IMPROVING DROUGHT TOLERANCE OF COTTON BY GLYCINEBETAINE APPLICATION AND SELECTION

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Abstract

Plants accumulate a variety of organic osmoprotectant solutes through a biochemical mechanism which improves their ability to withstand stresses. Of these solutes, betaines (fully N-methylated amino acids) appear to play a major role in conferring resistance to drought, salinity and temperature stresses. Stress tolerance research involving this biochemical mechanism has two approaches: ?1) External application of betaine: Cotton seed treatment with glycinebetaine (patented by CSIRO Australia) at 5 and 7.5 % increased seed cotton yield by 18 to 22%, respectively. 2) Breeding and/or genetic engineering: Cotton cultivars bred for rain fed environments accumulated significantly more glycinebetaine than cultivars adapted for irrigated cultivation. Selection for higher glycinebetaine has the potential to speed up breeding for drought tolerance.

Key words: *Water stress, drought, betaine, seed treatment, foliar application, selection.*

Water stress and salinity often limit seedling vigour and yield of crops, including cotton. These two stresses will become more significant because second-ary salinisation is increasing and more cotton is being grown under rain-fed conditions in Australia. It has been well established that plants accumulate a variety of osmo protectant solutes as an adaptive mechanism to environ-mental stresses such as salinity (4), water deficit (10) and temperature extremes (4). Osmoprotectant solutes include (A) sugars and sugar alcohols (polyols) (11), (B) proline (1), and (C) a number of quaternary ammonium compounds (betaines) and tertiary sulphonium com-pounds (10).

This basic research has been recently used in two ways to increase plant tolerance to stress: Firstly, external application as seed (6) and/or foliar treatments (2, B. Naidu, unpublished) of glycinebetaine under mild field stress conditions, resulting in yield increases of 10 to 50% of a variety of crops and pastures. Secondly, developing plants with higher levels of natural solute-accumulating capacity to increase stress tolerance by selection between cultivars or isogenic lines and/or by genetic engineering of plants to accumulate high levels of polyols (11), proline (5), and glycinebetaine (4).

In this paper we describe results obtained by using these two approaches on cotton.

Materials and methods

Field experiments with glycinebetaine treated seed and foliar application

Field experiments were conducted under rain fed conditions at three locations near Dalby (Qld) in October 1995 to test the effect of seed treatment of cotton (cv. Sicala V-2) and to determine whether the crop responds to further treatment with glycinebetaine by foliar applications.

Seed treatment

Methyl cellulose solution was used as a sticker to hold glycinebetaine on the seed surface. Five g of methyl cellulose powder was dissolv-ed in 100 ml of boiling water. After cooling and storing at 4^{o}C overnight, 50 g of this methyl cellulose solution was sprayed on to 1 kg of cotton seed while seed was being rotated in a long beaker. Mixing continued until a uniform coating was obtained and the layer dried to become sticky and slightly moist. Finely ground glycinebetaine was sprinkled at 0.0, 2.5, 5.0, and 7.5 % (w/w) on the methyl cellulose-coated seed. Glycine-betaine is highly hygro-scopic and, in order to dry the seed surface, 50 g of dried and powdered peat/kg of betaine-coated seed was also applied. One set of plots received only the seed treatment and no foliar applications. The second set received one glycinebetaine foliar application two weeks after emergence at 2 kg/ha in addition to the seed treatments. The third set received two foliar applications at 2 weeks after emergence and at square (flower bud) formation, each application at 2 kg/ha in addition to the seed treatments. Each plot was 10m long and consisted of 4 rows, with a seeding rate of 15 kg/ha.. The 4 levels of seed treatment, 3 foliar treatments were arranged in a randomised complete block design with 4 replications.?The middle two rows were mechanically harvested in April, 1996.

Water stress under controlled conditions and betaine accumulation

Nine cotton cultivars were subjected to water deficit to study cultivar differences for glycinebetaine accumulation. The cultivars were Siokra L-23, Siokra 1-4, Siokra S-101, Siokra V-16, Sicot 189, CS 50, Tamcot HQ95, Tamcot Sphinx, and Cascot 014 (Fig. 2). The first 6 cultivars were released by CSIRO, mainly for irrigated farming and the last 3 cultivars were released for drought affected conditions in Texas (USA). The experiment was conducted in a controlled environment with a temperature regime of $30/25^{o}C$, 14 h photo period, relative humidity of 60/95%, and a photon flux density of 500 mE m^{2}.

Five cotton seedlings were grown in each mini lysimeter (45 cm deep and 10 cm diameter) filled with 11 kg of soil. Each cultivar was replicated 5 times. Soil moisture was maintained at field capacity until the 3rd leaf stage and water was withheld thereafter. The lysimeters were weighed on alternate days to monitor soil moisture usage. One youngest fully expanded leaf was sampled from each plant at field capacity (control) and when soil moisture reached 5 % (water stressed). ?Leaves were cut longitudinally and one half were used for the measurement of relative water content (RWC). ?The other half was used for the measurement of glycinebetaine by high performance liquid chromato-graphy (7).

Results

Cotton seed treatment and yield

In one of the three rainfed field experiments seed cotton yield increased over control by 18 and 22% in response to 5.0 and 7.5% betaine seed treatment, respectively (Fig. 1). Foliar applications did not increase yield when seed was not treated with glycinebetaine. However, foliar application in combination with 2.5% betaine seed treatment was beneficial. Foliar application for seedlings that were treated with 7.5% betaine showed an adverse response which was greater when two foliar doses were applied (Fig 1).

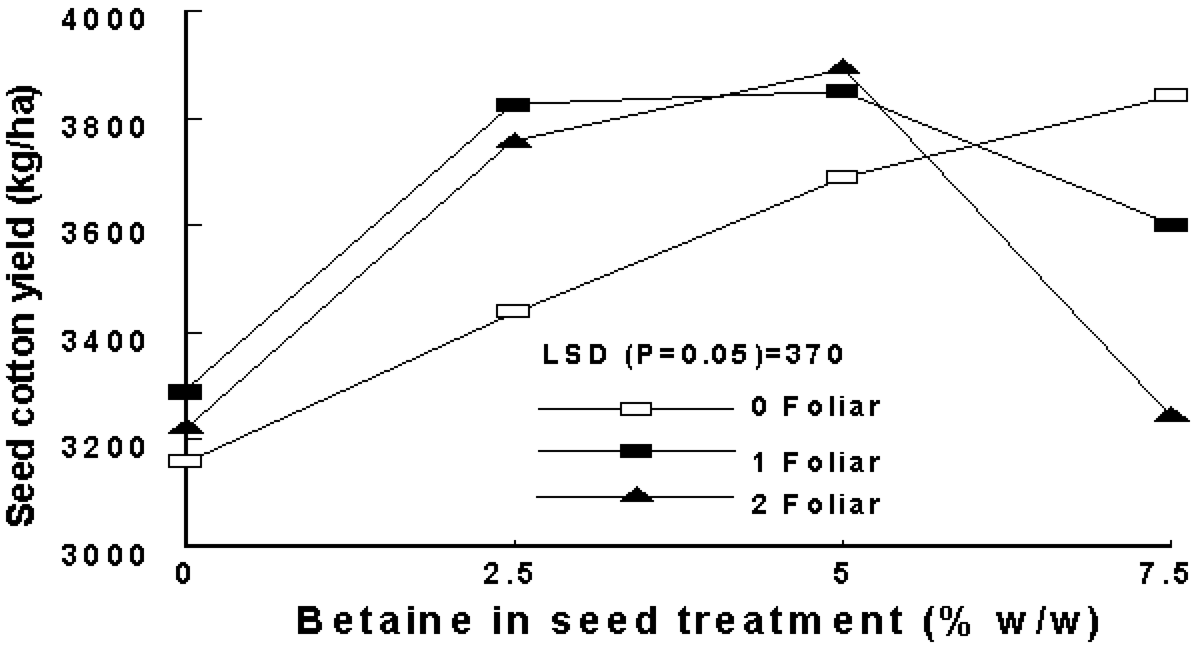

Figure 1

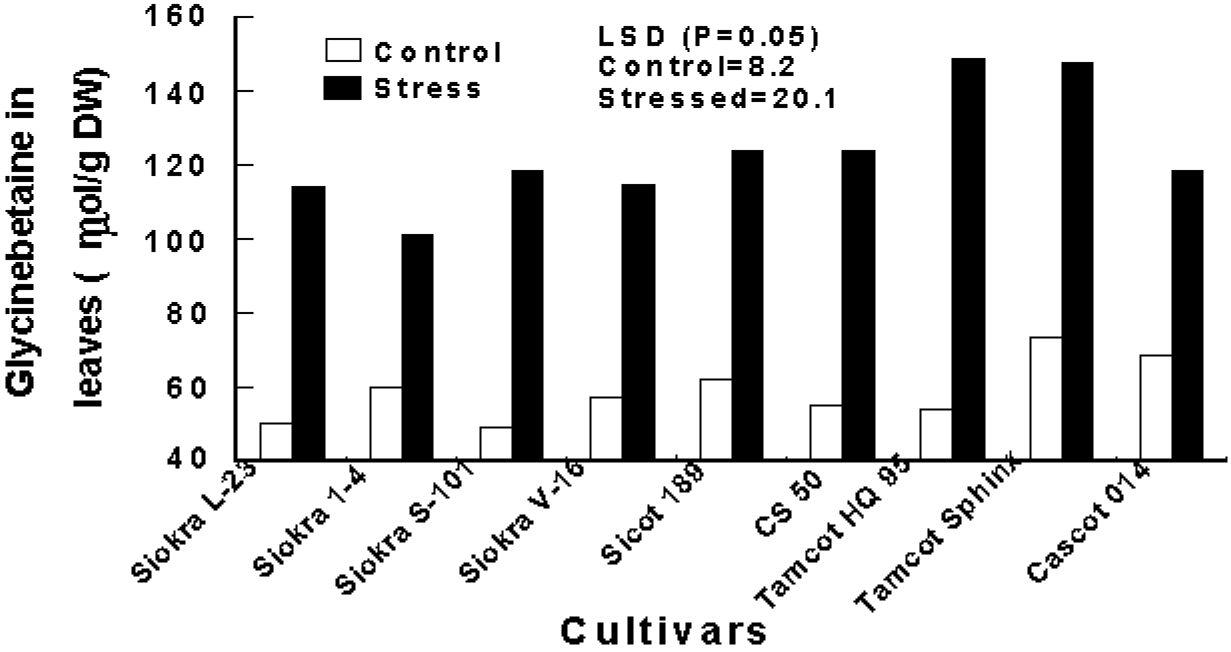

Figure 2

Cotton yield at the two other locations was not significantly affected by glycinebetaine seed treatment or foliar application. These two sites received more rainfall and were flooded for about 3 weeks compared to the site where yield responses to glycinebetaine were recorded.

Genotypic differences for glycinebetaine accumulation

Leaf RWC declined from about 90% at field capacity (control) to about 70% by the time most of the available soil moisture was utilised (data not presented). Glycine-betaine content differed significantly among cultivars at field capacity, being the highest in Tamscot Sphinx and the least in Siokra S101 (Fig. 2). Water deficit resulted in a significant increase in glycinebetaine content in all cultivars compared to their levels at field capacity. Here also, a significant variation was recorded for glycinebetaine level among cultivars (Fig. 2). When water stressed, the highest level of glycinebetaine was record-ed in two Texas cultivars: Tamcot HQ 95 and Tamcot Sphinx, bred for rainfed and water stress situations. CSIRO cultivars also showed a marked increase in glycinebetaine content in response to water deficit, but did not attain the high levels shown by Tamcot HQ 95 and Tamcot Sphinx.

Discussion and conclusions

This paper demonstrates that a fundamental biochemical mechanism such as glycinebetaine accumulation could be used as the basis for an agronomic treatment to increase the yield of cotton (Fig. 1). It also demon-strates, for the first time, that the cotton plant has the natural ability to accumulate glycinebetaine and that genotypic differences exists for this character (Fig. 2).

The benefits and stress-alleviating effects of glycine-betaine have been demonstrated under laboratory conditions, often on isolated enzymes (9). On the basis of the beneficial concentrations, the quantity of glycine-betaine required made it uneconomical to use on the field scale. However, recent work in controlled environment (6) and field experiments (2, 8) revealed that, in fact, whole plants growing in the field require only 1 to 5 kg/ha of glycinebetaine to provide stress tolerance or yield benefits. In the present experiment, the best option for utilising glycinebetaine was by seed treatment at 7.5% which requires 1.1 kg/ha. With a betaine cost of $20-25/kg and assuming there is little or no added cost for seed treatment, the net benefit of betaine treatment would be as high as $580/ha. A similar yield increase could also be obtained by glycinebetaine seed treatment with 2.5 or 5.0 % followed by a foliar application, but the glycinebetaine requirement in this case would be as high as 2.4 kg/ha.

Soil application of betaine increased germination and seedling vigour of cotton and wheat in pot experiments under saline conditions (6, 8). The findings from our field evaluations with cotton suggest a possibility of increasing cotton productivity under field conditions. ?Under rain fed conditions cotton yield increase in response to seed treatment was up to 22% over an untreated control (Fig. 1). In this situation, the yield increase may have come from increased water use efficiency (6) or photosynthetic efficiency. It was noted in the present study that cotton seed treatment resulted in stronger stems and roots, improved branching, earlier flowering, and a greater number of squares or bolls (data not presented). These responses suggest a hormone-like activity for glycinebetaine and similar effects have been noted in grapes (B. P. Naidu, unpublished). Glycinebetaine has been postulated to act as a non-toxic cytoplasmic osmoticum and protect enzymes and membranes from the debilitating ionic and dehydrating effects of salt (9). At least one of these two protective roles of glycinebetaine must have helped the seed-treated cotton to achieve higher yield under rain fed conditions. Caution must be taken not to apply higher than recommend-ed dose of glycinebetaine which may cause an yield depression (Fig. 1). Yield depression may have been due to osmotic and/or hormonal imbalances in the plants.

In addition to external applications of glycinebetaine, genetic variation in natural accumulating ability could also be used in plant improvement research. The level of glycinebetaine accumulation in cotton is comparable to that in high betaine accumulating species such as spinach and barley (10). Studies in a range of plant species have shown considerable genetic variation in the content of glycinebetaine and associated tolerance to environmental stresses (10). It is noteworthy that two of the Texas cultivars, which are adapted to water stress conditions (3, G. Constable, pers. comm.) accumulated higher glycinebetaine than the other cultivars.

It was confirmed here and in an another study (7) that glycinebetaine is the predominant osmoprotectant. ?Further work is essential to determine whether higher glycinebetaine accumulating ability could be used as a reliable index of stress tolerance in breeding programs as has been shown in barley, wheat, and maize. It may be possible to select, breed, or genetically engineer cultivars for higher glycinebetaine content (combined with other available mechanisms of stress tolerance such as transpiration efficiency) to improve crop performance in saline, and dry land conditions.

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