

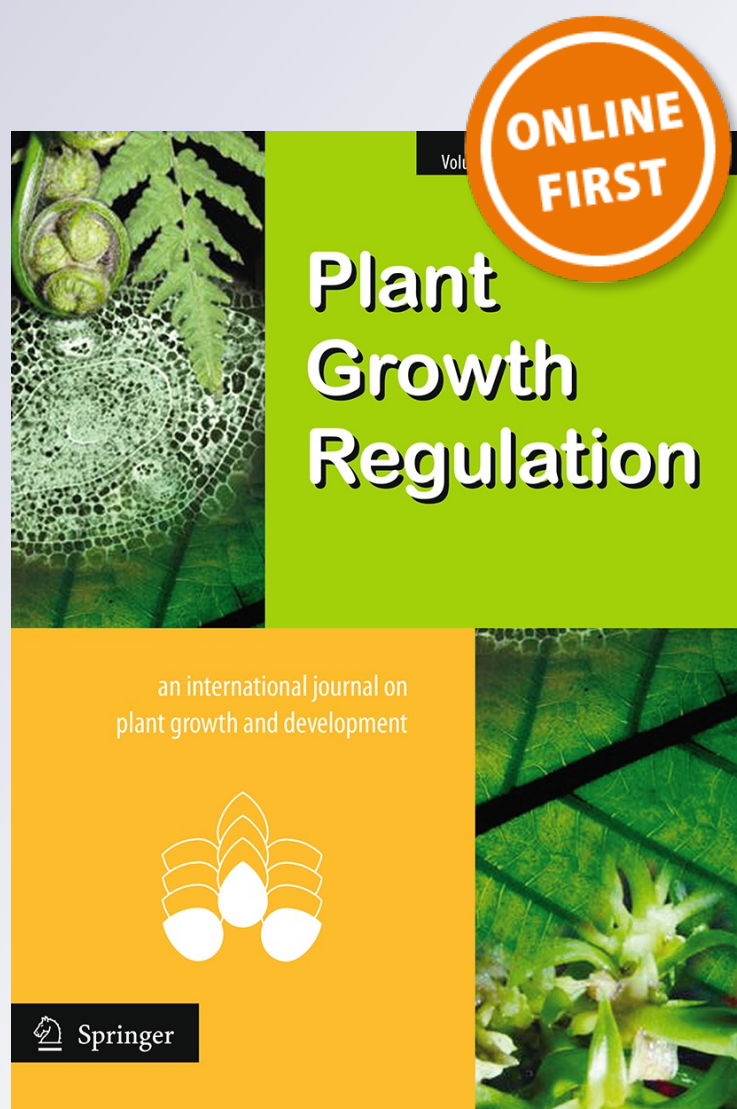
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Aminoethoxyvinylglycine (AVG) ameliorates waterlogging-induced damage in cotton by inhibiting ethylene synthesis and sustaining photosynthetic capacity

Ullah Najeed · Brian J. Atwell · Michael P. Bange · Daniel K. Y. Tan

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Abstract In this glasshouse study, we investigated the mechanisms of aminoethoxyvinylglycine (AVG)-induced waterlogging tolerance in cotton. Two cotton cultivars Sicot 71BRF (moderately waterlogging tolerant) and LA 887 (waterlogging sensitive) were grown in a clay-loam soil, and exposed to waterlogging at early squaring stage (53 days after sowing). One day prior to waterlogging, shoots were sprayed with AVG (ReTain[®], 830 ppm). Continuous waterlogging for 2 weeks accelerated the shedding of leaves and fruits. As the duration of waterlogging increased, shoot growth rate, biomass accumulation, photosynthesis (P_n) and stomatal conductance (g_s) were all reduced. Growth of LA 887 was more severely impaired than Sicot 71BRF, with a decline in leaf P_n and g_s after just 4 h of waterlogging. Waterlogging inhibited allocation of nitrogen (N) to the youngest fully expanded leaves, photosynthesis and biomass accumulation, while it accelerated ethylene production promoting leaf and fruit abscission. AVG blocked ethylene accumulation in leaves and subsequently improved leaf growth, N acquisition and photosynthetic parameters. In addition, AVG enhanced fruit production of both cotton cultivars under waterlogged and non-waterlogged conditions. Higher ethylene

production in cotton is linked with fruit abscission, implying that AVG-induced ethylene inhibition could potentially limit yield losses in waterlogged cotton.

Keywords Ethylene · Fruit shedding · Leaf wilting · Nutrient acquisition · Photosynthesis · Waterlogging

Abbreviations

ACC	1-Aminocyclopropane-1-carboxylic acid
AVG	Aminoethoxyvinylglycine
DAS	Days after sowing
DAW	Day of waterlogging
FID	Flame ionisation detector
g_s	Stomatal conductance
LAR	Leaf area ratio
LWC	Leaf water content
N_{area}	Nitrogen concentration per unit leaf area
NWL	Non-waterlogged
PAM	Pulse-amplitude modulated
PAR	Photosynthetically active radiation
P_n	Rate of photosynthesis
Post-WL	Post waterlogging
SLA	Specific leaf area
TDM	Total plant dry biomass
WL	Waterlogged

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Introduction

Excessive water content in soil (waterlogging) is a major constraint to crop production in many irrigated parts of the world including Pakistan, India and China (Crosson and Anderson 1992). In Australia, waterlogging-induced annual

crop production losses are estimated at A\$180 million p.a. (Price 1993). Waterlogging hinders O₂ diffusion into soils, causing hypoxic or even anoxic conditions in the rooting zone. Hypoxia not only reduces the availability of various nutrients in soil but also alters root plasma membrane H⁺-ATPase activity (Jackson et al. 2003) and thus capacity to take up inorganic nutrients (Colmer and Greenway 2011). Since the uptake of most of inorganic nutrients such as N, P, K and Mg is an energy-dependent process; partial depolarisation of root plasma membrane suppresses nutrient uptake (Steffens et al. 2005). Thus inhibited nutrient supply to leaves impairs many aspects of shoot metabolism, including photosynthetic competence, and impairs shoot growth in terrestrial plants (Jackson and Drew 1984).

Cotton (*Gossypium hirsutum* L.) is an important crop as a source of natural lint for clothing manufacture. In Australia, where cotton is cultivated on heavy clay soil and furrow irrigated, the crop can often experience yield losses due to soil waterlogging, especially following substantial rainfall events. In 2011 alone, heavy rainfall and waterlogging-induced damage to cotton industry was estimated at A\$300 million (CRC 2010–2011). Cotton roots are the first target of waterlogging-induced hypoxia, which exhibit growth inhibition under moderately hypoxic (O₂ < 10 %) conditions (Huck 1970), and influence various physiological processes leading to final yield reduction (Bange et al. 2004).

Soil O₂ deficiency also accelerates the biosynthesis of ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in cotton roots (Christianson et al. 2010), which is converted into ethylene upon arrival to the aboveground aerated parts. Increased ethylene accumulation facilitates different regulatory functions in plants including programmed death of selected tissues and cells (apoptosis), development of adventitious roots, air spaces and other physiological modifications (Sairam et al. 2008). In cotton, elevated ethylene levels induce square and boll abscission and reduce overall lint yield (Lipe and Morgan 1973). Development of genetic and management techniques that block ethylene induction or perception are thus of great interest to the cotton industry.

The impact of waterlogging in terms of ethylene-induced damage to yield requires analysis of two yield components; the retention of fruits that were initiated before waterlogging and the number of new fruit produced during waterlogging (Bange et al. 2004). Stress-induced ethylene accumulation and subsequent damage to plants have been reported in many species including cotton (Hall and Smith 1995). Since shedding of young fruits in stressed cotton is linked with higher ethylene accumulation, regulating ethylene production could improve cotton yield by limiting fruit abscission. Several chemical agents [e.g. aminoethoxyvinylglycine (AVG), aminoethoxycetic acid (AOA), 1-methylcyclopropene (1-MCP) and cobalt and silver ions] can regulate ethylene accumulation by blocking its biosynthetic pathway (Yang and

Hoffman 1984) or its action (McDaniel and Binder 2012). Earlier reports suggested a positive role of AVG on plants experiencing variety of stresses e.g. salinity (Hall and Smith 1995), drought (Beltrano et al. 1999) and waterlogging (Bange et al. 2010). In a 2-year field study, Brito et al. (2013) recorded positive effects of AVG on lint yield of field-grown cotton crop. Similar effects of 1-MCP have been investigated on drought- (Kawakami et al. 2010) and heat-stressed (Kawakami et al. 2013) cotton growth and yield.

Although the yield promoting role of these anti-ethylene agents in stressed or non-stressed crop has previously been studied, limited information is available on the mechanism of AVG action in waterlogged (WL) cotton. In addition, the relationship between waterlogging sensitivity and ethylene accumulation has not yet been explored. The aims of this study were to (1) investigate the role of ethylene accumulation in waterlogging sensitivity of WL and non-waterlogged (NWL) cotton and (2) to understand the mechanisms by which AVG prevents waterlogging damage to cotton. We used two cotton cultivars with contrasting sensitivity to soil waterlogging, and hypothesised that higher ethylene accumulation might be responsible for relatively higher waterlogging damage to the sensitive cultivar.

Materials and methods

Seeds of two cotton cultivars, Sicot 71BRF and LA 887 were surface cleaned with distilled water and planted into plastic pots after overnight imbibition. Sicot 71BRF is a commercial cotton cultivar [*G. hirsutum* L. (Bollgard II[®] Roundup Ready Flex[®]), CSIRO Australia] (Stiller 2008) that is widely cultivated in Australian cotton growing areas on heavy clay soils. It contains Cry 1 Ac and Cry 2 Ab genes encoded for resistance to lepidopteron insect pests and CP4—two copies of EPSPS for tolerance to glyphosate application (Monsanto, St. Louis, MO, USA). LA 887 is a *Fusarium* wilt disease-resistant cotton cultivar bred for Louisiana USA cotton production (Jones et al. 1991). LA 887 was relatively more waterlogging sensitive than Australian cultivars, as it was mainly grown on well drained soils in USA (Conaty et al. 2008).

The seeds were allowed to germinate in (30 × 24 cm; height × diameter) plastic pots each containing 4.5 kg finely mixed red silt loam Ferrosol soil from Robertson, NSW Australia. Fertiliser viz. (MgNO₃)₂, KNO₃, (NH₄)₂SO₄ and NH₄NO₃ and CaCO₃, was added to achieve the final nutrient composition as N 0.68, P 0.17, K 1.4, Ca 2.8, S 1.1 and Mg 0.41 g per kg of dry soil. Plants were grown under glasshouse conditions at 28/20 °C day/night temperature, and 14/10 h light/dark photoperiod under natural light. The light intensity during the day cycle was maintained to a minimum of 400 μmol m⁻² s⁻¹ using

supplemented light (Philips Contempa High Pressure Sodium lamps). Three plants per pot were germinated and thinned to one plant per pot after 2 weeks of germination. At 9–10 nodal stage (53 days after sowing, *DAS*), when plants initiated square formation (flower bud), they were exposed to soil waterlogging. One day prior to waterlogging, a single dose of AVG formulated as ReTain® (Sumitomo Chemicals Australia) was applied (ReTain® 830 ppm \approx AVG 125 g [ai] ha⁻¹) using a hand sprayer. AVG application rate and time was used on the basis of previous field experiments of Bange et al. (2010). Plants were exposed to waterlogging by immersing the pots into water-filled plastic tubs, whereas NWL pots were watered regularly to field capacity. Water level in the tubs was kept approximately 3 cm above soil surface. After 15 days of waterlogging, the pots were removed from tubs, and plants were allowed to recover for 7 days. The experimental layout was a completely randomised block design with 8 replicates of each treatment; four replicates were harvested at the termination of waterlogging, while the remaining four plants per treatment were harvested at the end of recovery period (7 days after termination of waterlogging).

Plant growth

Data on plant growth were recorded from the first day of waterlogging. Plants were subsequently mapped for height, nodes and fruit numbers on 3rd, 7th and 15th day of waterlogging (*DAW*) and at the end of recovery period (7 days after termination of waterlogging). Biomass accumulation and fruit retention were measured by harvesting a subset of plants i.e. at the termination of waterlogging and at the end of recovery period (four replicates each time). Plants were divided into leaves, stems and fruits, and were oven dried at 65 °C for 72 h to measure the dry biomass. Leaf area of each plant was measured from fresh leaves using a LICOR LA-3100 planimeter.

Fresh and dry biomass measurements were used to calculate specific leaf area (*SLA*), leaf area ratio (*LAR*) and leaf water content (*LWC*) of cotton plants as:

$$SLA = \frac{\text{Leaf area}}{\text{Leaf DW}}$$

$$LAR = \frac{\text{Leaf area}}{\text{Plant DW}}$$

$$LWC (\%) = \frac{(\text{Leaf FW} - \text{Leaf DW})}{\text{Leaf FW}} \times 100$$

Leaf gas exchange parameters

Leaf CO₂ exchange parameters such as photosynthesis (*P_n*), stomatal conductance (*g_s*) were measured from the youngest fully expanded leaves on the 1st (4 h after waterlogging),

3rd, 7th and 15th *DAW*, between 0900 and 1300 hours using the Li-6400 portable photosynthesis system (Li-Cor Ltd, Lincoln, NE, USA) with a pulse-amplitude modulated (*PAM*) leaf chamber head. The gas exchange measurements were recorded at 1800 μmol photon m⁻² s⁻¹ photosynthetically active radiation (*PAR*, saturating light conditions for cotton) (Zhang et al. 2011), 30 °C leaf chamber temperature (Burke et al. 1988), a constant level of CO₂ concentration (400 or 700 μmol mol⁻¹) and 1.5–2.0 kPa of vapour pressure deficit (*VPD*).

Nutrient acquisition

Dried samples of upper leaves (from top five nodes) were ground to powder and were analysed for leaf C and N content using a CHN analyser (Model CHN 900, LECO, St. Joseph, MI, USA). The samples were burnt in a tin capsule at 950 °C under pure (99.9 %) oxygen producing N₂ and N-oxides. The N content of the sample were then determined using a thermal conductivity detector (Leco Corporation 2008). The leaf N concentrations were expressed on leaf dry mass {(N%), leaf N contents/leaf dry weight, mg g⁻¹} and leaf area basis {(N_{area}), leaf N dry weight/leaf area, mg cm⁻²}.

Ethylene measurements

Ethylene accumulation was measured from the youngest fully expanded leaves and young squares (three leaves and three squares per plant) at the end of waterlogging (15th *DAW*). To measure ethylene production, leaves and squares were removed from the plants and transferred into 25 mL glass vials. The vials were immediately sealed with rubber septa. Gas samples (1 mL) were withdrawn from the vials after 20–30 min (Jackson and Campbell 1976). Ethylene concentrations were determined by injecting gas samples into PYE series 104 gas chromatograph fitted with a flame ionisation detector (*FID*) and equipped with activated aluminium coated glass column. The oven, detector and injector temperatures were set at 150, 120 and 120 °C, respectively, and ethylene was detected after 50 s. The fresh biomass of the leaf tissues was determined after ethylene detection, and ethylene synthesis rates were calculated as nmol g⁻¹ FW h⁻¹.

Data analysis

Data for different growth parameters was statistically analysed by JMP v. 9 (SAS Institute, Cary, NC, USA) statistical program. Linear mixed model REML (Residual Maximum Likelihood) was applied to assess the differences over time, while the respective means of the studied parameters were compared using the Tukey's HSD (honestly significant difference) test.

To identify the parameters that best describe the waterlogging effects on cotton, principal component analysis (PCA) was performed. Values of leaf P_n , N and ethylene (leaf and square) concentration of the two cotton cultivars under various treatment conditions were included in the PCA. This analysis estimates and then ranks principal components (PC) for contribution to the variation in data by consolidating the relationships among measured physiological variables. Eigenvectors generated by PCA were then used to identify the effect of these parameters on fruit production (final fruit number) in cotton using a Generalised Linear Model fit with a Poisson distribution and Log link function (SAS JMP program).

Results

Shoot growth and fruit development

As the duration of waterlogging increased, it reduced shoot height, biomass accumulation and fruit number in both cotton cultivars. Cultivar LA 887 was more sensitive to soil waterlogging than Sicot 71BRF. Visual leaf wilting and growth inhibition in LA 887 started from the 3rd day of waterlogging (DAW), while non-AVG-treated WL plants shed most of their leaves and squares at end of recovery period. On the other hand, Sicot 71BRF showed better resilience to soil waterlogging during first week, and then it started wilting leaves and the rates of photosynthesis dropped. An analysis of the data pooled across two cultivars showed that waterlogging significantly affected all the growth parameters of cotton at 7th and 15th day of waterlogging (Table 1). Significant interaction between waterlogging and cultivar ($P < 0.05$) at the end of first week of waterlogging suggested that waterlogging more severely inhibited shoot growth, P_n , and fruit numbers of LA 887

plants compared with Sicot 71BRF (Table 1). AVG increased the fruit number in WL as well as NWL cotton, and this effect was significant under long-term waterlogging (15th day of waterlogging) for both cultivars. AVG increased the P_n and g_s of LA 887 but not in Sicot 71BRF during first week of waterlogging (Figs. 2, 3; Table 1, waterlogging \times AVG \times cultivar $P < 0.05$).

Under NWL conditions, both cultivars exhibited a gradual increase in fruit production. Inhibitory effect of waterlogging on fruit development became significant at 7th and 15th DAW in LA 887 (Fig. 1a) and Sicot 71BRF (Fig. 1b), respectively, and the gap between the fruit number plant⁻¹ of WL and NWL plants grew wider during the recovery period (Fig. 1). Despite differences in growing conditions i.e. AVG-treated (WL or NWL) plants contained substantially more fruits during waterlogging and at the end of recovery period.

In non-AVG treatment condition, WL plants of both cotton cultivars contained significantly lower number of fruits compared with NWL plants at the end of waterlogging (post-WL) as well as at 7 days after termination of waterlogging (post-recovery). In addition to inhibited new fruit development, the WL plants had lower post-waterlogging ($P = 0.022$) and post-recovery ($P < 0.0001$) fruit retention than the NWL plants (Table 2). AVG increased fruit retention of both WL and NWL plants, and the effect was more evident during the recovery period ($P = 0.0013$), where it caused approximately 20 % improvement in the fruit retention of AVG-treated compared with non-AVG treated plants (Table 2). AVG application was relatively more effective in increasing fruit retention of WL than NWL plants, as was apparent from the interactive effect of waterlogging \times AVG ($P = 0.048$) (Table 2). The reduction in fruit number in WL cotton was associated with the development of fewer fruiting sites, as indicated by the lower node number and the higher fruit abscission rate. The

Table 1 Changes in plant growth attributes of two cotton cultivars under waterlogging and AVG treatment

Treatments	7th of waterlogging					15th day of waterlogging				
	P_n	g_s	Shoot length	Nodes number	Fruit number	P_n	g_s	Shoot length	Nodes number	Fruit number
Waterlogging	0.0003	<0.0001	0.0003	0.0004	<0.0001	<0.0001	<0.0001	0.0002	0.0005	<0.0001
AVG	0.082	0.092	0.218	0.119	0.082	0.056	0.076	0.434	0.178	0.041
Cultivar	0.007	<0.0001	0.0001	0.519	0.010	0.082	0.022	0.467	0.314	0.315
Waterlogging \times AVG	0.115	0.106	0.324	0.519	0.582	0.001	0.717	0.215	0.385	0.365
Waterlogging \times cultivar	0.044	0.097	0.403	0.024	0.045	0.229	0.893	0.684	0.681	0.038
AVG \times cultivar	0.435	0.214	0.646	0.129	0.232	0.026	0.0903	0.485	0.658	0.568
waterlogging \times AVG \times cultivar	0.037	0.039	0.759	0.519	0.442	0.922	0.0663	0.257	0.191	0.582

Data presented in the table was collected at 7th day and 15th day of waterlogging and summarises the significant differences (P values)

The significant ($P < 0.05$) effects are shown as bold in the ANOVA table

P_n = rate of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), g_s = leaf stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)

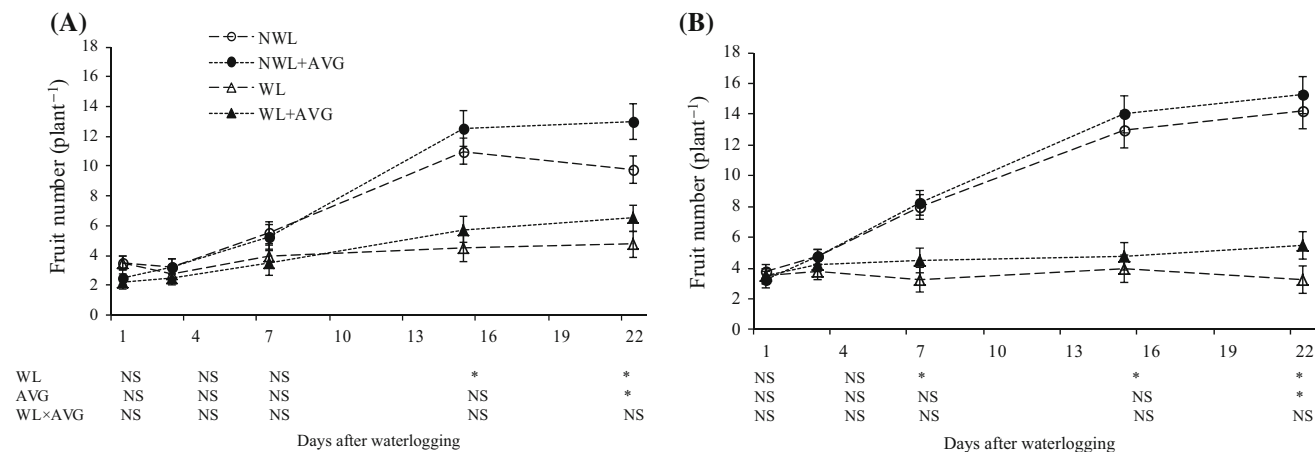


Fig. 1 Changes in fruit development with increasing inundation period **a** cotton cultivar Sicot 71BRF and **b** cotton cultivar LA 887. Vertical bars represent \pm SE of the mean of each treatment (four replicates). *NWL* non-waterlogged + non-AVG treated. *NWL + AVG* non-waterlogged + AVG treated, *WL* waterlogged + non-AVG

treated, *WL + AVG* waterlogged + AVG treated. *P* values were separately calculated for each time interval for pairwise comparison. *F test treatment effects are significantly different at $P < 0.05$. NS F test treatment effects are not significantly different ($P > 0.05$)

data indicated that AVG primarily restricted the fruit abortion in WL or NWL cotton, while having a limited role in shoot growth improvement.

Biomass accumulation and distribution

At the termination of waterlogging, total shoot dry biomass (*TDM*) of WL only plants (combined data of both cultivars) was 20 % lower ($P = 0.046$) compared with NWL only plants, and this reduction primarily was result of reduced (28 %, compared with NWL only plants) leaf dry matter accumulation. Further reduction ($P < 0.0001$) in *TDM* was recorded during post-recovery period, when the WL only plants had 43 % lower *TDM* compared with NWL only plants. This reduction in *TDM* was equally attributed to lower leaf, stem and fruit dry biomass (Table 2). Sicot 71BRF exhibited a better recovery in dry matter accumulation compared with LA 887 after termination of waterlogging, as was evident from the waterlogging \times cultivar interaction ($P = 0.012$). Waterlogging-induced shedding of leaves suppressed the shoot growth recovery by limiting light capturing capacity of LA 887.

AVG treatment to both WL and NWL plants, had a positive effect ($P = 0.044$) on dry biomass accumulation during the recovery period. AVG increased post-recovery *TDM* of WL and NWL plants by 18 and 13 %, respectively, compared with their respective non-AVG treated plants (Table 2).

Leaf morphology

Waterlogging significantly suppressed leaf growth, and reduction in leaf area continued during the recovery period. In the absence of AVG, WL plants had 55 and 64 % lower

post-WL and post-recovery leaf area, respectively, compared with NWL plants. Waterlogging variably influenced the post-recovery leaf area of cotton cultivars (waterlogging \times cultivar, $P = 0.023$), causing relatively more reduction in leaf area of LA 887 than leaf area of Sicot 71BRF (Table 3). Significant reduction in *SLA*, *LAR* and *LWC* of WL plants also occurred during the waterlogging and recovery period. An interactive effect ($P < 0.10$) of waterlogging \times AVG at post-WL leaf area, *SLA* and *LAR* showed that AVG variably influenced these leaf traits in WL and NWL plants (Table 3). For example, AVG reduced the leaf area, *SLA* and *LAR* in NWL plants, while they increased in WL plants.

Dynamics of gas exchange parameters during waterlogging

Under NWL conditions, both cotton cultivars showed little or no variation in the photosynthesis (P_n) of youngest fully expanded leaves throughout the experiment. In Sicot 71BRF, increasing waterlogging duration reduced the P_n of WL plants, causing significant reduction at 7th DAW, while AVG application had no significant effect on P_n of WL or NWL plants (Fig. 2a). Leaf P_n of LA 887 was more sensitive to soil waterlogging, decreasing within 4 h of waterlogging. The gap between leaf P_n of WL and NWL LA 887 plants grew wider with the increasing duration of waterlogging, as seen by the decrease in leaf P_n of WL plants to $5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at the termination of waterlogging (Fig. 2b). AVG significantly increased the leaf P_n of WL-LA 887 plants at 7th and 15th DAW, and AVG-treated LA 887 plants had approximately two fold higher P_n ($10.92 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) compared with non-AVG

Table 2 Post-waterlogging and post-recovery changes in dry biomass (g) of cotton under waterlogging and AVG treatment

Treatment	Post-WL dry biomass (g plant ⁻¹)			Post WL FR (%)			Post recovery dry biomass (g plant ⁻¹)			Post recovery FR (%)
	Leaf	Stem	Fruit	TDM	Leaf	Stem	Fruit	TDM		
NWL	10.86 ± 1.4	13.63 ± 1.2	1.74 ± 0.4	26.23 ± 2.1	82.21 ± 6.5	13.69 ± 1.1	5.71 ± 0.7	31.29 ± 2.5	77.35 ± 7.2	
NWL + AVG	11.13 ± 1.3	13.09 ± 1.4	3.15 ± 0.4	27.37 ± 2.6	84.18 ± 6.5	13.01 ± 1.1	6.18 ± 0.8	35.50 ± 2.8	79.51 ± 6.7	
WL	7.84 ± 0.8	11.00 ± 1.1	2.25 ± 0.2	21.09 ± 2.2	64.51 ± 5.7	6.24 ± 0.5	3.09 ± 0.5	17.97 ± 2.1	43.32 ± 6.4	
WL + AVG	8.18 ± 0.7	14.24 ± 1.2	2.09 ± 0.2	24.51 ± 2.2	71.47 ± 6.6	7.19 ± 0.4	4.92 ± 0.5	21.86 ± 2.1	54.64 ± 6.1	
<i>Cultivar</i>										
Sicot 71BRF	10.11 ± 1.1	13.36 ± 1.4	1.78 ± 0.2	25.25 ± 2.3	81.34 ± 6.8	10.19 ± 1.1	4.28 ± 0.5	27.18 ± 2.5	78.47 ± 6.8	
LA 887	8.89 ± 1.2	12.62 ± 1.3	2.83 ± 0.3	24.34 ± 2.4	82.52 ± 7.2	8.98 ± 1.1	5.98 ± 0.6	26.44 ± 2.6	77.67 ± 6.9	
<i>ANOVA (F test P values were calculated for main treatment and interaction effects)</i>										
Waterlogging	0.0021	0.296	0.842	0.046	<0.0001	<0.0001	0.006	<0.0001	<0.0001	
Cultivar	0.213	0.727	0.318	0.799	0.550	0.182	0.097	0.467	0.482	
AVG	0.580	0.763	0.514	0.540	0.037	0.237	0.587	0.044	0.001	
Waterlogging × cultivar	0.609	0.739	0.675	0.864	0.458	0.058	0.364	0.012	0.556	
Waterlogging × AVG	0.798	0.181	0.369	0.799	0.057	0.817	0.916	0.694	0.048	
Cultivar × AVG	0.701	0.122	0.729	0.537	0.521	0.574	0.036	0.147	0.642	
Waterlogging × cultivar × AVG	0.413	0.093	0.912	0.343	0.557	0.539	0.805	0.459	0.625	

The treatment data are mean ± SE of 8 individual plants (cultivars × replicates, 2 × 4), whereas cultivar data are mean ± SE of 16 individual plants (treatment × replicates, 4 × 4)

The significant (*P* < 0.05) effects are shown as bold in the ANOVA table

FR fruit retention, NWL + AVG non-waterlogged + AVG treated, NWL non-AVG treated non-waterlogged, WL + AVG waterlogged + AVG treated, WL non-AVG treated, waterlogged; TDM total plant dry matter

Table 3 Post-waterlogging and post-recovery changes in leaf growth of cotton under waterlogging and AVG treatment

Treatment	Post waterlogging				Post recovery			
	Leaf area (cm ²)	SLA	LAR	LWC	Leaf area (cm ²)	SLA	LAR	LWC
NWL	2,503.0 ± 51.4	230.4 ± 17.1	95.4 ± 11.2	77.7 ± 6.7	2539.5 ± 68.5	213.6 ± 15.5	81.2 ± 6.8	77.1 ± 4.5
NWL + AVG	2,206.9 ± 66.7	198.3 ± 18.5	80.6 ± 8.6	75.2 ± 6.8	2492.1 ± 66.7	191.5 ± 16.8	70.2 ± 6.4	74.0 ± 7.7
WL	1,130.6 ± 47.1	144.2 ± 13.5	53.6 ± 5.7	66.4 ± 5.7	917.1 ± 48.7	146.9 ± 14.5	51.0 ± 5.7	67.9 ± 6.7
WL + AVG	1,329.0 ± 58.3	162.5 ± 15.8	54.2 ± 6.7	69.7 ± 5.8	1029.2 ± 45.7	143.1 ± 13.6	47.1 ± 4.8	69.0 ± 4.4
<i>Cultivar</i>								
Sicot 71BRF	1,799.1 ± 84.1	177.9 ± 17.4	71.3 ± 7.2	72.3 ± 7.8	1776.7 ± 57.4	174.4 ± 16.4	65.4 ± 5.5	72.4 ± 5.4
LA 887	1,785.7 ± 87.7	200.9 ± 12.2	73.4 ± 7.5	72.6 ± 6.7	1,712.3 ± 61.4	190.7 ± 16.7	64.8 ± 4.5	71.6 ± 5.7
<i>ANOVA (F test P values were calculated for main treatment and interaction effects)</i>								
Waterlogging	<0.0001	<0.0001	<0.0001	0.005	<0.0001	<0.0001	<0.0001	0.005
Cultivar	0.925	0.045	0.962	0.711	0.646	0.059	0.644	0.725
AVG	0.731	0.067	0.122	0.101	0.693	0.123	0.463	0.627
Waterlogging × cultivar	0.436	0.644	0.234	0.998	0.027	0.789	0.411	0.858
Waterlogging × AVG	0.061	0.074	0.052	0.220	0.465	0.362	0.188	0.457
Cultivar × AVG	0.930	0.411	0.546	0.732	0.648	0.180	0.284	0.331
Waterlogging × cultivar × AVG	0.481	0.768	0.927	0.444	0.215	0.684	0.465	0.467

The treatment data are mean ± SE of 8 individual plants (cultivars × replicates, 2 × 4), whereas cultivar data are mean ± SE of 16 individual plants (treatment × replicates, 4 × 4). The significant ($P < 0.05$) effects are shown as bold in the ANOVA table

NWL + AVG non-waterlogged + AVG treated, NWL non-AVG treated non-waterlogged, WL + AVG waterlogged + AVG treated, WL non-AVG treated waterlogged, SLA specific leaf area (cm² g⁻¹ leaf dry weight), LAR leaf area ratio (cm² g⁻¹ plant dry weight), LWC leaf water contents (%)

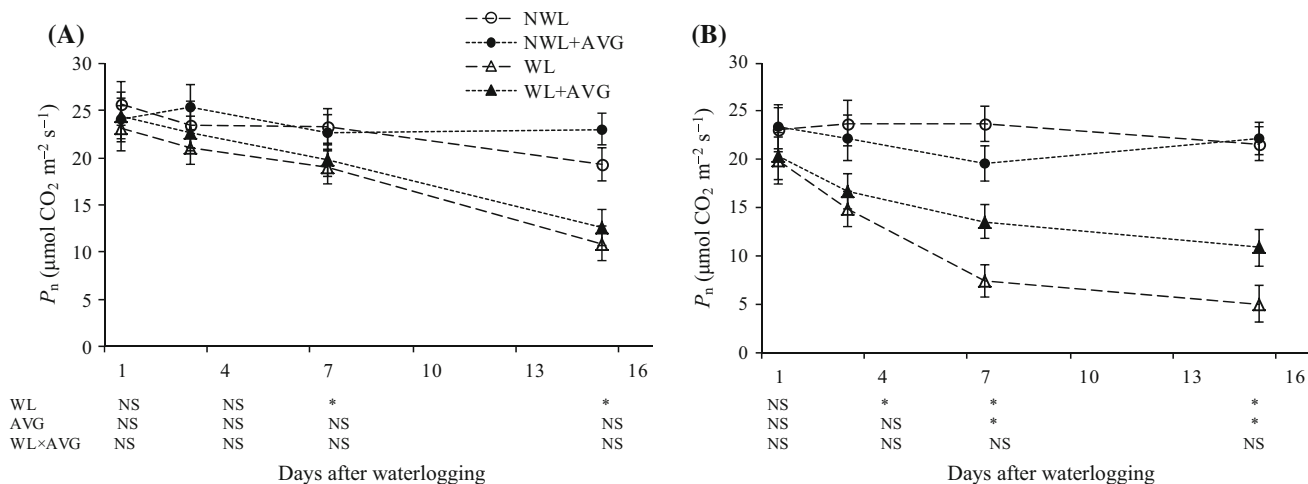


Fig. 2 Changes in rate of photosynthesis of cotton under waterlogging and AVG application; **a** cotton cultivar Sicot 71BRF, **b** cotton cultivar LA 887. Vertical bars represent \pm SE of the mean of each treatment (four replicates). *NWL* non-waterlogged + non-AVG treated, *NWL + AVG* non-waterlogged + AVG treated, *WL* waterlogged +

non-AVG treated, *WL + AVG* waterlogged + AVG treated. P_n = rate of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). P values were separately calculated for each time interval for pairwise comparison. *F test treatment effects significantly different at $P < 0.05$. NS F test treatment effects not significantly different ($P > 0.05$)

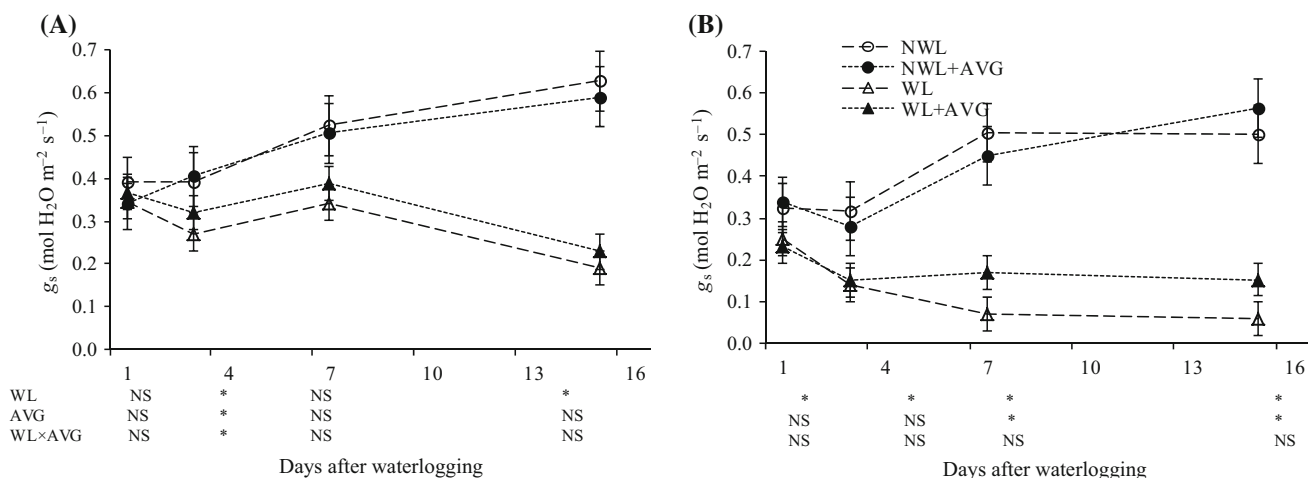


Fig. 3 Changes in leaf stomatal conductance of cotton under waterlogging and AVG application; **a** cotton cultivar Sicot 71BRF, **b** cotton cultivar LA 887. Vertical bars represent \pm SE of the mean of each treatment (four replicates). *NWL* non-waterlogged + non-AVG treated, *NWL + AVG* non-waterlogged + AVG treated, *WL* waterlogged +

non-AVG treated, *WL + AVG* waterlogged + AVG treated. g_s = leaf stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). P values were separately calculated for each time interval for pairwise comparison. *F test treatment effects are significantly different at $P < 0.05$. NS F test treatment effects not significantly different ($P > 0.05$)

treated plants ($5.08 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at the termination of waterlogging (Fig. 2b), although AVG had no significant effect on leaf P_n of NWL LA 887 plants.

Under NWL conditions, cotton plants of both cultivars exhibited a slow gradual increase in leaf stomatal conductance (g_s) over time (Fig. 3). No significant change in g_s of WL-Sicot 71BRF plants was observed during first week of waterlogging; however, it fell during later stage of waterlogging (Fig. 3a). In contrast, g_s of WL-LA 887 started falling just after initiation of waterlogging, and continued to fall till the end of waterlogging (Fig. 3b). AVG

improved the g_s of WL-Sicot 71BRF plants during the initial waterlogging period only (Fig. 3a). By contrast, in WL-LA 887, after an initial fall in g_s , AVG-treated plants maintained significantly higher g_s compared with WL-only plants during later period of waterlogging (Fig. 3b).

Leaf N and C content

Waterlogging significantly inhibited N acquisition (calculated on % leaf DW basis) in the youngest fully expanded leaves of both cotton cultivars, and WL plants did not

recover the nutrient levels to that of NWL plants a week after termination of waterlogging (Table 4). LA 887 contained relatively higher ($P = 0.046$) post-WL N (% leaf DW) compared with Sicot 71BRF but the difference was non-significant at post-recovery. On the other hand, Sicot 71BRF contained significantly higher C (% leaf DW) content compared with LA 887. The effect of waterlogging on leaf C and N contents was non-significant, when expressed on leaf area (mg cm^{-2}) basis reflecting an associated reduction of leaf area in the upper part of the plant. Leaf area reduction was attributed to reduced leaf size and higher leaf abscission (Table 3). Leaf C contents of cotton were relatively less sensitive to soil waterlogging and exhibited no significant change (% leaf DW or mg cm^{-2}) under any treatment of waterlogging or AVG. Relatively higher impact of waterlogging on leaf N compared with C contents in turn significantly increased the leaf C/N ratio in WL plants.

Ethylene accumulation

Significant increase in ethylene production from the youngest fully expanded leaves of both cotton cultivars was recorded at the termination of waterlogging. Despite variation ($P = 0.048$) in leaf ethylene synthesis of two cotton cultivars under various treatment conditions (Fig. 4a), the leaf ethylene production followed a similar pattern of change i.e. waterlogging increased ethylene production, which was reduced by AVG application. Ethylene production from the leaves of WL-LA 887 and Sicot 71BRF plants was approximately two- and three-fold higher, respectively, compared with their respective NWL plants (Fig. 4a). Foliar-applied AVG inhibited ($P < 0.0001$) ethylene production from the leaves of both cotton cultivars. AVG-induced reduction in ethylene synthesis was 54 and 27 % in the leaves of WL and NWL plants, respectively. Significantly higher ethylene production was observed from the young squares of WL-LA 887 plants only, which was suppressed ($P < 0.0001$) by AVG (Fig. 4b). In contrast, Sicot 71BRF showed no significant change in ethylene production from squares under any treatment (Fig. 4b).

Role of leaf photosynthesis and ethylene in fruit production

To study the mechanism of waterlogging damage, relationships among the major yield affecting variables i.e. leaf P_n , g_s , ethylene and fruit numbers (post-WL) were developed. Higher fruit numbers in NWL cotton plants were positively associated with leaf P_n and negatively associated with ethylene production (Fig. 5a, b). Although AVG-induced decrease (50 %) in ethylene production was

relatively greater than the increase in fruiting production (30 %), limiting ethylene production in WL cotton increased total number of fruit produced (Fig. 5b). Leaf P_n was positively associated with g_s and negatively associated with ethylene production (Fig. 6a, b). A sharp reduction both in leaf P_n and g_s in the first part of graph indicates the potential role of stomatal resistance in photosynthetic inhibition under WL environment (Fig. 6a). Higher ethylene production in WL cotton reduced leaf P_n (Fig. 6b), which in turn inhibited fruit production (Fig. 5a). Thus, higher levels of ethylene in cotton tissues can induce yield losses either directly by increasing fruit abortion and/or indirectly by impairing photosynthesis and fruiting node development (Fig. 7).

Principal component analysis (PCA)

Principal component analysis (PCA) was used to estimate the relative changes in leaf P_n , N contents, and leaf and square ethylene concentrations in two cotton cultivars under various treatments. The loading matrix of PCA indicated a strong positive correlation between P_n , N, which were negatively correlated with tissue (leaf and square) ethylene production (Fig. 8b). The first principal component (PC1) explained most of the variation (75.3 %) followed by second principal component (PC2), which accounted for 13.8 % of variation (Fig. 8). The eigenvectors for PC1 and PC2 were

$$\text{PC}_1 = 0.4839X_1 + 0.5481X_2 - 0.5053X_3 - 0.4583X_4, \text{ and} \\ \text{PC}_2 = 0.647X_1 + 0.1138X_2 + 0.0614X_3 + 0.7514X_4$$

where X_1 is leaf N; X_2 is leaf P_n ; X_3 is leaf ethylene production and X_4 is square ethylene production.

Coefficients of PC1 (eigenvectors) leaf P_n and N contents were both positive, while leaf and square concentrations were negative. Significantly higher values of eigenvectors for P_n and leaf ethylene indicated that PC1 is an index of good plant health with higher P_n and lower ethylene production in cotton leaves, and it mainly separated the plants on the basis of WL and NWL treatments (Fig. 8b). Under NWL conditions, both cultivars were grouped together (the right hand side of the axis; Fig. 8a) indicating a similar behaviour of cultivars irrespective of AVG treatment. On the other hand, WL cotton plants were grouped to the left hand side of the axis. It shows that NWL plants had higher leaf P_n and N compared with WL plants, while higher tissue ethylene synthesis in non-AVG treated WL plants was mainly attributed to the variance in the data set for this treatment.

The response of two cultivars to applied AVG, however, varied under WL conditions. For example, under WL

Table 4 Post-waterlogging and post-recovery changes in C and N concentrations of upper cotton leaves under waterlogging and AVG treatment

Treatment	Post waterlogging nutrient content						Post recovery nutrient content												
	% leaf DW			mg cm ⁻² leaf area			C/N			% leaf DW			mg cm ⁻² leaf area			C/N			
	N	C		N	C		N	C		N	C		N	C		N	C		
NWL	3.30 ± 0.4	41.22 ± 3.4	0.24 ± 0.02	2.96 ± 0.2	12.43		3.53 ± 0.4	41.97 ± 3.5	0.28 ± 0.02	3.24 ± 0.3	11.89								
NWL + AVG	3.87 ± 0.3	41.18 ± 3.6	0.28 ± 0.03	3.02 ± 0.3	10.64		3.41 ± 0.5	41.00 ± 3.4	0.29 ± 0.03	3.51 ± 0.4	12.02								
WL	2.75 ± 0.2	40.53 ± 3.7	0.20 ± 0.02	3.15 ± 0.4	15.55		2.48 ± 0.3	41.78 ± 3.5	0.35 ± 0.03	4.09 ± 0.3	16.85								
WL + AVG	2.43 ± 0.3	40.82 ± 3.6	0.22 ± 0.02	3.45 ± 0.4	15.87		2.41 ± 0.3	41.39 ± 3.6	0.26 ± 0.02	4.74 ± 0.4	17.17								
Cultivar																			
Sicot 71BRF	2.95 ± 0.2	41.63 ± 3.5	0.25 ± 0.03	3.49 ± 0.3	13.73		2.89 ± 0.3	41.73 ± 3.5	0.26 ± 0.02	3.60 ± 0.3	14.44								
LA 887	3.23 ± 0.3	40.25 ± 3.5	0.26 ± 0.03	3.13 ± 0.3	12.18		3.03 ± 0.4	41.34 ± 3.4	0.30 ± 0.02	4.55 ± 0.4	13.64								
<i>ANOVA (F test P values were calculated for main treatment and interaction effects)</i>																			
Waterlogging	<0.0001	0.249	0.657	0.125	<0.0001		<0.0001	0.795	0.246	0.103	<0.0001								
Cultivar	0.046	0.004	0.639	0.487	0.284		0.317	0.315	0.268	0.286	0.154								
AVG	0.357	0.781	0.357	0.331	0.110		0.473	0.080	0.757	0.880	0.352								
Waterlogging × cultivar	0.534	0.002	0.475	0.991	0.642		0.701	0.346	0.268	0.255	0.130								
Waterlogging × AVG	0.003	0.709	0.118	0.918	0.847		0.855	0.441	0.682	0.812	0.522								
Cultivar × AVG	0.357	0.393	0.908	0.564	0.687		0.865	0.620	0.764	0.856	0.827								
Waterlogging × cultivar × AVG	0.481	0.098	0.571	0.506	0.528		0.057	0.245	0.739	0.771	0.158								

The treatment data are mean ± SE of 8 individual plants (cultivars × replicates, 2 × 4), whereas cultivar data are mean ± SE of 16 individual plants (treatment × replicates, 4 × 4)

The significant (*P* < 0.05) effects are shown as bold in the ANOVA table

DW dry weight, NWL + AVG non-waterlogged + AVG treated, NWL non-AVG treated non-waterlogged, WL + AVG waterlogged + AVG treated, WL non-AVG treated waterlogged

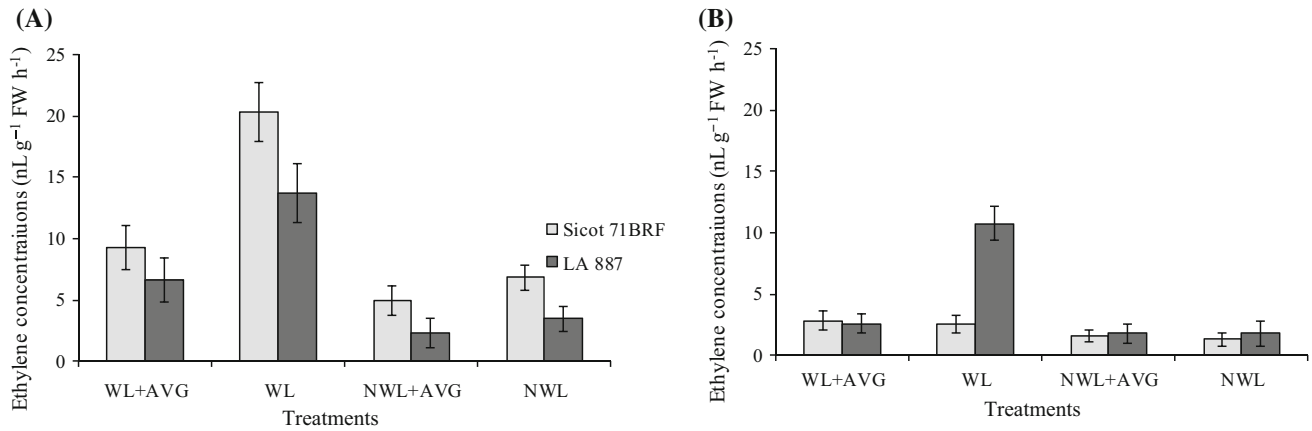


Fig. 4 AVG-induced changes in ethylene production from cotton **a** leaves and **b** squares at the end of the waterlogging period. Vertical bars represent \pm SE of the mean of each treatment (four replicates).

NWL non-waterlogged + non-AVG treated, NWL + AVG non-waterlogged + AVG treated, WL waterlogged + non-AVG treated, WL + AVG waterlogged + AVG treated

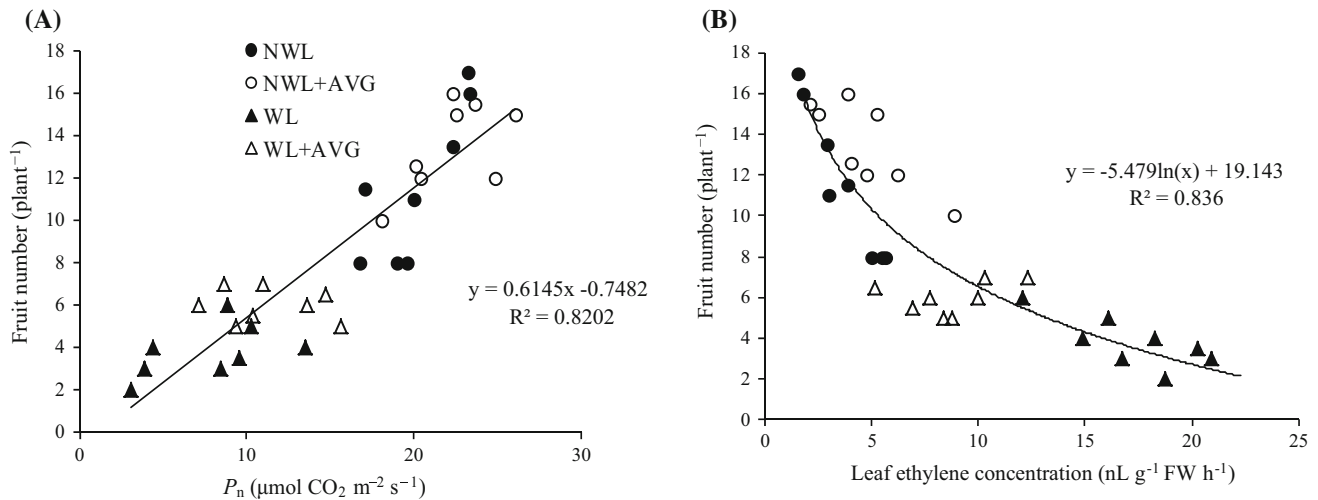


Fig. 5 Effect of leaf photosynthesis (**a**) and ethylene (**b**) on fruit number of cotton plants under waterlogging and AVG treatment. P_n = rate of photosynthesis. NWL non-waterlogged + non-AVG

treated, NWL + AVG non-waterlogged + AVG treated, WL waterlogged + non-AVG treated, WL + AVG waterlogged + AVG treated

treatment, non-AVG treated LA 887 plants were grouped away from the main axis showing a positive association with PC2 (top left corner of the axis), compared with Sicot 71BRF and AVG-treated LA 887 plants, which were grouped close to the main axis (bottom left corner of the axis, Fig. 8a). The eigenvectors values of PCA indicated that PC2 was an index of the subtle differences in square ethylene and leaf N content, and thus could explain the variable grouping of WL-LA 887 plants in terms of ethylene production from squares, which increased in non-AVG treated plants only.

As PC1 explained most of the variance in cotton under different treatments, we used it to estimate the effect of various growth components such as P_n , N and ethylene (square and leaf) on fruit production. Log-linear regression showed a highly significant relationship between the fruit

number and PC1 in both cotton cultivars, suggesting that higher P_n and N or lower ethylene (square and leaf) production lead to higher fruit production (Fig. 9). It also suggested that waterlogging-induced lower fruit production (either due to higher fruit abscission or inhibited fruiting node development) was mainly the result of higher ethylene synthesis, or impaired leaf P_n and N acquisition and these processes are inter-connected to affect plant response to soil waterlogging.

Discussion

The first objective of the study was to investigate the relationship between waterlogging sensitivity and ethylene production in WL cotton. Relatively higher waterlogging

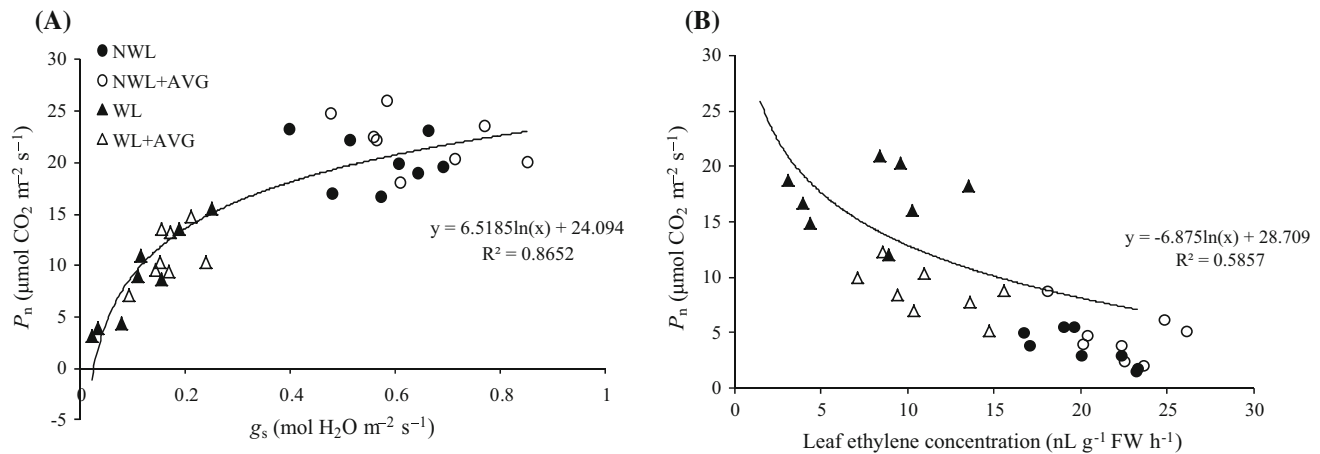


Fig. 6 Effect of leaf stomatal conductance (a) and ethylene (b) on rate of photosynthesis in cotton plants under waterlogging and AVG treatment. *NWL* non-waterlogged + non-AVG treated, *NWL + AVG*

non-waterlogged + AVG treated, *WL* waterlogged + non-AVG treated, *WL + AVG* waterlogged + AVG treated. P_n = rate of photosynthesis, g_s = stomatal conductance

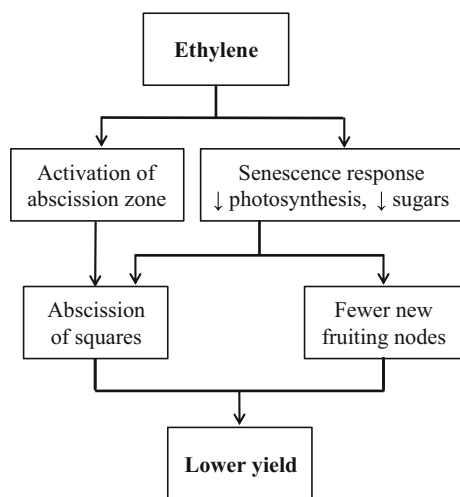


Fig. 7 Possible mechanism of ethylene-induced yield reduction in cotton under waterlogged environment

sensitivity of LA 887 compared with Australian cotton cultivars (Conaty et al. 2008) was attributed to growth environments i.e. LA 887 is cultivated in well drained soils compared with Australian cultivars, which are adapted to heavy clay soils. Both cotton cultivars experienced significant reduction in shoot growth, N acquisition and fruit development, while leaf ethylene synthesis increased with waterlogging. Cultivar LA 887 was relatively more sensitive to soil waterlogging and showed shoot growth and photosynthesis inhibition at 3rd DAW, compared with Sicot 71BRF, which exhibited less damage during the first week of waterlogging.

A rapid decline in leaf P_n of LA 887 compared with Sicot 71 BRF under waterlogging indicated that waterlogging sensitivity in LA 887 is more closely associated

with leaf photosynthetic parameters than the ethylene production rate. Although significantly higher ethylene production from the young squares in WL-LA 887 indicated that waterlogging sensitivity might be associated with ethylene production by squares rather than the leaves, and accelerated fruit abortion. On the other hand, rapid photosynthetic reduction in WL-LA 887 could be associated with the timing of ethylene production or its perception by plant tissues rather than the production rate. For example, ACC biosynthesis by the roots, or ethylene production in shoots of LA 887, might be accelerated earlier than in Sicot 71BRF; further studies are needed to confirm this hypothesis.

Contrary to the previous studies, where photosynthetic inhibition in WL cotton was independent of stomatal closure (Ashraf et al. 2011; McLeod 2001); we observed a parallel drop in leaf g_s of WL plants, suggesting involvement of stomatal closure in photosynthetic inhibition by limiting intracellular CO_2 supply (Malik et al. 2001). Waterlogging-induced P_n and g_s inhibition has been reported in many waterlogging-sensitive crops (Christianson et al. 2010; Meyer et al. 1987) but the exact mechanism of this closure is still unknown. WL plants exhibited stomatal closure, yet leaf turgidity was unable to be maintained and the plants started wilting at 7th DAW, indicating that it is not the higher transpiration causing leaf wilting but restricted water supply from WL roots that stimulated stomatal closure (Hebbar and Mayee 2011). The significant drop in *LWC* of WL plants also affirmed that impaired root hydraulic conductance and lower water supply from cotton roots affected leaf morphology. Waterlogging-induced cytoplasmic acidification of root aquaporins (Tournaire-Roux et al. 2003) might be responsible for impeded water uptake and root conductance, leading to stomatal closure

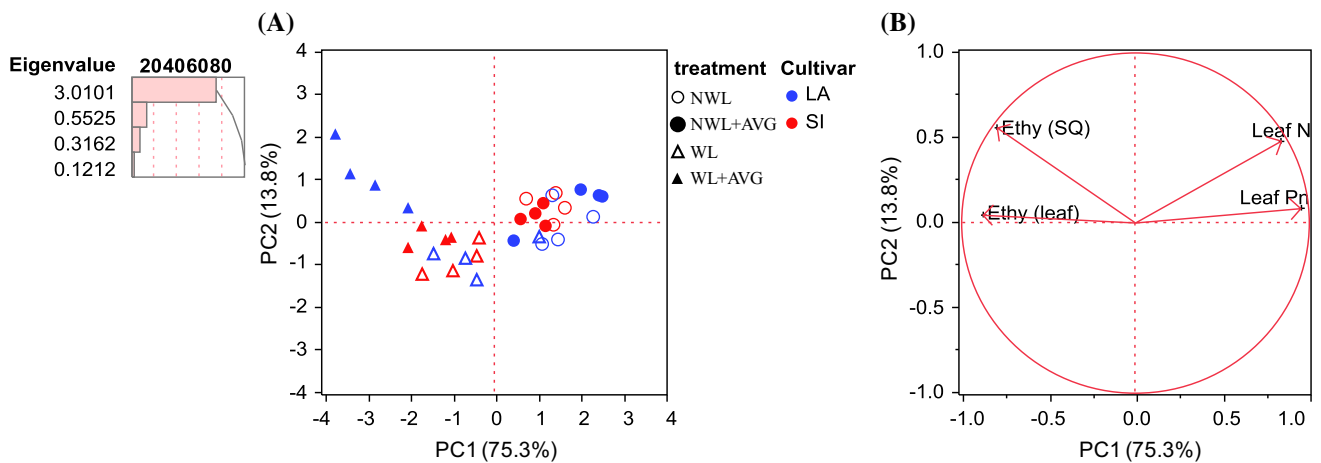


Fig. 8 Principal component analysis (PCA) of cotton cultivars Sicot 71 BRF and LA 887 subjected to various treatments i.e. waterlogging only (WL) and waterlogging + AVG (WL + AVG), non-waterlogged only (NWL) and non-waterlogged + AVG (NWL + AVG); **a** score plot showing distribution of treatment in relation to each other, with an adjusted value for the mean and standard deviation and **b** loadings

plot showing two-dimensional loadings of various variables i.e. *Ethy (leaf)* ethylene concentration youngest fully expanded leaves, *Ethy (SQ)* ethylene concentrations in young squares, *Leaf Pn* rate of photosynthesis in youngest fully expanded leaves, *Leaf N* nitrogen concentrations (mg g^{-1} leaf dry weight) in upper cotton leaves (top five nodes)

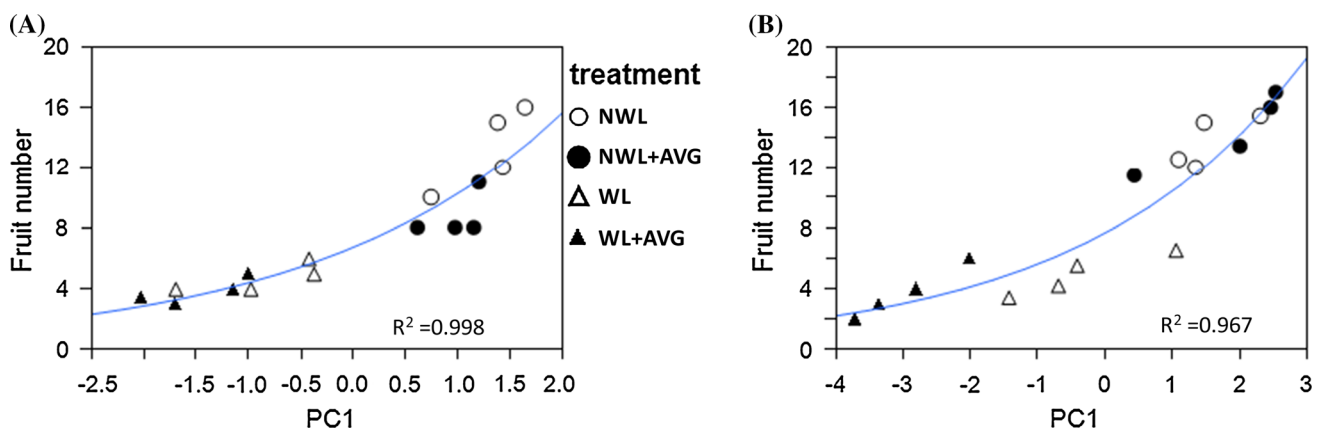


Fig. 9 Generalised log-linear regressions fitted for the first principal component (PC1) and cotton fruit number per plant to predict fruit production in cotton cultivars Sicot 71BRF (a) and LA 887 (b) under

various treatment conditions i.e. waterlogging only (WL-NA) and waterlogging + AVG (WL-A), non-waterlogged only (NWL-NA) and non-waterlogged + AVG (NWL-NA)

(Else et al. 2001). In addition, the role of ethylene in regulating stomatal closure cannot be ruled out e.g. ethylene can stimulate abscisic acid-driven stomatal closure (Ahmed et al. 2006).

Soil waterlogging significantly reduced N contents in upper plant leaves, and the plants were unable to compensate N acquisition after 1 week of recovery. Limited or no N recovery in WL cotton 7 days after termination of waterlogging indicated reduced N availability in WL soils, thus additional post-waterlogging N fertilisation may enable plants to recover N acquisition (Hodgson and MacLeod 1987; Milroy et al. 2009). In addition, inhibited N acquisition in WL cotton plants could be the result of waterlogging-induced root growth inhibition and increased root ACC concentration affecting nitrate assimilation and

biosynthesis of organic compounds (Bloom et al. 2010; Kawakami et al. 2012). Exogenously applied ACC significantly halted root development and N acquisition by down-regulating the expression of genes (*BnNrt2.1*) involved in N metabolism (Leblanc et al. 2008). On the other hand, no significant change in leaf N_{area} of WL plants suggested a parallel reduction in leaf growth and SLA that masked N deficiency in plants (Singh et al. 2013; Taub and Wang 2008). Previous studies also suggested that waterlogging could inhibit SLA and leaf N concentration in plants, without influencing leaf N_{area} (Gardiner and Krauss 2001; González et al. 2009).

In addition to its potential role in stomatal closure and photosynthetic reduction (Ahmed et al. 2006; Pallas and Kays 1982), elevated ethylene synthesis could regulate

synthesis of enzymes (pectinase and cellulase) responsible for abscission of squares and young bolls (Guinn 1982). In the present study, WL cotton plants retained 3–4 times fewer fruits than control plants with a higher leaf ethylene production suggesting that accelerated ethylene synthesis in WL cotton inhibited fruiting node development and increased fruit abscission. Lower final boll production was the major reason of yield reduction in WL cotton, caused by a combination of inhibited fruiting node development and fruit retention. Our data are consistent with Bange et al. (2004) who found that 15 % reduction in main-stem node number could cause 20–30 % lower fruit number in WL cotton. Ethylene-induced growth inhibition in WL cotton could be result of increased leaf shedding and photosynthetic inhibition. Significant negative association of leaf ethylene concentrations with P_n also supported the view that elevated ethylene production from cotton tissues reduced plant yield either directly through accelerating fruit abortion and/or indirectly by limiting node and subsequent fruiting site development.

The role of anti-ethylene agents has been suggested for improving plant tolerance to abiotic stresses (Kawakami et al. 2010). In the present study, AVG application increased total fruit number and retention by blocking ethylene biosynthesis in cotton tissues under WL as well as NWL conditions. A strong negative correlation between ethylene (leaf) and fruit numbers revealed that higher ethylene production reduces the fruit number in cotton, while blocking ethylene biosynthesis by AVG promoted fruit retention. Similar positive effects of AVG on cotton growth and yield have already been reported under WL (Bange et al. 2010) and NWL conditions (Brito et al. 2013). Although AVG applied to WL plants reduced ethylene production rate to almost half to non-AVG treated plants, the ethylene levels in WL plants were still higher than the NWL plants. It indicates that AVG possibly restricted the ethylene production to just below the threshold level of damage, thus further yield improvement could be expected by terminating ethylene biosynthesis or perception in cotton through transgenic techniques. A degree of success in regulating ethylene production (70 % reduction) and waterlogging tolerance have been achieved through development of transgenic tomato plants over-expressing ACC deaminase (an enzyme that cleaves ACC) activity (Grichko and Glick 2001) but no information is available in cotton.

In addition to increasing fruit retention, AVG had a positive effect on growth of WL and NWL plants; possibly through increasing N acquisition and photosynthesis (Khan et al. 2014). Positive effects of AVG on leaf P_n and g_s were more obvious in LA 887, where it significantly improved P_n and g_s during the late waterlogging period (7th DAW), signifying its role in plant survival under severe stress. A

positive effect of AVG on leaf growth and chlorophyll contents of drought-stressed wheat has also been reported (Beltrano et al. 1999). Comparatively lower *LWC* in AVG-treated NWL cotton plants indicated some role of ethylene in regulating stomatal behaviour, as AVG blocked ethylene accumulation and consequently reduced *LWC*. However, no significant improvement in stomatal conductance of AVG-treated cotton plants under WL conditions, suggested that AVG increased *LWC* via changes to root physiology without influencing stomatal behaviour. Higher ACC concentration has been found to induce structural and functional damage in roots (Leblanc et al. 2008), while AVG may reverse the damage by blocking ACC biosynthesis.

No significant recovery of WL plants in terms of growth or fruit development after termination of waterlogging indicated an irreversible damage to cotton. On the other hand, AVG more effectively improved the leaf growth, biomass accumulation and fruit retention of WL plants once the waterlogging was terminated, suggesting that AVG led to reversal of otherwise terminal damage. Khan et al. (2014) proposed that AVG could protect plants from waterlogging-induced injury by up-regulating the biosynthesis of metabolites involved in stress tolerance (glycinebetaine and methionine) but this remains to be confirmed as a mechanism for recovery.

Conclusions

We found that long-term soil waterlogging restricted the N acquisition and accelerated ethylene accumulation in cotton leaves. Lower N concentrations in leaves impaired photosynthesis, which in turn inhibited shoot growth, node and fruit development. Waterlogging also increased leaf and fruit abscission through inducing higher ethylene production in cotton tissues, while AVG increased leaf and fruit retention by blocking ethylene biosynthesis. However, limited role of AVG on shoot growth and nutrient uptake of WL cotton suggested that blocking ethylene biosynthesis alone is not adequate to mitigate waterlogging tolerance in cotton. An integrated approach of soil and fertiliser management along with AVG application could be more effective in ameliorating waterlogging-induced damage in cotton.

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Conflict of interest All the authors of the manuscript certify that they have no affiliations with, or involvement in any organization or entity with any financial in the subject matter or materials discussed in the manuscript.

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