

Treatment with Glycinebetaine to Increase Seed Germination, Seedling Vigour and Yield of Cotton*

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Abstract: Glycinebetaine is a compound that some plants accumulate as a defence against stress conditions. In this paper we demonstrate that the application of glycinebetaine to cotton seed increased plant yield. Seed germination and seedling vigour in a saline soil were increased by 64 and 68%, respectively by seed treatment with glycinebetaine @ 5% (w/w). Cotton yield was increased by 18 to 22% by the seed treatment @ 5 and 7.5 % , respectively. Additional foliar application of glycine betaine was beneficial only at low levels of seed treatment.

Introduction: Water stress and salinity limit seedling vigour and yield potential of many crops, including cotton. These two stresses will become more significant as secondary salinisation increases and more cotton is grown under rain-fed conditions in Australia. Plants accumulate a variety of low molecular weight solute osmoprotectants in a biochemically adaptive mechanism which enables them to withstand stress. Of these solutes, **betaines** (fully N-methyl amino acids) have a major role in increasing tolerance to drought, salinity and cold stress (Wyn Jones and Storey, 1981; Zao *et al.* 1992; Naidu *et al.* 1996).

Stress tolerance research at our Division involving this biochemical mechanism has two approaches:

(a) External application of betaine: Foliar application of betaine to field grown plants has resulted in yield increases of 10 to 50% in a variety of crop and pasture plants (B. P. Naidu, unpublished data; Naidu *et al.* 1992; Campbell *et al.* 1996).

(b) Developing plants with high levels of natural betaine-accumulating capacity to increase tolerance to stress: Plants with a greater ability to accumulate glycinebetaine are more tolerant of stress than those with a lower ability (Grumet and Hanson 1986; Yang *et al.* 1995). There have been recent reports of genetic engineering of tobacco for glycinebetaine accumulation with the transgenic plants showing greater ability to withstand stress (eg. Rathinasabapathi *et al.* 1994).

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In this paper we report on external application of betaine for the improvement of cotton seed germination and seedling vigour in a saline soil. We have also shown that seed treatment with glycinebetaine increased cotton yield in a non-saline situation.

2. Materials and Methods:

2.1 Seed treatment: A 5% solution of methyl cellulose was used as a sticker to hold glycinebetaine on the seed surface. Fifty grams of methyl cellulose solution was sprayed on to cotton seed while seed was being rotated in a long beaker. Mixing continued until a uniform coating had dried to a sticky and slightly moist layer. Finely ground glycinebetaine was then added @ 0.0, 2.5, 5.0, and 7.5 % (w/w). Since glycinebetaine is highly hygroscopic, 50 g of dried and powdered peat/kg of betaine coated seed was also applied in order to dry the seed surface.

2.2 Field germination of cotton: A salinity-affected soil [EC(saturation) 14 mScm⁻¹] near Dalby in Queensland (c/o Mr. Greg McVeigh) was selected to test the effect of glycinebetaine seed treatment. Cotton (cv. Siokra L23) seed, treated with glycinebetaine @ 0, 2.5, 5.0, and 7.5% w/w, was sown in soil with moisture content of about 15%. Fifty seeds from each treatment were sown in a plot of 2 x 1m at a depth of 5cm. All treatments were replicated 4 times in a completely randomised block design (RBD). Seedling emergence was assessed 2 weeks after planting. At the same time, all seedlings were cut at the collar region and dried in an air-forced oven at 60° C for 24 h before dry weights were recorded.

2.3 Field experiments with glycinebetaine treated seed and foliar application: An experiment in a non-saline soil near Dalby (c/o Mr. Rob Town) tested the effect of seed treatment and crop response of cotton to foliar applications of glycinebetaine. The crop was rain fed. Cotton (cv. V2) seed was treated with glycinebetaine @ 0, 2.5, 5.0, and 7.5% (W/W) as above. One set of treatments received only seed treatment and no foliar applications. The second set received a glycinebetaine foliar application of 2kg/ha two weeks after emergence. The third set received a foliar application of 2 kg/ha at 2 weeks after emergence and again at square formation. Each plot was 10m long and consisted of 4 rows. The experiment was laid out in an RBD with 4 replications. The middle two rows were mechanically harvested 6 months after sowing to determine yield of cotton.

3 Results: When cotton seed with a germination potential of about 90% was sown in saline field conditions, only 50% of the seeds germinated (Table 1). Glycinebetaine treated seed @ 2.5 or 5.0% germinated more than 80%. However, higher betaine concentration was not beneficial with germination similar to that of the control.

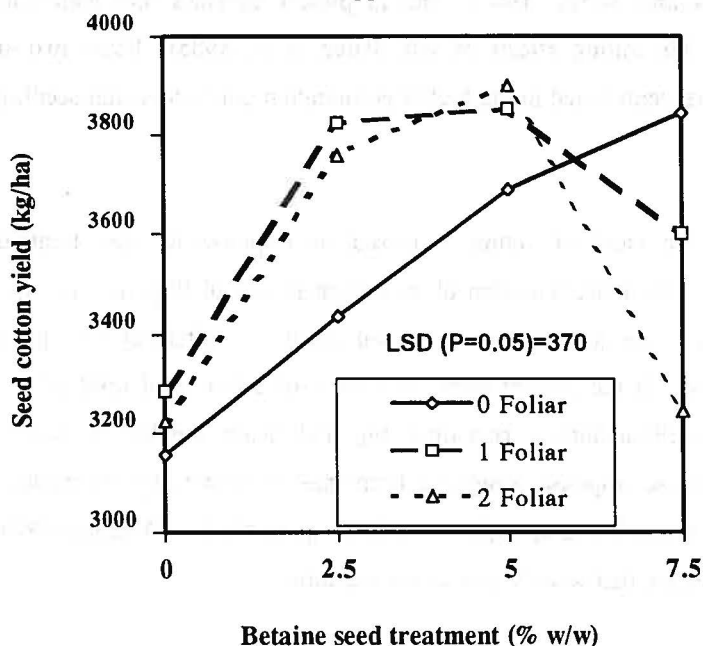
Table 1: Germination percentage and dry matter yield (g/seedling) as a result of glycinebetaine seed treatment of cotton (cv. Siokra L23) in a saline soil [EC(saturation) 14 mScm⁻¹]

Seed treatment level (% glycinebetaine, w/w)	Germination (percentage)	Seedling DM yield (g/plant)
0.0	50	0.25
2.5	75	0.33
5.0	82	0.42
7.5	55	0.24
Least Significant Difference (P=0.05)	12	0.06

The dry weight of seedlings treated with betaine @ 5% was the highest and it was 68% greater than the seedlings grown from untreated seed (Table 1). As with germination, 7.5% betaine seed treatment showed no increase in dry matter compared to control.

In the field experiment at the non-saline site there was a significant increase in seed-cotton yield (Fig. 1) in response to 5 and 7.5% betaine seed treatment. The yield increase over control was 18 and 22 % in response to 5.0 and 7.5% betaine seed treatment, respectively. Foliar applications

Fig. 1: Seed-Cotton (cv. V2) yield as influenced by betaine seed treatment and foliar applications in a non-saline soil



did not increase yield when seeds had not been treated with glycinebetaine. However, foliar application in combination with 2.5 or 5.0% 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.

4 Discussion & Conclusions: This paper demonstrates for the first time that addition of small quantities of glycinebetaine could be used as the basis for an agronomic treatment to increase germination, seedling vigour and yield of cotton. The benefits and stress-alleviating effects of glycinebetaine have been demonstrated previously under laboratory conditions on isolated enzymes (Paleg *et al.* 1985) or on whole plants in short duration experiments (Zao *et al.* 1992). These experiments required a very high concentration of glycinebetaine to provide protection against stress effects. When an agronomic use was considered, the quantity of glycinebetaine required, based on the early experiments, suggested it would be uneconomical to use on the field scale. However, recent work in controlled environment (B. P. Naidu, unpublished) and field experiments (Naidu *et al.* 1992; Campbell *et al.* 1996) 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.

Application of betaine to the soil increased germination and seedling vigour of cotton and wheat in pot experiments under saline conditions (Naidu, 1995). The findings from our field evaluations with cotton suggest considerable potential for increasing cotton productivity in saline and non-saline conditions. In a saline soil, germination percentage and seedling dry matter production increased by 64 and 68%, respectively, in response to 5% seed treatment using glycinebetaine (Table 1). Glycinebetaine has been postulated to act as a non-toxic cytoplasmic osmoticum (Wyn Jones and Storey, 1981) and to protect enzymes and membranes from debilitating ionic and dehydrating effects of salt (Paleg *et al.* 1985). These two suggested protective roles may have contributed to the higher germination percentage and seedling vigour (Table 1).

In a non-saline soil, the yield of cotton increased in response to seed treatment with glycinebetaine by up to 22% more than that of an untreated control (Fig. 1). In this situation yield increase may have been the result of increased water use efficiency (Naidu, 1995) or photosynthetic efficiency. In the present study treatment of cotton seed resulted in stronger stems and roots, improved branching, earlier flowering, and 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). Wheeler (1973) in fact suggested that glycinebetaine had activity similar to cytokinins.

In the present experiment, the amount of glycinebetaine required to obtain significant yield responses was in the range 0.75 - 1 kg/ha. Depending on the commercial cost of betaine and costs of seed treatment, the gross benefit of betaine treatment could be as high as \$580/ha.

While agronomic applications of glycinebetaine are exciting, genetic variation in natural accumulating ability could be used in plant improvement research. Cotton is a natural accumulator of glycinebetaine. It may be possible to select, breed, or genetically engineer cultivars for higher glycinebetaine content to increase crop performance in saline, dry land conditions, and also to increase the water use efficiency of irrigated cotton.

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