Glycinebetaine Treatment Improves Cotton Yields in Field Trials in Pakistan

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ABSTRACT

This study was designed to test the effectiveness of glycinebetaine (an osmoprotectant purified from sugar beet and applied as a spray of 'Greenstim') in improving the yields of cotton under normal cultivation conditions in the Punjab of Pakistan. Field trials were conducted in replicated small plots at two sites over two years to determine the optimal timing and dose of glycinebetaine. CIM-240 was sown in 1996 at the Postgraduate Agricultural Research Station (PARS), Faisalabad but was badly affected by whitefly and cotton leaf curl virus. CIM-443 was sown in 1996 and 1997 at the Central Cotton Research Institute (CCRI), Multan. This was resistant to leaf curl virus, and was sown at PARS in 1997. Generally, glycinebetaine concentrations and time of application had little effect on physiological parameters. In 1996, glycinebetaine concentrations were significantly increased in the trial at Faisalabad, but not at Multan. In 1996 the highest yield of seed cotton at Faisalabad (136 % of the control) was obtained with 3-kg/ha glycinebetaine applied at squaring. At Multan, seed cotton yields were highest with 3 and 6 kg/ha glycinebetaine applied at squaring. Glycinebetaine dose and time of application had highly significant (P<0.001) effects on yield, total dry matter and bolls per plant. Glycinebetaine-treated plants also had more nodes at maturity than the controls. Mean boll weight, % flower and boll shedding were not affected. In 1997 the highest yields were obtained when glycinebetaine was applied at first flowering at PARS, but at the pre-squaring stage at CCRI.

Introduction

Glycinebetaine is a quaternary ammonium dipole that is compatible with cytoplasmic enzyme activity and has some osmo-protectant properties (Gorham, 1995). It is a bye-product of the sugar beet refining industry and its exogenous application has been studied in a variety of crops (Agboma, 1997). Cotton naturally contains quite high concentrations of glycinebetaine, up to 100 mol m^{-3} in sap (Gorham, 1996). We report here on yield increases obtained by spraying glycinebetaine on field-grown cotton in Pakistan.

Materials and Methods

Field experiments in 1996 and 1997 were performed at two sites in the main cotton-growing area of Pakistan, the Postgraduate Agricultural Research Station (PARS) of the University of Agriculture, Faisalabad (Latitude 31.25º N, Longitude 73.6º E, Altitude 185 m), and the Central Cotton Research Institute (CCRI), Multan (Latitude 30.1ºN, Longitude 71.29º E). The cotton varieties used were CIM-240 (Faisalabad 1996), and CIM-443, both developed at CCRI. CIM-240 was sensitive to cotton leaf curl virus and was replaced by CIM-443 in 1997. Sowings were made in late May or early June in completely randomized small plots ranging from 15 x 10 feet to 30 x 12.5 feet. Treatments consisted of factorial combinations of glycinebetaine ('Greenstim' in Europe) doses and application times (Table 1).

In 1997 the main experiment at PARS (Faisalabad) suffered from poor establishment because of unfavourable conditions after sowing, and a smaller trial consisting of a control and 3-kg/ha glycinebetaine at three application times was resown. Spraying was by hand at low wind speeds in volumes equivalent to 200 litres/ha. At CCRI cotton seed oil was added to the spray to decrease drying times. Agronomic practices (fertilizer, irrigation, pest control) were normal for the area.

Leaf samples were taken from the plants one week after spraying, and again in September or October. These samples were frozen in 1.5 ml microcentrifuge tubes and taken to Bangor for analysis of inorganic ions (by ion chromatography) and glycinebetaine (by periodide or reineckate precipitation, or by HPLC) (Gorham, 1996).

In September/October a number of physiological measurements were made in the field, including rates of photosynthesis and transpiration (by infra-red gas analysis), stomatal conductance (by porometry), leaf temperature (infra-red thermometer), chlorophyll content (Minolta SPAD meter) and chlorophyll fluorescence analysis (OS100 modulated fluorometer). Final harvesting was performed in December, when a variety of morphological characters and yield components were recorded. At this stage a number of plants from each plot were mapped for fruiting positions and bolls at each main stem node.

Results

1996

At Multan, seed cotton yields were highest with 3 and 6 kg/ha glycinebetaine applied at squaring (Fig. 1). Analysis of variance showed that dose and time of application had highly significant ($P<0.001$) effects on yield. Glycinebetaine dose and time of application also significantly ($P<0.001$) increased total dry matter production and the number of bolls per plant. Glycinebetaine-treated plants also had more nodes at maturity than the controls. Characters that were not affected included mean boll weight and % flower and boll shedding, so that the increase in yield could be entirely attributed to greater growth with glycinebetaine treatment. Greenstim concentration and time of application effects on most physiological parameters were insignificant taken over the whole series of measurements.

Chlorophyll fluorescence analysis using dark-adaptation leaf cuvettes gave Fv/Fm ratios that were higher (although not significantly) in the glycinebetaine-treated plants than in the controls. Stomatal conductance values and transpiration rates were slightly lower than in the Faisalabad trial, and again it is not possible to separate environmental and varietal contributions to this difference.

Photosynthesis (carbon dioxide exchange rate) and transpiration were slightly stimulated by glycinebetaine treatment. Glycinebetaine concentrations were higher than in CIM-240 at Faisalabad, but were not increased by glycinebetaine application. Sodium concentrations were higher than at Faisalabad, and magnesium and calcium concentrations lower. Analysis of variance showed no significant effects of glycinebetaine dose or time of application on glycinebetaine or cation concentrations in September. Neither time of application nor dose significantly affected anion concentrations. Compared with Faisalabad, the plants at Multan had lower chloride and malate, and higher sulphate concentrations.

The highest yield of seed cotton at Faisalabad was obtained with 3-kg/ha glycinebetaine applied at squaring (Fig. 2). Yields were lower and more variable than at Multan because of cotton leaf curl virus infection. Varietal and environmental differences may also have contributed to the lower yield at Faisalabad.

The number of bolls at harvest was not significantly affected by glycinebetaine concentration, but was significantly higher with later application. Ginning outturn and staple length were unaffected by glycinebetaine. Glycinebetaine concentrations (measured with a modified periodide assay) were significantly increased with glycinebetaine treatment. The values are normal for unstressed, field-grown cotton (In drought-stressed plants at the same site glycinebetaine concentrations approached 100 mol m^{-3}). Sodium concentrations were increased, although not significantly, by glycinebetaine treatment, but other cations were not affected. The Faisalabad site is slightly more saline than the Multan site, and the plants had higher sodium and lower magnesium and calcium concentrations than at the latter site. Glycinebetaine significantly increased malate concentrations, but the dose or time of application of glycinebetaine did not affect other anions.

1997

All glycinebetaine treatments produced higher yields at Multan, although the seed treatment (with dry glycinebetaine) was not considered very practical. In Multan the higher yields were associated with higher boll numbers in the glycinebetaine-treated plants. The time of spraying was not critical (Figure 3). The number of bolls per plant was the only significant contributor to the higher yield of the glycinebetaine-treated plants. Other yield components were not affected by glycinebetaine (data not shown). Mapping of fruiting points and mature bolls showed increased fruiting points in the 13-29 node region for plants sprayed at squaring, and in the 16-36 node region in the pre-squaring treatment. Boll numbers were higher at nodes 20-30 (pre-squaring and seed treatment) and at nodes 16-20 and 28-31 (squaring). No clear effect of glycinebetaine could be detected when sprayed at first flowering.

Glycinebetaine application, particularly the foliar sprays, significantly reduced stomatal conductance measured with the AP4 porometer, but had no effect on leaf temperature. No effects of glycinebetaine spray on glycinebetaine or cation concentrations were observed in leaves collected in August or in October. Glycinebetaine, sodium and calcium concentrations were higher in October than in August. Potassium concentrations were higher in August.

At PARS, Faisalabad the yields of plants sprayed at 50% squaring and at 1st flowering were significantly higher than the controls (Figure 4). Height (93 - 97.5 cm) and mean internode length (3.8 cm) were not significantly affected by the treatments (data not shown). Data from the mapping of the fruiting points and mature bolls were examined as linear regression lines which gave good fits ($r_2 > 0.9$) to all of the data sets. There were differences in slope between the controls and the treated plants In both the total number of fruiting sites and the number of mature bolls at harvest, with the differences increasing in the following order of spraying: pre-squaring (14th July) > squaring (21st July) > 1st flowering (31st July). Sympodial branches on the lower nodes of sprayed plants had more fruiting points and bolls than the controls, while for the upper nodes the reverse was true. Control plants had bolls at higher nodes than the sprayed plants. Spraying with glycinebetaine had no effect on internode lengths at nodes lower than 25. Glycinebetaine and sodium concentrations were higher in the glycinebetaine-treated plants in August, but not significantly so in October. Concentrations of glycinebetaine were higher in October than in August, while those of sodium and magnesium were highest in August. Spraying with glycinebetaine had no significant effect on other inorganic ions.

Conclusions

Foliar sprays of glycinebetaine can increase the yield of cotton in Pakistan. The optimum dose appears to be 3 kg ha-1, and the optimum time around the squaring to first flowering stages.

References

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Table 1. Factorial combinations of glycinebetaine ('Greenstim' in Europe) doses and application times.

Experiment	Glycinebetaine doses (kg/ha)	Application times[1]	Reps.
PARS 1996	0,1,3,6	Squaring, pre-flowering, flowering	6
PARS 1997[2]	0,3	pre-squaring, pre-flowering, flowering	6
CCRI 1996	0,1,3,6	Squaring, pre-flowering, flowering	4
CCRI 1997	0,3	Seed, pre-squaring, pre-flowering, flowering	6

[1] Pre-squaring (first squares just visible); squaring (>%50 plants with 1 square) preflower (1st flower open); flowering (all plants flowering); Seed (seed coated with glycinebetaine). Sprays were applied during July or early August.

[2] Incomplete factorial design.

Figure 1. Seed cotton yield, CCRI, Multan, 1996.

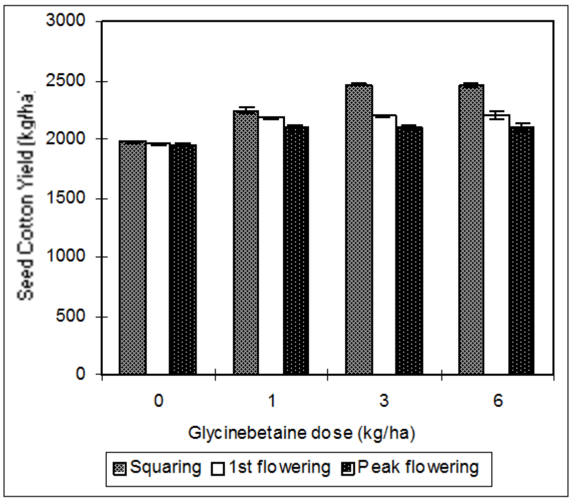

Figure 2. Seed cotton yield, PARS, 1996.

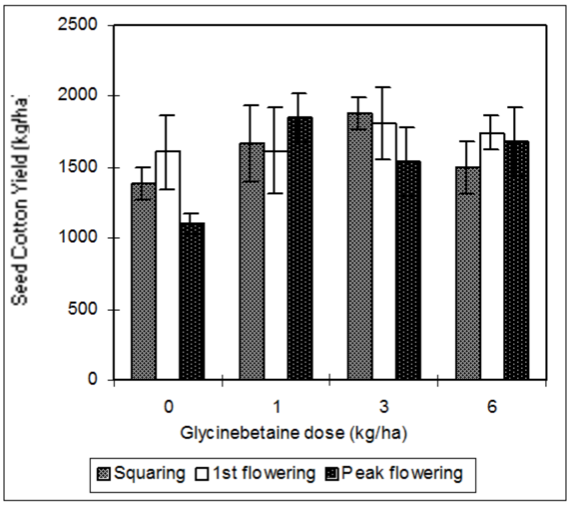

Figure 3. Seed cotton yield, CCRI, Multan, 1997.

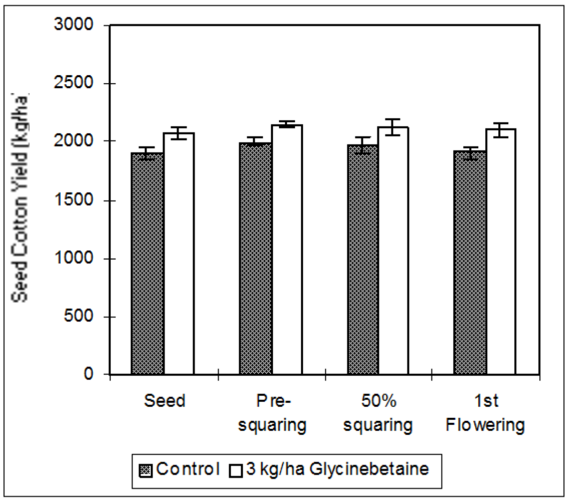

Figure 4. Seed cotton yield, PARS, 1997.