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## "Can we induce thermo-tolerance in cotton by regulating ethylene biosynthesis?" Introduction:

Ethylene, a simple organic molecule, is responsible for regulating the key plant responses to various biotic and abiotic stresses (Abeles et al., 1992). Cotton plants generally produce ethylene from different tissues throughout their life, while the process is accelerated by various growth and environmental factors ((Hyodo, 1991, Glick, 2005). Increased ethylene concentrations in cotton tissues can trigger fruit and flower shedding and overall yield reduction (Guinn, 1982). Our previous glasshouse and field studies indicated that yield losses in stressed (waterlogged) cotton can be reduced by restricting ethylene production (Najeeb et al., 2015). As abscission of fruits is a major cause of yield reduction in cotton crops exposed to periods of elevated temperature (Reddy et al., 1992), we anticipated that regulating ethylene biosynthesis can increase fruit retention and yield. To reduce the impact of ethylene damage, we used an anti-ethylene agent aminoethoxyvinylglycine (AVG) and a cotton mutant line (5B). This mutant line was found less sensitive to ethylene-injury (Najeeb et al. 2015, unpublished data).

**Methods**: A commercial cotton cultivar Sicot 71 BRF and 5B mutant line (lintless) were used in the study. The plants were grown under glasshouse conditions. At 10-11 nodal stage (60 days after sowing, early flowering), the plants were exposed to heat shock ( $45/30^{\circ}$ C, day/night temperature) for 7 days. A separate set of plants were exposed to ramping high temperature ( $30-42^{\circ}$ C, with an increment of  $3^{\circ}$ C / day, and finally at  $45^{\circ}$ C for 7 days). One day prior to heat stress, AVG (125 g [ai] ha<sup>-1</sup>) was applied using a hand sprayer. After heat treatment, the plants were allowed to recover for 2 weeks and data on ethylene production, fruit development, pollen germination and leaf membrane damage were regularly collected during and after the treatment.

**Results and discussion**: Both heat shock or ramping temperature caused shedding of nonpollinated cotton flowers. At the end of the treatment, the heat-treated plants contained no pollinated flowers. Flower abscission was the result of inhibited pollen development. Pollen development completely ceased at temperatures above 36°C. Heat stress induced leaf membrane injury, reduced leaf photosynthesis, impaired photosystem II activities and overall plant growth (Figures 1, 3 and 5). Elevated temperature reduced ethylene release from cotton tissues, and consequently, no significant effect of AVG was observed on fruit development in cotton under heat stress (Figures 2 and 4). The study indicated that pollen abortion was the major reason for heat-induced damage, and it was independent of ethylene concentrations (Figure 6). Relatively greater damage was observed on plants exposed to ramping high temperature compared with heat shock, possibly due to accumulative exposure to elevated temperature, suggesting a limited ability of these cultivars to acclimatise to ramping temperature. Photosynthetic inhibition and PSII impairment were possibly related to heat-induced leaf membrane damage.

**Conclusions:** Elevated temperature induces injury to cell membrane and impairs PSII functioning leading to growth inhibition. Yield reduction in cotton plants under high temperature is primarily result of pollen abortion and abscission of non-pollinated flowers. On the other hand, no significant effect of blocking ethylene through AVG and in the lintless mutant, in this study suggested a limited role of ethylene in yield reduction in cotton crop under heat stress.

## References

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**Figure 1:** Changes in photosystem II yield (PSII) and non-photochemical quenching (NPQ) of Sicot 71BRF and lintless mutant (5B) leaves of plants exposed heat shock and ramping high temperature. PAR = Photosynthetically active radiation



Figure 2: Effect of heat shock, ramping high temperature and AVG on fruit development in Sicot 71BRF and lintless mutant (5B) cotton.



**Figure 3**: Changes in gas exchange parameters of Sicot 71BRF and lintless mutant (5B) leaves in response to heat shock and ramping high temperature.  $P_n$  = Rate of photosynthesis; Tr = transpiration rate



**Figure 4:** Changes in ethylene release from Sicot 71BRF and 5B leaves in response to heat shock and ramping high temperature.



**Figure 5:** Effect of heat shock, ramping temperature and AVG on relative cell membrane injury in (A) Sicot 71BRF and (2) lintless mutant (5B) leaves.



**Figure 6:** Pollen germination and pollen tube length of (A) Sicot 71BRF and (B) lintless mutant (5B) in response to changes elevated temperature.