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# STUDIES ON INDUCTION OF DROUGHT TOLERANCE BY SEED HARDENING IN Bt COTTON

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## Abstract

An experiment was conducted to study the effect of seed hardening with different chemicals *viz*. CCC, $CaCl_2$, $ZnSO_4$, $KNO_3$, water soaking and untreated (unsoaked) control on drought tolerance in Bt cotton hybrid. The results reveal that, hardening of cotton seeds with $CaCl_2$ (2%) or CCC 100 ppm or CCC 150 ppm recorded significantly more yield when compared to water soaking and untreated control. In general, seed hardening resulted in increased germination, better crop establishment and enhanced early vigour and ultimately seed cotton yield. Germination percentage, total dry matter (TDM), relative water content (RWC), leaf chlorophyll content and gas exchange parameters, number of bolls, boll weight and seed cotton yield were significantly high. This may be due to favorable effect of $CaCl_2$ on seed germination and seedling vigour, growth and yield of cotton. Seed hardening with $CaCl_2$ (2%) also recorded highest benefit cost ratio.

***Key words :*** Cotton, drought tolerance, germination, seed hardening, seedling vigor.

## Introduction

In India, nearly 70% of cultivated land is rainfed and accounts about 42% of the total quantity of food grains produced. The low productivity under rainfed condition is due to soil moisture deficit, low and erratic rainfall, use of poor quality seeds, poor crop establishment and improper crop management. Seed hardening is a practice adopted to alleviate the moisture stress or making the plant resistant to moisture stress through proper germination and crop establishment. There are several studies done to show the beneficial effects of presowing seed hardening in different crops. For example, Pothiraj and Sankaran (1984) and Rathinavel and Dharmalingam (2000) in cotton; Pawar *et al.* (2003) in sunflower; Bhatnagar *et al.* (1975) in moong; Parvatikar *et al.* (1975), Nayeem and Bhapat (1976) in sorghum; Sheela and Alexander (1995) in rice; Singh *et al.* (1975) in barley. Also there are reviews done by Kulkarni and Chittapur (2003) and Solaimalai and Subburamu (2004) on seed hardening in different crops. The inorganic salts like NaCl, $Na_2SO_4$ KCl, $KH_2PO_4$, $CaCl_2$ and $MgSO_4$; organic acids like succinic acid, CCC and auxins are used as pre-hardening agents. Seed hardening will modify the physiological and biochemical nature of seeds, so as to get the characters that are favorable for drought tolerance. Although, it varies from crop to crop, the principle remains same.

Beneficial effects of seed hardening includes accelerated rapid germination and growth rate of seedling, hardened plants recover much more quickly from wilting than those from untreated plants, induces resistance to salinity and drought condition, seeds withstand higher temperature for prolonged period, flowering is slightly accelerated, compete more efficiently with weeds due to early emergence and results in more yield (Solaimalai and Subburamu, 2004). Soaking the seeds in water for 6 to 12 hours and drying the seeds in shade to its original moisture condition had profound effect on germination and preventing chilling injury (Thomas and Christiansen, 1971).

Seed cotton yield increased significantly with application of cycocel @ 80g ai/ha over control at 40 days after sowing (Karnail Singh, 1976). Increased germination and emergence of cotton seedlings were observed due to 6 hours water soaking at 30°C (Cole and Christiansen, 1975). Soaking of seeds for 24 hours in 50 and 100 ppm of CCC, malic hydrazides (MH), diamin,

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ozides and AMO-1618 revealed that higher yield was recorded by diaminozide soaking at 50 ppm and the effects of other compounds were highly variable (Antably, 1976). Soaking seeds for 24 hours in CCC under saline condition resulted an increased in seed cotton yield (Gabr and Ashkar, 1977). Germination was 93% for water soaked seeds compared to 66% for the control. Water soaking improved the seedling vigour as measured by mean root length and dry matter (Dharmalingam and Basu, 1978).

With this background an investigation was made to know the effect of seed hardening in Bt cotton by using different seed hardening chemicals.

## Materials and Methods

An experiment was conducted during 2009-10 and 2010-11 at Agricultural Research Station, University of Agriculture Sciences, Dharwad, Karnataka in medium deep black soil (with 62% clay content) to know the effect of seed hardening on germination, seedling vigor, growth and yields in Bt cotton (NCH 145 Bt BG-II). Annual rainfall received was 750 and 882 mm during the year 2009 and 2010, respectively. The initial soil moisture content was recorded gravimetrically. The soil moisture content of the experimental plot at the time of sowing was 28% and 33% during 2009 and 2010, respectively.

The experiment was laid out in RBD with nine treatments and three replications. A day before sowing, the Bt cotton hybrid NCH-145 Bt BG-II obtained from the market were soaked for six hours separately in water and solution of $CaCl_2$ (1% and 2%), CCC (100 and 150 ppm), $ZnSO_4$ (0.5%) and $KNO_3$ (0.5% and 1%). Later seeds were dried under shade to its original moisture. The seeds were sown in field by digging and followed all the normal cotton packages of practices includes agronomic practices and plant protection measures for the crop.

To know the effect of seed soaking on germination and early seedling vigour, 100 seeds were sown in each plot (6 rows of 5.4 m length). The germination count was taken from these plots. From this the percentage germination was worked out. The seedlings were thinned out at 8 days. The observations on percent germination, root length, shoot length seedling vigour were recorded at the beginning of crop growth. The observations on total dry matter, leaf area index, chlorophyll content and gas exchanging parameters were recorded at 50% flowering and harvest. The gas exchange parameters were recorded using infrared gas analyser (LI-6400). The boll weight at harvest was recorded by selecting 20 bolls randomly from each plot covering top, middle and bottom positions on the plant and their average weight was worked out. The periodical soil moisture, weather parameters were recorded at an interval of one month.

## Results and Discussion

The pooled analysis of the data obtained from the experiments conducted during 2009-10 and 2010-11 are presented in tables 1 to 5.

1. **Rainfall :** The rainfall received 10 days prior to sowing and 15 days after sowing is presented in the fig. 1. The data shows that after sowing up to 15 days there was no rainfall received and hence there was stress at germination and seedling growth in both the years 2009 and 2010 (fig. 1).
2. **Germination :** The percent germination was significantly high in treatment receiving 2% $CaCl_2$ (93.0%) compared to untreated control (82.7%) (table 1). Other treatments recorded germination in between these values (figs. 2 & 3). Germination was 84.7% for water soaked seeds. Water soaking improved the seedling vigour as measured by mean root length and dry matter (Dharmalingam and Basu, 1978). In the present study, the number of days taken for seedling emergence was significantly low (early) in $CaCl_2$ treatment (5.43 days) when compared to control (7.96 days). The improvement in germination by seed hardening due to number of physicochemical changes within the cytoplasm leading to improvement in seed viability, vigour, root length, shoot length and root to shoot ratio of field crops has been recorded (Solaimalai and Subbaramu, 2004) and reviewed by many workers (Rathinavel & Dharnalingraju, 2000; Kulkarni and Chittapur, 2003).
3. **Leaf area index :** The leaf area index recorded at 50% flowering showed significant difference among the treatment. The treatment $CaCl_2$ (2%) recorded significantly high LAI (2.71) compared to untreated control (1.92) (table 1). Similarly, there is an increased leaf area due to $CaCl_2$ (0.2%) of KCL (2%) has been recorded in cotton compared to control (Thandapani and Subbarayalu, 1980).
4. **Total dry matter :** The data on total dry matter, RWC and water potential recorded at harvest (table 1). The total dry matter recorded at 50% flowering and at harvest was significantly high in $CaCl_2$ (2%) treatment (87.6 g/plant and 123.6 g/plant, respectively). Whereas in control, it was significantly low (69.0 g/plant at flowering and 80.4 g/plant at harvest) (table 1). Similarly, water soaking increased the cotton seedling vigour and total dry matter

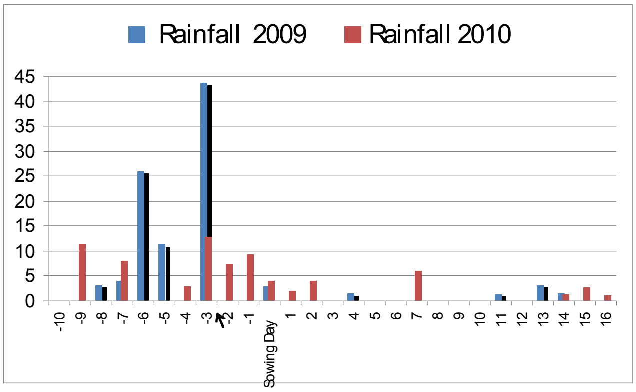


**Fig. 1 :** Rain fall (mm) received 10 days prior (-) and 15 days after sowing in 2009 and 2010 during experimentation.

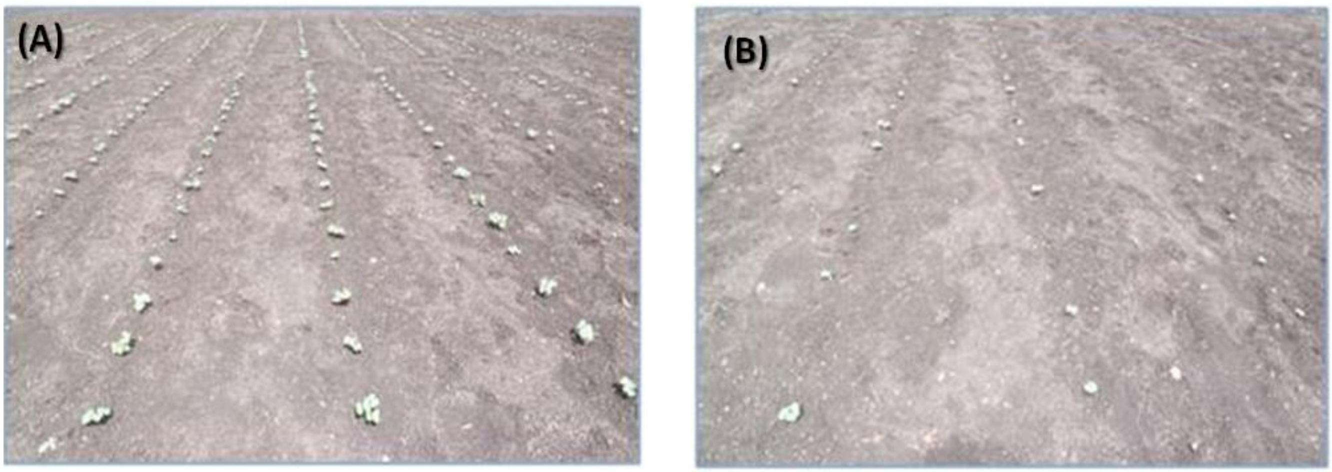


**Fig. 2 :** Experimental plot view at 8 days after sowing (A): $CaCl_2$ (2%) seed hardened Cotton plot (B): Control cotton plot.


**Fig. 3 :** $CaCl_2$ (2%) treated cotton plot and control (showed in arrow mark) plot at 15 days after sowing.

(Dharmalingam and Basu, 1978).

5. **Rate of photosynthesis :** The data on the rate of photosynthesis and leaf chlorophyll content, SPAD meter readings and recorded at 50% flowering (table 2). Seed hardening with $CaCl_2$ (2%) enhanced the photosynthetic rate at 50% flowering (22.68 µmole $CO_2 m^{-2} s^{-1}$ in untreated control to 26.3 µmole $m^{-2}$ $sec^{-1}$ in 2% $CaCl_2$ treatment. Thus 15% enhancement in photosynthetic rate was observed due to seed hardening in Bt cotton (table 2).

6. **Chlorophyll content :** Among the treatments seed hardening with $CaCl_2$ (2%) recorded highest chlorophyll content (from 1.98 mg/g fresh weight in untreated control to 3.04 mg/g fresh weight in $CaCl_2$ followed by $KNO_3$ (2.61 mg/g fresh weight). The SPAD readings recorded highest values in treatment receiving $CaCl_2$ (2%) (43.0) as compared to control (34.6). Similar result on significant increase in chlorophyll content due to seed hardening with $CaCl_2$ and CCC was observed in cotton (Thandapani and Subbarayalu, 1980).

7. **Relative water content :** The RWC differed significantly among the treatments. Seed hardening with $CaCl_2$ (2%) recorded significantly more RWC

**Table 1 :** Effect of presowing seed hardening with agro-chemicals on germination, number of days for seedling emergence and leaf area index, total dry matter in Bt cotton.

| S. no. | Treatment | Germination (%) | No. of days for seedling emergence | LAI at 50% flowering | TDM at 50% flowering (g/plant) | TDM at harvest (g/plant) |
|---|---|---|---|---|---|---|
| 1 | $CaCl_2$ 1% | 89.7 | 5.49 | 2.43 | 83.1 | 105.3 |
| 2 | $CaCl_2$ 2% | 93.0 | 5.43 | 2.71 | 87.6 | 123.6 |
| 3 | CCC 100 ppm | 91.3 | 5.83 | 2.54 | 84.5 | 107.3 |
| 4 | CCC 150 ppm | 88.7 | 6.49 | 2.15 | 82.2 | 100.0 |
| 5 | $ZnSO_4$ 0.5% | 87.7 | 6.83 | 2.45 | 79.9 | 95.1 |
| 6 | $KNO_3$ 0.5% | 88.0 | 6.16 | 2.40 | 81.6 | 101.9 |
| 7 | $KNO_3$ 1.0% | 86.7 | 7.16 | 2.31 | 78.6 | 95.4 |
| 8 | Water Soaking | 84.7 | 7.83 | 2.33 | 73.3 | 87.0 |
| 9 | Control | 82.7 | 7.96 | 1.92 | 69.0 | 80.4 |
| | **SEm±** | **1.11** | **0.31** | **0.15** | **3.06** | **5.41** |
| | **CD at 5%** | **3.34** | **0.96** | **0.46** | **9.16** | **16.26** |

**Table 2 :** Effect of presowing seed hardening with agro chemical on the rate of photosynthesis, chlorophyll content and SPAD readings, relative water content and water potential at 50% flowering in Bt cotton.

| S. no. | Treatment | Photosynthetic rate (µmol $CO_2$ $m^2s^{-1}$) | Chlorophyll content (mg $g^{-1}$ fr. wt) | SPAD reading | RWC (%) | Water potential (- bars) |
|---|---|---|---|---|---|---|
| 1 | $CaCl_2$ 1% | 25.21 | 2.52 | 39.0 | 84.2 | 16.2 |
| 2 | $CaCl_2$ 2% | 26.30 | 3.04 | 43.0 | 86.7 | 15.0 |
| 3 | CCC 100 ppm | 25.72 | 2.62 | 40.71 | 85.3 | 15.3 |
| 4 | CCC 150 ppm | 24.44 | 2.46 | 37.72 | 83.3 | 15.7 |
| 5 | $ZnSO_4$ 0.5% | 23.38 | 2.35 | 36.35 | 80.4 | 16.9 |
| 6 | $KNO_3$ 0.5% | 24.18 | 2.61 | 37.15 | 81.9 | 16.1 |
| 7 | $KNO_3$ 1.0% | 23.53 | 2.32 | 36.55 | 81.3 | 17.1 |
| 8 | Water Soaking | 23.01 | 2.15 | 35.30 | 77.4 | 18.1 |
| 9 | Control | 22.68 | 1.98 | 34.6 | 75.8 | 19.6 |
| | **SEm±** | **0.53** | **0.11** | **0.92** | **1.79** | **0.69** |
| | **CD at 5%** | **1.59** | **0.34** | **2.77** | **5.39** | **2.09** |

**Table 3 :** Effect of presowing seed hardening with agro chemicals on yield and yield components, economics of presowing seed hardening with agrochemicals in Bt cotton (Note: Basic cost of cultivation=Rs. 26000/-, Cost of CCC 60 ppm=Rs.1200/-, Price of seed cotton = Rs. 4500/q).

| S. no. | Treatment | Yield (kg/ha) | No of bolls /plant | Boll wt (g/boll) | TCOC | Gross return | Net Returns | B.C. Ratio |
|---|---|---|---|---|---|---|---|---|
| 1 | $CaCl_2$ 1% | 2518 | 31.05 | 5.65 | 29105 | 104310 | 75205 | 3.58 |
| 2 | $CaCl_2$ 2% | 2629 | 33.65 | 5.95 | 29205 | 109305 | 80100 | 3.74 |
| 3 | CCC 100 ppm | 2557 | 31.61 | 5.75 | 29406 | 105885 | 76479 | 3.60 |
| 4 | CCC 150 ppm | 2492 | 29.95 | 5.27 | 29508 | 102825 | 73323 | 3.53 |
| 5 | $ZnSO_4$ 0.5% | 2465 | 28.15 | 4.95 | 29107 | 99450 | 70341 | 3.41 |
| 6 | $KNO_3$ 0.5% | 2427 | 28.85 | 5.38 | 28905 | 101340 | 72435 | 3.40 |
| 7 | $KNO_3$ 1.0% | 2392 | 28.50 | 5.27 | 28980 | 98325 | 69345 | 3.39 |
| 8 | Water Soaking | 2338 | 26.92 | 4.75 | 28708 | 93015 | 64307 | 3.24 |
| 9 | Control | 2276 | 25.80 | 4.47 | 28505 | 88200 | 59695 | 3.09 |
| | **SEM±** | **31.9** | **0.66** | **0.27** | **-** | **-** | **-** | **-** |
| | **CD at 5%** | **96.9** | **2.04** | **0.81** | **-** | **-** | **-** | **-** |

at flowering (86.7%) as compared to control (75.8%) (table 2). There was increase in relative water content in cotton as recorded by Thandapani and Subbarayalu (1980).

8. **Water potential :** The water potential increased significantly from -19.6 bars in untreated control to -15.0 bars in $CaCl_2$ (2%) treatment (table 2). In cotton seed hardening helps for the accumulation of solutes in cytoplasm, induces lowering of osmotic potential and helps in maintenance of turgor (Turner, 2007).

9. **Yield and yield components :** The data on seed cotton yield, number of bolls per plant and boll weight recorded at harvest are presented in table 3. The seed cotton yield increased significantly from 2276 kg/ha in control to 2629 kg/ha in $CaCl_2$ (2%) treatment followed by CCC 100 ppm (2557 kg/ha). Thus, there was significant enhancement of yield to an extent of 15.51% due to seed treatment (hardening) with 2% $CaCl_2$. The treatment CCC 1000 ppm also recorded 12.3% increase in seed cotton yield, which was significantly high.

Many studies on the improvement of growth and yield due to pre sowing seed hardening are documented (Solaimalai and Subbarmanu, 2004; Meek and Oosterhugs, 2005). Soaking the seeds for 24 hours in CCC under saline condition resulted an increase in seed cotton yield (Gabr and Ashkar, 1977). A yield increase of 25% in cotton was obtained when the seeds were treated with CCC (1000 ppm) for 6 hours compared to untreated dry seeds (Pothiraj *et al.*, 1984).

The number of bolls per plant increased significantly from 25.8 in untreated control to 33.65 in $CaCl_2$ (2%) treatment respectively. The boll weight increased significantly from 4.47 g/boll in control to 5.95 g/boll in $CaCl_2$ (2%) treatment. The improvement in yield and total dry matter in many crops has been attributed to the beneficial effects of seed hardening due to increased bound water content, triggering of biosynthesis of nucleic acids and rapid germination and growth of seedlings resulting in increased uptake of nutrients and the ability of the treated seeds to with stand high temperature for prolonged periods under dry condition (Swaminathan and Sujatha, 2001).

**Economics :** The results on the economics of use of presowing hardening on the monitory benefit in Bt cotton are presented in table 3. The treatment receiving 2% $CaCl_2$ has recorded maximum benefit cost ratio (3.74) followed by CCC 100 ppm (3.60) whereas untreated control and water soaking recorded benefit cost ratio 3.09 and 3.24, respectively.

## Conclusion

Presowing seed hardening in cotton with different inorganic salt solutions /growth retardant (CCC) brought increase in germination, seedling vigour, growth and yield components including seed cotton yield. There was an enhancement in yield to an extent of 12 to 15% with $CaCl_2$ or CCC in the present study.

The result shows that there is an increase in 10 to 13 per cent yield in the seed hardening treatment with $CaCl_2$ and CCC compared to control.

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