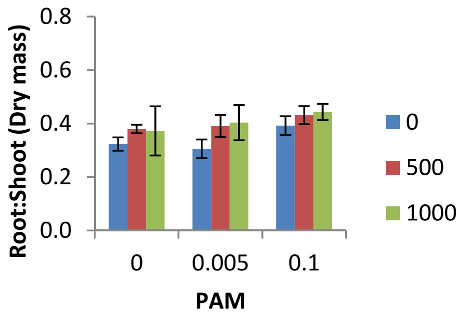

Figure 5: Harvest 1 root:shoot (dry mass) according to MC rate (mg/kg) and PAM treatment (% weight)

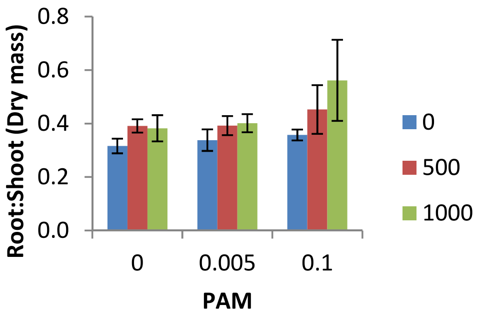

Figure 6: Harvest 2 root:shoot (dry mass) according to MC rate (mg/kg) and PAM treatment (% weight)

Error bars are ± 1 standard deviation (n=4).

5. Conclusion:

The results clearly show a decrease in plant height with the MC treatment of the seeds. This corresponds to the use of the chemical in the field, as well as with trials testing the suitability of seed treatments (Yeates, Constable and McCumstie 2005). Shoot biomass also followed a similar trend, although it was less significant.

It was found that PAM did not improve plant growth compared to the control soil. This impacted on the ability to test for responses in plant growth to sodic soil structural constraints. Possible reasons for the lack of improvement with PAM soil treatment may include an alteration in the soil-plant water relations, or an impact on the root-soil contact due to the coating of the aggregates with the polymers. A test to determine the air-dry and field capacity moisture contents of various PAM rates was run (data not shown). We found no effect of increasing PAM rate on field capacity. The lack of benefit from PAM may also have been due to the moderate sodicity level of the soil, which may not have produced significantly constraining growth conditions. Alternatively, the bulk density of the soil in the pot was very low (0.9 g/cm^3) compared to field conditions and may not have been adequate to restrict root growth.

Mepiquat chloride did not improve root growth. The increase in dry mass root:shoot with increasing MC rate is attributed to the reduced shoot growth, since root growth did not increase.

These results differ from others' results (Xu and Taylor 1992; Iqbal *et al.* 2005; Duan *et al.* 2007), who found that MC did increase root growth or alter root morphology. In each case, a non-structurally limiting growth medium (sand, sandy loam, and glass culture plates, respectively) was used. Due to the completely different root environment of the medium-clay Vertosol used in this experiment, the incongruous results may be attributable to soil type structural differences.

MC did not increase root growth in a sodic Vertosol soil, although the lack of an effective comparison soil treatment disallows confirmation of conclusions as to whether it is the sodic status of the soil that has produced this result in apparent contradiction to other studies. Further investigations are required to understand the responses of cotton roots to mepiquat chloride under different soil structural conditions, including sodic and non-sodic soils. It would also be informative to describe the soil conditions, if any, under which PAM treatment can improve plant growth.

6. Highlights:

- Mepiquat chloride did not increase root growth of cotton grown in pots of sodic vertosol soil.
- Increased root:shoot with MC rate was attributed to decreased shoot growth.
- Differences in root response to MC to that in other studies were attributed to the greater soil structural limitation in this study.

7. Presentations and public relations:

n/a

8. References:

Dodd, K., Guppy, C., Lockwood, P. & Rochester, I. 2010, The effect of sodicity on cotton: plant response to solutions containing high sodium concentrations, *Plant and Soil*, vol. 330, pp. 239-249.

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Iqbal, M., Nisar, N., Khan, R.S.A. & Hayat, K. 2005, Contribution of mepiquat chloride in drought tolerance in cotton seedlings, *Asian Journal of Plant Sciences*, vol. 4, no. 5, pp. 530-532.

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Reddy, A.R., Reddy, K.R. & Hodges, H.F. 1996, Mepiquat chloride (PIX)-induced changes in photosynthesis and growth of cotton, *Plant Growth Regulation*, vol. 20, pp. 179-183.

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Yadav, S., Irfan, M., Ahmad, A. & Hayat, S. 2011, Causes of salinity and plant manifestations to salt stress: A review, *Journal of Environmental Biology*, vol. 32, pp. 667-685.

Yeats, S.J., Constable, G.A. & McCumstie, T. 2005, 'Cotton growth and yield after seed treatment with mepiquat chloride in the tropical winter season', *Field Crops Research*, vol. 93, pp. 122-131.

9. Executive Summary:

(provide a one paragraph summary of the Summer Scholarship, suitable for posting on the Cotton CRC web site)

Sodic soils are a major constraint for much of Australia's cotton growing regions. These soils particularly reduce the establishment of cotton seedlings due to reduced water availability. In order to improve drought tolerance on these soils, seedlings need to have a quickly established root system. The Summer Scholarship project investigated whether mepiquat chloride, a commercially available plant growth regulator for cotton, can increase cotton seedling root growth in a sodic vertosol soil. A sodic vertosol with an exchangeable sodium percentage of 9% was treated with three rates of polyacrylamide; 0, 0.005, 0.1% soil weight; while cotton seeds were soaked with three treatment rates of mepiquat chloride; 0, 500, 1000 mg/kg. Two harvest groups were established in a glasshouse, with four replications of each treatment in both harvests. After 19 and 28 days, the pots were harvested and root and shoot measurements taken.

The results showed that PAM did not increase plant growth, so the treated soil could not be used to compare with the sodic soil. Plant height decreased with MC rate, as did shoot dry mass to a lesser extent. Root:shoot increased with MC rate. The increased root:shoot was therefore attributed to reduced shoot mass. MC did not increase root growth in a sodic vertosol soil, probably due to the structural constraints of the soil. Further research is required to describe the responses of cotton roots to plant growth regulators and their interactions with soil structural constraints.