Opportunities to reduce the impact of water-logging on cotton

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Abstract

Cotton is poorly adapted to water-logged conditions which can cause significant yield loss. Two potential contributors to yield loss under water-logged conditions are: (1) cotton's inability to form aerenchyma and so aerobic respiration cannot proceed in the roots and thus must rely on anaerobic fermentation, and (2) an expected increase the production of ethylene by the cotton plant which may contribute to increased fruit abscission. Field experiments were conducted in two seasons in which cotton was subjected to water-logging. We firstly assessed the performance of transgenic genotypes over-expressing Pyruvate decarboxylase (*Pdc*) and alcohol dehydrogenase (*Adh*) proteins. Increased expression of these proteins may increase alcohol fermentation enabling the plant to maintain ATP production thus providing some insurance against the effects of intermittent water-logging. Secondly, we applied a plant ethylene inhibitor (AVG, aminoethoxyvinylglycine) applied to the crop just prior to water-logging to reduce fruit abscission.

The use of AVG improved yields in water-logged conditions thus highlighting the potential role of ethylene in cotton water-logging. Although the over-expression of *Pdc* or *Adh* did not reduce the impact of water-logging compared with the control genotype (non-transgenic), the relative reduction in yield between the water-logged and non-water-logged treatments for each individual genotype was consistent with ethanol production found in other studies. This suggests that there may have been some response of genotypes to water-logging but too small in light of other impacts on crop growth associated with water-logging.

Key Words

cotton, water-logging, yield, hormones, ethylene, transgenic

Introduction

Water-logging is considered to be one of the major problems in global cotton production (Gillham et al. 1995) and cotton is known to be poorly adapted resulting in reduced lint yields (Bange et al. 2004, Hodgson 1982). In Australia, production of cotton is concentrated on vertosols which have low drainage rates. Combined with furrow irrigation and a summer dominant rainfall pattern, this results in a significant risk of intermittent water-logging. The problem can be exacerbated by poor soil structure, poor land forming (such as excessive field length, inadequate slope, poor leveling or bed formation), or by substantial rainfall after irrigation.

Cotton does not produce functional aerenchyma under water-logged conditions (Leonard and Pinckard 1936, Huck 1970), and aerobic respiration cannot proceed. The plants rely on anaerobic fermentation, leading to the production of ethanol which enables the plant to maintain ATP production albeit with a reduced energy yield. Two key enzymes in the alcoholic fermentation pathway are pyruvate decarboxylase (*Pdc*) and alcohol dehydrogenase (*Adh*) (Drew 1997). However, the endogenous levels of alcohol dehydrogenase in cotton are low (Dennis et al. 1992). It is therefore conceivable that increasing the levels of the enzymes for alcohol fermentation in cotton may provide some insurance against the effects of intermittent water-logging.

Ethylene induction in cotton plants is associated with a wide range of injuries and stresses (Christiansen 1986) and can result in increased rates of abscission of young fruit (squares and bolls) (Guinn 1976). However, its involvement with the impact of water-logging on cotton has not been explicitly researched. Aminoethoxyvinylglycine (AVG) is a known ethylene inhibitor (Jackson 1985) that has been used in horticulture crops and may reduce the effects of water-logging on fruit abscission in cotton.

In researching ways to reduce the effects of water-logging on cotton yield, in two seasons transgenic genotypes that over-express *Pdc* and *Adh* proteins were exposed to water-logging over the whole period of crop growth, and AVG was applied prior to a single water-logging event at peak-squaring in both seasons.

Methods

Experiments were conducted at Narrabri, Australia (30.31?S 149.78?E), in the 1999-2000 and 2000-2001 seasons. In each season AVG was evaluated on water-logged treatments (+WL) using cultivar Sicala V2*i* in experiments described in Bange et al. (2004). Briefly the plots were 5m in length with treatments (AVG rates) arranged in a RCBD with four replications. In the same seasons transgenic genotypes were evaluated using separate experiments using a split-plot design with four replicates. Main plots were non-water-logged control (–WL) and water-logged (+WL) and the genotypes were sub plots. Each plot was 7 m long and six rows wide. An additional five rows were included on either side of each –WL plot to provide a buffer against lateral water movement through the soil from the +WL plots. The irrigation and water-logging treatments for all experiments are described in Table 1. Water-logging was generated by extending the duration of irrigation events. Experiments were sown on 29 Sep. 1999 and on 29 Oct. 2000.

Table 1. Details of irrigation and water-logging treatments across field experiments in this study. (–WL, non-water-logged treatments; +WL, water-logged treatments). –WL irrigation treatments were applied on the same starting dates as the +WL treatments.

Irrigation No.	-WL (h) all years	1999/2000		2000/2001	
		DAS	+WL (h)	DAS	+WL (h)
1st	8	47	52	51	72
2nd	8	74	72	65	72
3rd	8	87	72	80	72
4th	8	101	72	112	72
5th	8	125	72	128	72

Four rates of AVG were applied using a hand sprayer the day before the second water-logging event which was at the peak squaring stage. Rates were 0, half (62.5 g a.i./ha), full (125g a.i./ha) and double (250 g a.i./ ha) the recommended rate used in horticultural crops of 125 g a.i./ha.

Transgenic cotton genotypes with increased levels of alcohol fermentation through the insertion of extra copies of the *Adh* and *Pdc* genes using Agrobacterium transformation were used (Ellis et al. 2000). Three genotypes (B1, TA, B4) were used with increased *Adh* activity and three other genotypes (A3, A4, A5)

were used with increased *Pdc* activity. In addition an antisense genotype (C5) was used to limit the activity of *Adh* and thereby the production of ethanol. The parent/control genotype was cv. Coker 315.

Seed cotton yield from the AVG treatments was handpicked from 2 m^2 per plot and total bolls were recorded. Seed cotton yield in the alcohol fermentation experiments was determined from 7 m^2 (machine picked) in the first season and 2 m^2 (handpicked) in the second season per plot.

Results

Following single applications of AVG, significant ($P < 0.05$) quadratic relationships were measured between the rate of application and (i) final boll number and (ii) seed cotton yield (Figure 1). In both seasons the 0 rate of AVG had the lowest yield, while the double rate had less yield than both the half and full rates of AVG.

In experiments evaluating transgenic genotypes, seed cotton yields were significantly reduced by water-logging. Across genotypes there was a 15% reduction in 1999/2000 and 32% in 2000/2001 (Table 2). There were no significant differences between genotypes in both seasons, and no treatment by genotype interactions.

Discussion

This is the first study of which we are aware that has assessed the use of AVG as an ethylene inhibitor to reduce the effects of water-logging in cotton. While the role of ethylene in water-logged cotton is not fully understood, Christianson et al. (2010) found changes in the level of expression of genes associated with ethylene in cotton after water-logging. These findings, along with results of this study, emphasise the potential role of ethylene in mediating responses to water-logging. Differences in the effect of AVG rate on seed cotton yield were reflected in changes in boll number which, in turn, may have been caused by differences in the level of fruit abscission resulting from changes in ethylene. Ethylene is known to be associated with stress in cotton and can result in increased rates of abscission of young fruit (squares and bolls) (Guinn 1976). Reductions in cotton yield by water-logging are strongly associated with reduced boll number (Bange et al. 2004, Hodgson 1982, Hodgson and Chan 1982). While growth analysis by Bange et al. (2004) showed that the reduction in boll number was commensurate with reductions in total crop dry weight and total fruiting site development, fruit abscission per se was not measured. They acknowledge along with Hodgson (1982) that differences in fruit abscission may have contributed to differences in final boll number. However, Conaty et al. (2008) studying genotypic differences in water-logging tolerance, found that fruit abscission did differ between genotypes following water-logging, supporting the idea that the difference in fruit number that we found were due to differences in abscission. The lower yield in the double rate of AVG was not unexpected as toxic effects of high levels of AVG have been reported elsewhere (Jackson 1985).

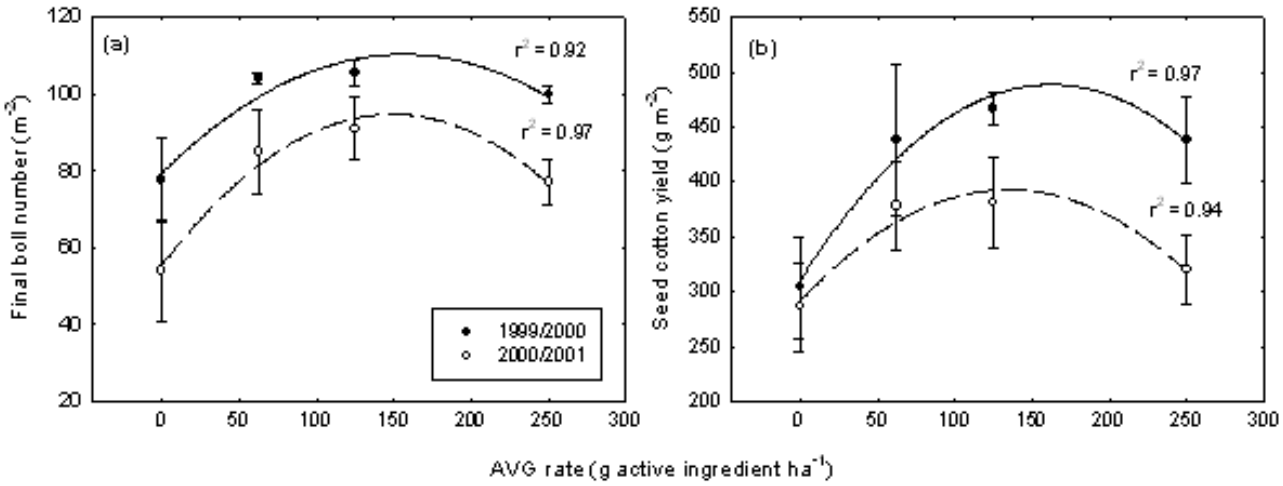

Figure 1. The effect of AVG rate on (a) final boll number and (b) seed cotton yield applied prior to water-logged treatments at the 2nd irrigation in both season (see Table 1). Error bars are two standard errors of the mean.

Table 2: Seed cotton yields (g/m^2) of transgenic genotypes and Coker control (CK). (–WL, non-water-logged treatments (TRT); +WL, water-logged). [a]Mean of the genotypes. [b]Standard error of the difference between the means. [c]Non-significant. * Significant at P < 0.05; ** Significant at P < 0.01

Season	+PdcA3	+Pdc A4	+Pdc A5	+Adh B1	+Adh B4	+Adh TA	-Adh C5	CK	Mean[a]	S.E.D.[b] TRT S.E.D. GEN
1999/2000	349	371	370	368	375	378	321	386	365	TRT 11*
-WL										GEN 29 n.s.[c]
+WL	317	344	266	316	313	323	275	340	312	
% Reduction	9	8	28	14	17	15	14	12	15	
2000/2001	283	290	304	287	322	278	298	299	296	TRT 9**
-WL										GEN 23 n.s.
+WL	211	219	196	232	192	203	148	194	196	

% Reduction	26	25	36	19	40	27	50	35	32

The use of transgenic genotypes that over-express *Pdc* and *Adh* proteins did not significantly improve seed cotton yield under water-logged conditions. This result were in contrast to Ellis et al. (2000) who demonstrated in controlled conditions when oxygen was limited to the roots that these genotypes differently (up to 80% increase) expressed ethanol compared to the control genotype Coker 315. While there may have been differences in the production of ethanol in the field, it is most likely that any effect associated with ethanol production resulting from the transgenics when considered in the context of the other impacts of water-logging may not have been great enough to generate adequate differences in genotype field performance. Waterlogging involves a complex interaction of factors affecting both the soil environment and plant growth. In companion studies conducted at the same time as these experiments plant nutritional differences (Bange et al. 2009) and direct impacts on growth (Bange et al. 2004) were identified as the main reasons for impacts on cotton yield.

Conclusion

The use of AVG in this study was able to reduce the effects of water-logging and suggests that ethylene production in the roots or shoots may play a significant role in water-logging in cotton. More research is needed to understand the physiological mechanisms of ethylene and AVG, as well as investigating in more detail the practicality of applying AVG as a commercial management practice when the risk of imminent water-logging is known.

In this study the use of transgenic genotypes did not significantly improve responses to water-logging. It highlighted that for this approach to be effective, it requires the degree of physiological response (e.g. expression of both *Adh* and *Pdc* proteins together) to be substantially greater in the face of other interacting effects associated with water-logging. Indeed, only when cotton resilience to water-logging problems associated with other plant, soil and nutritional issues (e.g. Na, N and Fe) are resolved, may the transgenic approach presented in this study offer some opportunity.

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