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# Biotic activation of abiotic temperature stress tolerance in transgenic BT and wild type cotton

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**Activation of abiotic temperature stress tolerance in transgenic Bt and non-Bt cotton was studied using *Pseudomonas fluorescens* as biotic regulator. Seeds germinated at different temperatures after the bacterial treatment exhibited variation at the temperatures of 30°C and 37°C as measured in terms of percent seed germination in both Bt and non-Bt cotton. *Pseudomonas* treatment enhanced the seed germination in non-Bt cotton at 30°C. Enhancement of seed germination in Bt cotton was significant at 37°C after bacterial treatment. At elevated temperature of 45°C, neither Bt nor non-Bt seeds germinated, irrespective of *Pseudomonas* treatment. The temperature of 30°C supported better shoot and root growth of seedlings as compared to other temperatures tested. *Pseudomonas* treatment resulted in enhanced shoot and root length of Bt cotton seedlings at 30°C, whereas at 37°C had negligible influence. Non-Bt cotton seeds treated with bacteria failed to show significant variation in root and shoot growth compared to untreated control. Activity gel electrophoresis carried out to study the expression of superoxide dismutase revealed that the SOD activity in both Bt and non-Bt cotton was enhanced due to *Pseudomonas* seed treatment.**

**Key Words:** Bt cotton; Germination; Non-Bt cotton; *Pseudomonas*; SOD

Increase in atmospheric $CO_2$ concentration is thought to be largely responsible for recent increases in global mean surface temperature which rose by 0.6°C from 1900-2000 and are projected to increase by another 1.4 to over 5°C by 2100. In addition to mean increase in temperatures, there will also likely be increases in the frequency, duration and severity of periods with an exceptionally high temperatures (i.e., acute heat stress or heat waves). Thus, in future, plants will likely experience increases in acute heat stress. Understanding mechanisms of plant adaptation to heat stress would facilitate the development of heat tolerant cultivars for improving productivity in warm climatic regions. To sustain the agricultural productivity with increase in temperature could be achieved either by developing crop varieties tolerant to stress or using exogenous agents to induce stress tolerance.

The soil borne fluorescent pseudomonads have received particular attention throughout the globe because of their catabolic versatility, excellent root colonizing ability and their capacity to produce a wide range of enzymes and metabolites that favor the plant withstand under varied biotic and abiotic stress conditions (Ramamoorthy et al., 2001; Vivekananthan et al., 2004; Mayak et al., 2004).

Transgenic Bt cotton conferring resistance to insect pests have been under extensive evaluation for the other qualities like response to biotic and abiotic stresses. *P. fluorescens* has been used successfully for the biocontrol of pests and pathogens in cotton. However, the effect of this endophytic bacteria in inducing abiotic stress tolerance in cotton, particularly against temperature stress is largely unknown. Hence, the present study has been designed to evaluate *Pseudomonas* treatment of Bt and non-Bt wild type cotton seedlings against temperature stress.

## Materials and Methods

### Sample Collection

Cotton seeds (transgenic Bt and non-Bt RCH hybrids) were obtained from the Central Institute for Cotton Research, Coimbatore, India. Talc based powder formulation of *P. fluorescens* strain PF1 was obtained from Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India.

### Seed treatment

Seeds were soaked in sterile water overnight. The talc based formulation of *P. fluorescens* at the rate of 1 g per 100 g of seeds was mixed with few drops of sterile distilled water to make a paste. Cotton seeds were coated with the bacterial formulation paste in Petri dishes and incubated at room temperature for an hour. Untreated control seeds were soaked in sterile water. After the incubation period, seeds were carefully transferred to sterile filter paper kept in Petri plates under aseptic conditions. About 10 seeds were placed per plate and spread at equal distance. The filter papers were wetted with 2 mL of sterile water and incubated at different temperatures *viz.,* 30, 37 and 45°C.

### Growth observations on seedlings

The number of seeds germinated after a week of incubation was counted and recorded. Visual observations were made on the overall seedling growth, appearance, robustness, leafy structures and abnormalities. The root and shoot lengths of the seedlings were measured.

### Protein extraction

Seed coat was removed from the cotyledons using sterile foreceps under aseptic conditions and about 0.5 g of tissues were weighed and homogenized in 1 mL of 0.1M potassium phosphate buffer, pH 7.8 containing 0.5% Tween 20. The extracts were then centrifuged at 8000 rpm for 20 min at 4°C. The supernatant was collected and stored at -80°C until further use.

### Activity gel electrophoresis

Superoxide dismutase activity in the heat stressed seedlings of Bt and non-Bt cotton was analyzed on native polyacrylamide gel as described (Babu et al., 2005). Briefly, the extracted proteins were quantified using Bradford method (Bradford, 1976). Protein samples (100 µg) were loaded on nondenaturing polyacrylamide gels (4% stacking and 15% separating), and electrophoresis was carried out at 100 V at 4°C. Gel was incubated in 100 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 200 µL of *N,N,N′,N′*-tetramethylethylenediamine (TEMED), 1 mL of 10 mM ethylenediaminetetraacetic acid

(EDTA), 150 mg of riboflavin, and 15 mg of NBT. After incubation for 30 min in dark, the gels were submerged in the above phosphate buffer and illuminated under 40 W fluorescent bulbs until maximal contrast between clearer SOD bands against a blue background was attained. The gel was further destained in water overnight, and the experiment was repeated twice.

**Results and Discussion**

**Seed germination**

The results of seed germination experiment are presented in Table 1. About 70% of the wild type cotton seeds germinated irrespective of bacterial treatment whereas, 70% germination was observed in untreated Bt and 20% in *Pseudomonas* treated Bt seeds. At 37°C, 50% of the wild type untreated seeds germinated and by bacterial treatment, the germination increased to 70%. Poor germination to the level of 10% was recorded in untreated Bt at 37°C whereas, after bacterial treatment, Bt seeds failed to germinate at that temperature. At a still elevated temperature of 45°C, neither Bt nor wild type cotton seeds germinated. The germination experiment results indicate that 30°C is conducive for cotton seed germination. Although the overall percentage of germination is low in wild type cotton, the germination was more after *Pseudomonas* treatment. This indicates possible role of *Pseudomonas* in temperature tolerance and thus enhancing seed germination even under elevated temperatures. *Pseudomonas* treatment has resulted in germination comparable with germination at optimal and normal temperature.

Not much work has been done on the effect of beneficial rhizosphere microflora on the seed germination of Bt or non-Bt cotton. However, Pangrikar et al. (2009) reported the effect of culture filtrates of rhizosphere microbes obtained from Bt and non-Bt cotton on the seed germination. They observed that the culture filtrates obtained from Bt rhizosphere microbes inhibited the seed germination of cereals and oilseeds compared to the non-Bt rhizosphere microbes. This suggests that the germination of crop seeds are influenced by the exudates of the microbes in the rhizosphere region. Our study demonstrated that *Pseudomonas*, which is also a popular rhizosphere microbe can enhance seed germination. Therefore, mass multiplication of this beneficial bacteria and application as seed or soil treatment may increase its competency in the root region and thus eliminate the harmful effects of exudates of other harmful microbes.

*Pseudomonas* are reported to produce growth promoting substances and enhance plant growth (Leeman et al., 1995; Liu et al., 1995; Wei et al., 1996; Nandakumar et al., 2001; Viswanathan and Samiyappan, 2002).The germination of Bt, particularly RCH hybrid are reported to be either poor or slow as compared to non-Bt (Annual Report 2006, CICR, Coimbatore). The poor germination observed in the present study is thus supported by previous reports on RCH Bt hybrids. However, under optimum temperature, untreated Bt recorded germination of 70% as like non-Bt. This could be attributed to several factors including differential colonization abilities of *Pseudomonas* on Bt and non-Bt. This hypothesis is supported by the observation of zero germination in *Pseudomonas* treatment as against 10% germination in untreated under elevated temperature of 37°C.

**Seedling growth**

Results of seedling growth experiments are presented in Table 2. The temperature of 30°C supported better shoot and root length when compared to 37°C. Complete arrest of seed germination was observed at 45°C. The average shoot length was 5.5 and 5.3 cm in Bt and non-Bt cotton respectively. *Pseudomonas* treatment resulted in 8.9 and 5.3 cm of the same at 30°C. The average shoot lengths recorded at 37°C are 2.9 and 1.5 cm for non-Bt and Bt respectively.

**Table 1. Germination of Bt and non-Bt cotton seeds under different temperatures**

| Hybrids and treatments | Percentage germination of seeds* | | |
|---|---|---|---|
| | 30°C | 37°C | 45°C |
| *Pseudomonas* treated non-Bt cotton | 70% | 70% | 0% |
| Untreated non-Bt cotton | 50% | 70% | 0% |
| *Pseudomonas* treated Bt cotton | 0% | 20% | 0% |
| Untreated Bt cotton | 10% | 70% | 0% |

*Mean of three replications

**Table 2. Seedling growth of Bt and non-Bt cotton under different temperatures**

| Seedling parameters (cm)* | Temperature | Bt cotton | | Non-Bt cotton | |
|---|---|---|---|---|---|
| | | *Pseudomonas* treated | Untreated | *Pseudomonas* treated | Untreated |
| Shoot length | 30°C | 8.9 | 5.6 | 5.3 | 5.4 |
| | 37°C | 1.0 | 1.5 | 3.1 | 3.0 |
| | 45°C | - | - | - | - |
| Root length | 30°C | 4.6 | 3.6 | 1.2 | 3.2 |
| | 37°C | 1.2 | 1.0 | 2.1 | 1.2 |
| | 45°C | - | - | - | - |

*Mean of three replications

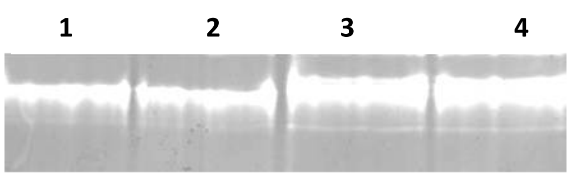


**Fig. 1. SOD activity in Bt and non-Bt cotton seedlings treated with *P. fluorescens*.** Lanes 1- Non-Bt cotton untreated; 2 – Bt cotton untreated; 3 – Non-Bt cotton treated with *P. fluorescens*; 4 – Bt cotton treated with *P. fluorescens*

Interestingly, bacterial treatment did not support root length in Bt cotton. Except for Bt, the bacterial treatment was found to enhance shoot length at 30°C and 37°C. In Bt cotton, there was a two fold increase in the shoot length at 30°C. The results obtained are supported by the documented evidences that *Pseudomonas* is a plant growth promoting rhizobacteria as it produces growth hormones. Plant growth promoting rhizobacteria were reported to enhance growth by increasing seedling emergence (Dunne et al., 1998), plant height (Nandakumar et al., 2001; Raupach and Kloepper, 1998), leaf number (Wei et al., 1996) and yield of crop plants (Duffy et al., 1996; Radjacommare et al., 2002). Seed treatment with native *P. fluorescens* has been reported to enhance growth and increase the yield in tomato (Sakthivel et al., 2009).

The bacteria coated on the seeds colonize the hypocotile region of germinated seeds. Later, since it is an endophytic microbe, it establishes systemically throughout the plant system. The enhanced shoot and root length after *Pseudomonas* treatment as observed in the present study supports the previously reported ecological and *in planta* behavior of this beneficial bacteria. In addition to the physical characteristics like root and shoot growth, much variation in robustness of the seedlings were noticed in *Pseudomonas* treated seedlings. The seedlings were healthier and greener in bacterial treated when compared to control. Leaf texture also showed variation between Bt and non-Bt.

**SOD analysis**

SOD activity was observed as isoform bands in the activity gel electrophoresis (Fig. 1). Based on the intensity of the bands, the activity of the enzyme was found to be more in *Pseudomonas* treatment both in Bt and non-Bt cotton. Oxygen free radicals like superoxide are produced during normal germination and hence the constitutive SOD scavenges these oxygen species. This constitutive expression was observed to be similar in Bt and non-Bt cotton seedlings (Fig. 1). However, upon bacterial treatment, the activity of the enzyme is high in both cases suggesting the induced antioxidant SOD by the bacteria to alleviate temperature stress related oxidative stress. *Pseudomonas* is reported to induce systemic resistance in many crop plants (Nandakumar et al., 2001). In cotton also, *P. fluorescens* is successfully used as biocontrol agent against pests and diseases. Root colonizing *P. fluorescens* has also been reported to produce its own catalase and superoxide dismutase (Katsuwon and Anderson, 1990). The increased activity of SOD as observed in the present study is whether due to induction of plant SOD or contribution by bacterial SOD is yet to be unraveled. As the bacteria is known to induce many other defense enzymes including SOD, further investigation may provide insights into the actual defense mechanism and enzymes involved in the temperature tolerance mediated by this bacteria in cotton.

Our study clearly indicates that *Pseudomonas* biocontrol agent treatment could induce defense against temperature stress in cotton. The Bt cotton although had constitutive defense mechanisms against stress, bacterial treatment is likely to enhance its performance. Rapid increase in the global temperature due to emission of toxic gases as a result of urbanization and industrialization is a growing menace. It is mandatory to design strategies for commercial agriculture under such environmental situation for sustainable crop production. Biodynamic farming is a new concept which involves ecofriendly, cost effective agriculture using novel biotechnological approaches for increased productivity. The transgenics like Bt cotton and the ecofriedly bioagents like *P. fluorescens* are best candidates for crop-microbe combination for biodynamic farming under temperature stress conditions.

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