Cold-tolerance screening for cotton cultivars using germination chill protocols

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Abstract

Cotton (*Gossypium hirsutum*) is sensitive to cold conditions during germination and establishment. Identifying cultivars with cold tolerance may provide growers with increased flexibility for sowing date options and allow for better establishment under cool conditions, reducing the costs associated with possible replanting. This study utilised germination chill protocols, and investigated their applicability in determining genetic variation in seedling chilling tolerance. Seeds of ten cultivars (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) were germinated at four temperatures (14, 18, 22 and 30?C) and germination probability was determined after 4, 7 and 10 d. Ten seedlings were selected randomly and the length from the hook of the hypocotyl to the tip of the radicle (seedling length) was measured. An electrolyte leakage test was also conducted after seed imbibition in 5?C water for 24 h. These laboratory tests were correlated to an early planted (August) field experiment conducted 40 km west of Narromine, central west NSW. Seedling length provided a better indication of cold tolerance than germination probability. The best correlation with field emergence was with the Cool Warm Seedling Length (SWSL) test (an average of seedling length between cool temperature 14?C at Day 7 and warm temperature 30?C at Day 4) which provided an R^2 of 0.73. The electrolyte leakage test provided a negative correlation with field emergence (R^2 = 0.43). There was no relationship between the weight of 200 seeds and field emergence. The laboratory and field experiments indicated that there was genetic variation in the cotton cultivars tested, with Namcala, DP16, Sicot 75, Sicala 350B and Siokra V-18 showing some degree of cold tolerance. Further research is needed to test more cultivars and to validate these tests.

Key Words

Cotton, cold tolerance, germination, establishment, cold germination assays, electrolyte leakage

Introduction

Poor germination of cotton (*Gossypium hirsutum* L.) can limit yield potential either through delays in crop development or patchy stands causing non-uniform growth. Cotton is sensitive to cold during germination and establishment (Wanjura *et al.* 1969). To germinate and establish successfully, cotton needs soil temperatures of 14**?**C or greater for three consecutive days (Constable and Shaw 1988). Chilling injury can occur in cotton seedlings when the temperature drops below 15 to 20**?**C during germination (Cole and Wheeler 1974). Prolonged exposure to temperatures below 15**?**C will slow seed metabolic activity and make the seed susceptible to plant pathogens and other stresses when soil temperatures start to rise (Buxton and Sprenger 1976). Identifying cultivars with chilling tolerance will allow cultivars to withstand the cold shocks during early cotton growth (15th September to 30th November in south-eastern Australia), allowing increased flexibility for sowing date options and allow for better establishment under cool conditions, reducing the costs associated with possible replanting. While some research has been conducted on American cultivars (Duesterhaus 2000; Schulze *et al.* 1996), few germination chill experiments have been carried out on modern Australian cultivars to assess their potential for cold tolerance. This study utilised germination chill protocols, and investigated their applicability in determining genetic variation in seedling chilling tolerance.

Methods

The experiments consisted of laboratory germination experiments with ten cotton cultivars (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) at the Australian Cotton Research Institute in Narrabri and a field experiment in Narromine.

Laboratory experiments

The germination protocol used four replicates of 50 seeds placed on two sheets of wet paper towel with another sheet placed on top and rolled (Duesterhaus 2000). The rolled sheets were placed in covered plastic containers and germinated at four temperatures 14?C, 18?C (hereafter referred to as cool germination tests), 22?C and 30?C (hereafter referred to as warm germination tests). Seedlings with a radicle length of 3 mm or longer were counted as germinated (Wanjura and Buxton 1972) and germination counts were made at Day 4, 7 and 10. In addition, ten seedlings were selected randomly and the length from the hook of the hypocotyl to the tip of the radicle was measured (hereafter referred to as seedling length). A combination of the warm and cool germination tests was reported to be a reliable indicator of field performance. For the Cool-Warm Vigour Index (CWVI) (Equation 1), also known as the "Texas Cool Test", the germination probabilities of the cool (14?C, or 18?C) and warm (22?C, or 30?C) germination tests are added together and divided by two for each cultivar (Bird and Reyes 1967):

$$Cool\ Warm\ Vigour\ Index = \frac{Cool\ Temperature + Warm\ Temperature}{2} \quad (1)$$

For the Cool Warm Seedling Length (CWSL) (Equation 2), the cool temperature seedling length (14?C, or18?C) is added to the warm temperature seedling length (22?C, or 30?C) and divided by two:

$$Cool\ Warm\ Seedling\ Length = \frac{\begin{matrix}Cool\ Temperature\\ Seedling\ Length\end{matrix} + \begin{matrix}Warm\ Temperature\\ Seedling\ Length\end{matrix}}{2} \quad (2)$$

The weight of 200 seeds was determined to see if seed weight was related to chilling tolerance. An electrolyte leakage test was also conducted during seed imbibition as an indicator of cellular damage during chilling and a lack of cold tolerance. Fifty seeds from each cultivar were washed with 30 mL deionised water twice. The seeds were placed in 30 mL of water and allowed to imbibe for 24 h at 5?C (Schulze *et al.* 1996). After 24 h, electrical conductivity of water from the tube was measured at room temperature (20?C).

Field experiment

The laboratory assays were correlated to a field experiment sown on 23 August 2008 (two months earlier than normal sowing) in a Grey Vertosol cotton field, 40 km west of Narromine (32?13'55'' S, 148?14'23'' E). The ten cultivars were sown in a randomised complete block design with four replicates. Fifty seeds of each cultivar were planted in a 5 m row and emergence counts were taken 14, 21, 28 and 35 days after planting until there was no change in emergence. Soil temperature at 10 cm depth was recorded at 9 am daily at Trangie Research Station located 40 km north of the experimental site (32?01'55'' S, 147?59'02'' E). Soil temperature was below 14?C for the first 19 days after sowing. Final field emergence was counted at 28 days after sowing (hereafter referred to as field emergence). Field emergence and laboratory germination counts were analysed using binary logistic regression (Genstat 10th Ed, VSN International Ltd). Data on field emergence and laboratory germination were presented as emergence or germination probabilities, respectively. Seedling length, electrolyte leakage and seed weight were analysed using analysis of variance (ANOVA). Relationships between laboratory assays and field emergence were analysed using regression analysis.

Results and discussion

Germination probability in warm germination tests was >0.9 in all cultivars tested which suggests that viability and quality of seed was generally good. Germination probability in the warm and cool germination tests alone did not have any relationship (*P*>0.05) with field emergence (data not shown) in this study, which also agrees with other studies (Bolek 2006; Schulze *et al.* 1996). There were also no relationships (*P*>0.05) between the CWVI, and field emergence in this study with any temperature combination (see Table 1). Other studies have reported that the CWVI was a good predictor of field stand establishment (R^2 = 0.8) when there were warm planting conditions, while cool planting conditions resulted in no correlation (R^2 = 0.22) as was the case in this study (Duesterhaus 2000). There was no relationship between the weight of 200 seeds and field emergence (data not shown) which agrees other studies (Hopper *et al.* 1994).

The coefficient of determination (R^2) for the CWSL and field emergence was higher at 0.73 at cool germination temperature of 14?C, and only 0.53 at 18?C. Hence, the CWSL at a cool temperature of 14?C on Day 7 and warm temperature of 30?C on Day 4 provided the best predictor of field emergence in this study (Figure 1a). Similarly, seedling height of lucerne (*Medicago sativa*) seedlings measured in a laboratory at suboptimal temperatures was a better trait to predict field emergence and height than germination time under cool field conditions (<15?C soil temperature at 10 cm) (Klos and Brummer 2000). There was also a negative linear relationship (*P*<0.05) between electrical conductivity after seed imbibition at 5?C and field emergence (Figure 1b) which agrees with other work on cotton and soybean (Schulze *et al.* 1996; Yaklich *et al.* 1979). CWSL and to a lesser extent, electrolyte leakage tests predicted low field emergence in Pima A-8 and Sicot 289RR and relatively higher field emergence in Namcala, DP16, Sicot 75, Siokra V-18 and Sicala 350B (>38 mm CWSL) under cold (<14?C) soil temperatures (Figures 1a and 1b). Sicot 289RR was bred for a tropical climate, and hence, has low chilling tolerance. Chill sensitivity has also been reported in many Pima cultivars (Bolek 2006; Buxton *et al.* 1976). Namcala, with a CWSL > 40 mm, has good chilling tolerance as it was bred in an arid climate in Arizona, USA that experiences extreme hot and cold temperatures. Hence, there is potential for developing these laboratory assays further to screen for chilling tolerance in cultivars and breeding lines in a cotton breeding program. Further research is needed to test more cultivars and to validate these tests.

Table 1. Relationship between field emergence at Day 28 and various combinations of CWVI and CWSL laboratory assays. Combinations with warm germination data at 22?C were not included due to non-significant *P* values for both combinations. * represents *P*<0.05, ** represents *P*<0.01, * represents *P*<0.001 and n.s. represents not significant at *P*=0.05**.

Cool		Warm		CWVI		CWSL	
Day	Temperature	Day	Temperature	R^2	*P* value	R^2	*P* value
4	14	4	30	0.2661	n.s.	0.6326	**
4	14	7	30	0.2873	n.s.	0.2467	n.s.
4	14	10	30	0.2923	n.s.	0.1767	n.s.
7	14	4	30	0.0221	n.s.	0.7293	**
7	14	7	30	0.0497	n.s.	0.3371	n.s.

7	14	10	30	0.0381	n.s.	0.2473	n.s.
10	14	4	30	0.1031	n.s.	0.6175	**
10	14	7	30	0.1402	n.s.	0.3669	n.s.
10	14	10	30	0.1374	n.s.	0.2687	n.s.
4	18	4	30	0.0322	n.s.	0.5741	*
4	18	7	30	0.0161	n.s.	0.3626	n.s.
4	18	10	30	0.0344	n.s.	0.2535	n.s.
7	18	4	30	0.0718	n.s.	0.5283	*
7	18	7	30	0.1229	n.s.	0.5494	*
7	18	10	30	0.1194	n.s.	0.4291	*
10	18	4	30	0.1413	n.s.	0.4684	*
10	18	7	30	0.1991	n.s.	0.5305	*
10	18	10	30	0.2133	n.s.	0.5352	*

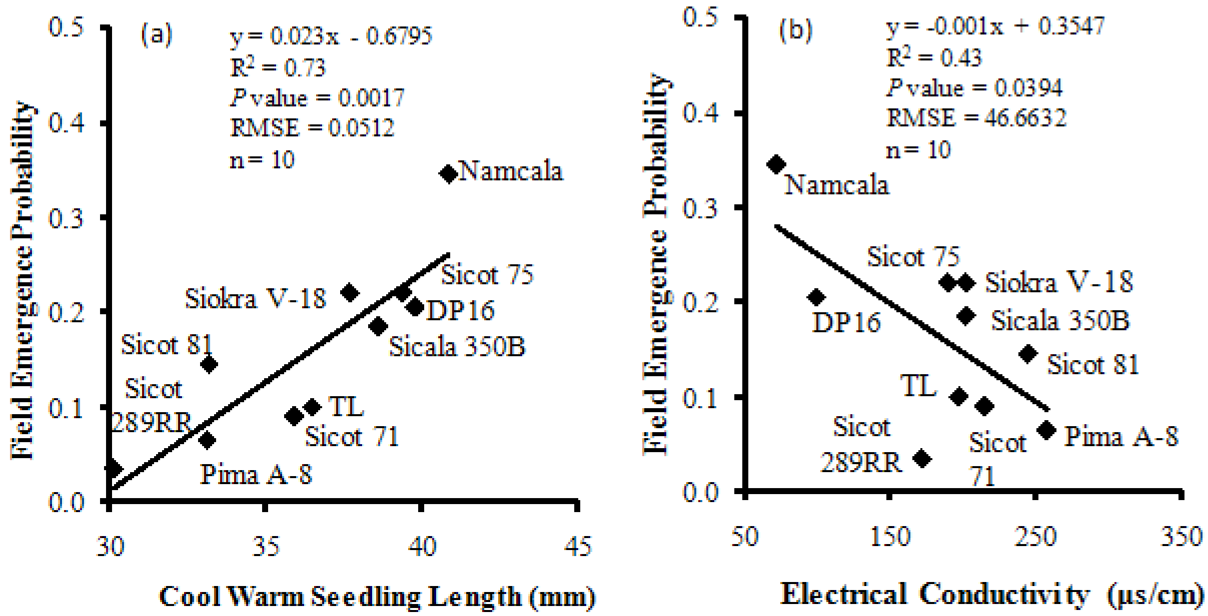

Figure 1. Relationship between field emergence probability at Day 28 and (a) CWSL (average of warm seedling length at 30?C on Day 4 and cool seedling length at 14?C on Day 7) and (b) electrical conductivity after imbibition of seed at 5?C for 24 h.

Conclusions

The CWSL (cool temperature of 14?C on Day 7 and warm temperature of 30?C on Day 4) and electrolyte leakage tests provided a better prediction of field emergence on Day 28 under cool (<14?C) soil conditions than the traditional CWVI based on germination probability.

Acknowledgements

Partial financial support by Cotton Research and Development Corporation and the Cotton Catchment Communities CRC is gratefully acknowledged and the assistance of technical support of CSIRO at ACRI.

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