Australian Journal of Crop Science AJCS
AJCS 7(2):241-248 (2013) ISSN:1835-2707

Intensified *Alternaria* spot disease under potassium deficiency conditions results in acceleration of cotton (*Gossypium hirsutum* L.) leaf senescence

Jingqing Zhao[1], Fuqiang Zhao[2], Guiliang Jian[1], Yuxia Ye[2], Wenwei Zhang[1], Jiasheng Li[2], Fangjun Qi[1*]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2, West Yuan Ming Yuan Road, Beijing 100193, PR China
[2]Agricultural Scientific Research Institute of Xinjiang Production and Construction 7th Agricultural Division, Kuitun City, Xinjiang, 833200, PR China

***Corresponding author: fjqi@ippcaas.cn**

Abstract

Premature senescence in cotton has been occurring at increasing frequencies and causes serious reduction in yield and quality. In this study, the relationship between potassium (K) deficiency and *Alternaria alternata* infection, causing leaf senescence in cotton, was investigated. All experiments were performed under controlled laboratory conditions, in which cotton seedlings were treated with various concentration of K for 10 days, followed by inoculating the third and fourth true leaves with *A. alternata.* Subsequently, both physiological indices reflecting leaf senescence and disease index were measured. The results showed that cotton leaves treated with K deficiency were more susceptible to *A. alternata* in two aspects. First, the appearance of disease lesions were emerged 1-4 days earlier in cotton leaves treated with various K deficiencies compared to control. Secondly, the disease lesions developed continuously and expanded in treated leaves showing more disease lesions, the size of which increased with decreasing concentrations of K in a dose-dependent manner. In addition, the symptoms of cotton leaf senescence were pronounced in these infected leaves. Acceleration of leaf senescence also reflected in various physiological indices such as increase in electrolyte leakage and MDA content, significant decrease in chlorophyll content and reduction of Fv/Fm ratio. These results provide evidence that the combination of K deficiency and *A. alternata* infection accelerates cotton leaf senescence. Understanding the key factors causing and promoting cotton leaf senescence should greatly help the management of cotton crops by preventing premature leaf senescence.

Keywords: cotton; potassium deficiency; Alternaria spot disease; leaf senescence.
Abbreviations: MDA, malondialdehyde; Fv/Fm, maximum photosystem Ⅱ quantum yield; PDA, potato-dextrose agar.

Introduction

Cotton (*Gossypium hirsutum* L.), the most important fiber producing crop, is a perennial, indeterminate plant that is cultivated as an annual in agronomic production systems. However, premature leaf senescence in cotton has been occurring at increasing frequencies in many cotton-growing countries particularly in China, which causes serious reduction in yield and quality (Dong et al., 2005a). Premature leaf senescence results in reduced lint yield and poor fiber properties, constituting an important constraint to cotton yield and quality (Wright, 1998). Loss in lint yield can be as high as 20% in USA (Cassman et al., 1989). In China, about 20-50% reduction in yields was recorded during the years of severe premature leaf senescence (Liu et al., 2010). In recent years premature leaf senescence has become an important barrier for cotton production in China.

The factors causing premature leaf senescence in cotton are still unclear. As reported previously, premature leaf senescence is the result of a complex interaction between developmental age of crop and environmental factors such as nutrient deficiency (Wright, 1998), drought (Radin and Ackerson, 1981; Marani et al., 1985), elevated carbon dioxide (Kakani et al., 2004), and salinity (Ganieva et al., 1998), or internal factors such as phytohormones (Dong et al., 2005c; Yong et al., 2000). As reported in Australia, large boll load and potassium (K) deficiency are likely the most important factors, causing premature leaf senescence in cotton (Wright, 1999; Pettigrew, 2003).

Potassium (K) is one of the major mineral nutrients impacting cotton growth, development, formation of lint yield and fiber quality (Kerby and Adams, 1985; Cassman et al., 1990). In recent years, the Chinese cotton production has encountered an increase in K deficiency (Zhan et al., 2012; Dong et al., 2005b). Compared to other field crops, cotton appears to be more sensitive to K availability and shows symptoms of K deficiency much earlier, when grown in soil limited in K (Cope, 1981; Cassman et al., 1989). Many studies have revealed that K deficiency occurs along with premature senescence, which is characterized by early chlorophyll degradation (Li et al., 2012) and reduced photosynthesis (Bednarz et al., 1998; Zhao et al., 2001) in mature leaves, that results in a lower lint yield (Pettigrew, 2003) and poor fiber quality (Pettigrew et al., 1996). In addition, K deficiency weakens the leaves and making them susceptible to fungal infection (Amtmann et al., 2008).

Alternaria alternata is not usually considered as a primary pathogen of cotton but is a weak fungal pathogen infecting young and senescent cotton plants (Watkins, 1981). Alternaria leaf spots on cotton are known to occur in most

cotton growing countries (Cotty, 1987) where at least two pathogens, *A. macrospora* and *A. alternata* are considered to be the main causative agents (Bashan and Hernandez-Saavedra, 1992). Although, there is no report of the impact of this disease on cotton production in China, a 37% yield loss has been reported in India (Padaganur et al., 1989) and 25% in Israel (Bashi et al., 1983). Most of upland cultivars are normally not susceptible to severe infection by these two pathogens, they may become predisposed to infection by several stress factors such as nutrient imbalance and water stress (Miller, 1969), chilling stress (Zhao et al., 2012), or nematode attack (Sciumbato and Pinckard, 1974). In general, infection of upland cultivars by *Alternaria spp*. was more severe under conditions of K deficiency (Hillocks and Chindoya, 1989), and the occurrence of Alternaria disease was often accompanied with leaf senescence (Ishida and Kumashiro, 1988). The combination of K deficiency and *A. alternata* infection on cotton leaf senescence is still unknown. Although the effect of K deficiency on senescence has been studied intensively, there are no reports yet on the cumulative effect of K supply in nutrition and *A. alternata* infection on leaf senescence. Therefore, the objectives of this study were: (1) to evaluate the effect of K deficiency on *A. alternata* infection in cotton leaves and (2) to evaluate the effect of K deficiency and *A. alternata* infection on cotton leaf senescence. The results clearly showed that potassium deficiency increased the intensity of *A. alternata* infection and these two combined stresses accelerated cotton leaf senescence.

Results

Influence of K deficiency on A. alternata infection and disease development

In control plants grown with adequate K, small necrotic lesions appeared on day 7 after inoculation with *A. alternata*, but the lesions hardly expanded and developed. This suggests that cotton leaves grown with adequate K in their nutrition were resistant to *A. alternata* infection to a certain degree (Fig 1, control leaves for XLZ13 and XLZ33 inoculated with *A. alternata*). The cotton leaves exposed to K deficiency were more susceptible to *A. alternata* in two respects: First, disease lesions appeared earlier in leaves exposed to K deficiency than in controls (at 6-7 days, 4-5 days and 3 days for 0.1 mM, 0.02 mM, 0 mM K deficiency treatments, respectively, compared to day 7 in control) (Table 1); second, the disease lesions developed continuously and expanded in leaves exposed to K deficiency. In addition, size of disease lesions increased with decreasing concentrations of K in a dose-dependent manner (Fig 1).

The disease index also indicated that K deficiency could promote the development of leaf spot disease. As shown in Table 1, the disease index detected was only 9.0 for control leaves of XLZ13 on day 15 after inoculation, while the index was significantly higher in all K deficiencient treatments with the values of 26.0, 58.0, 95.0 for 0.1 mM, 0.02 mM, 0 mM K concentrations, respectively.

In addition, K deficiency treatments had similar effects on leaf spot disease development for XLZ33, although differences in progress and severity of the leaf spot disease were observed between the XLZ13 and XLZ33 cultivars. Compared to XLZ13, disease lesions appeared one day later in XLZ33 leaves under K deficiency of 0.1 mM and 0.02 mM, and the detected disease index for XLZ33 was significantly lower in all K deficiency treatments (Table 1). These differences indicated that cultivars that are resistant to leaf senescence may also be resistant to leaf spot disease under K deficient conditions.

Change of electrolyte leakage and malondialdehyde (MDA) content during Alternaria disease progression is promoted by K deficiency treatments

Electrolyte leakage and MDA content are routinely used as indicators to assess the integrity of cell membranes (Kramer et al., 1991). Both electrolyte leakage and MDA content were relatively stable (Fig 2a, 3a line 2, 4) in control cotton leaves grown without K deficiency. Inoculation with *A. alternata* did not cause any significant change in electrolyte leakage and MDA content (Fig 2a, 3a, line 1, 3). This is consistent with the fewer lesions that developed in control leaves inoculated with *A. alternata*. These results also indicate that *A. alternata* did not infect cotton leaves under sufficient K supply and; therefore, could not cause permanent structural or functional damages in cell membrane that would eventually lead to cell death.

In both XLZ13 and XLZ33 cultivars exposed to K deficiency treatments, electrolyte leakage and MDA increased with decreasing concentrations of K from 0.1 to 0 mM on day 10 (Fig 2b-d, 3b-d). When the K deficiency is prolonged, electrolyte leakage and MDA content increased further (Fig 2b-d, 3b-d, line 2, 4). This indicates that K deficiency causes irreversible damaged to the cell membrane that eventually results in cotton leaf senescence as shown in Fig 1.

Susceptibility to infection was increased when leaves of both cultivars inoculated with *A. alternata* on day 10 after exposion to K deficiency (Fig 1). In addition, Alternaria spot disease intensified (Fig 1). A sharper increase in electrolyte leakage and MDA content (Fig 2b-d, 3b-d, line 1, 3) were also observed. As shown in Fig 2c, d and Fig 3c, d (lines 1, 3) on day 5 after inoculation (15 day after initiating K deficiency treatments), increase in electrolyte leakage and MDA content changed sharply when compared to non-inoculated leaves (Fig 2c-d, 3c-d, lines 2, 4). When exposed to 0.02 mM, 0 mM of K deficiencies and *A. alternata* inoculations, the electrolyte leakage in XLZ13 and XLZ33 cultivars increased to 85.2% and 78.9% on day 15 after inoculation (25 days after K deficiency treatments). These values were about 1.8 and 2.0-fold higher than single K deficiency treatments, and about 5.3 and 4.4-fold higher than healthy leaves with adequate K levels. Similarly, the MDA content increased to 67.2 nmol/g FW and 61.2 nmol/g FW in XLZ13 and XLZ33 cultivar on day 15 after inoculation (25 day after K deficiency treatments). These values were also 1.5 and 1.4-fold higher than sole K deficiency treatments, and about 3.1 and 2.7-fold higher than healthy leaves with adequate K levels. These observations are consistent with the previous reports, indicating that under severe K deficiency, disease symptoms such as high level of chlorotic lesions and visible yellowing are pronounced in leaves. We concluded that exposure to a combination of K deficiency and *A. alternata* infection causes more damage to cell membrane on leaves, when compared to K deficiency stress alone.

Changes in chlorophyll content is promoted by K deficiency treatments during Alternaria disease progression

Chlorophyll is an extremely important and critical biomolecule in photosynthesis that is required for light absorbance and light energy transformation. Chlorophyll degradation is a key indicator for senescence in plant leaves. As shown in Fig 4c-d (lines 2, 4) K deficiency resulted in a

Table 1. Influence of potassium (K) on occurrence and development of Alternaria disease.

K treatments	Initial day of appearance after inoculation (d)		Disease index at 15 days after inoculation	
	XLZ13	XLZ33	XLZ13	XLZ33
0.5 mM	7	7	9.0 a a	8.0 a a
0.1 mM	6	7	26.0 b a	19.0 b b
0.02 mM	4	5	58.0 c a	45.0 c b
0 mM	3	3	95.0 d a	85.0 d b

Cotton seedlings with four true leaves were treated for 10 days with nutrient solution containing various K concentrations. The third and fourth true leaves from each treatment were then inoculated by lightly brushing 1.2×10^4 conidial/mL inoculum which is a suspension of *A. alternata* from isolate A1 and continued growing in nutrient solutions with various K concentrations. Alphabets behind the disease index indicate significant differences with a *P* value of 0.05. The first letter indicates the comparison between different concentrations of K treatments and the second letters indicates the comparison between XLZ13 and XLZ33 cultivars

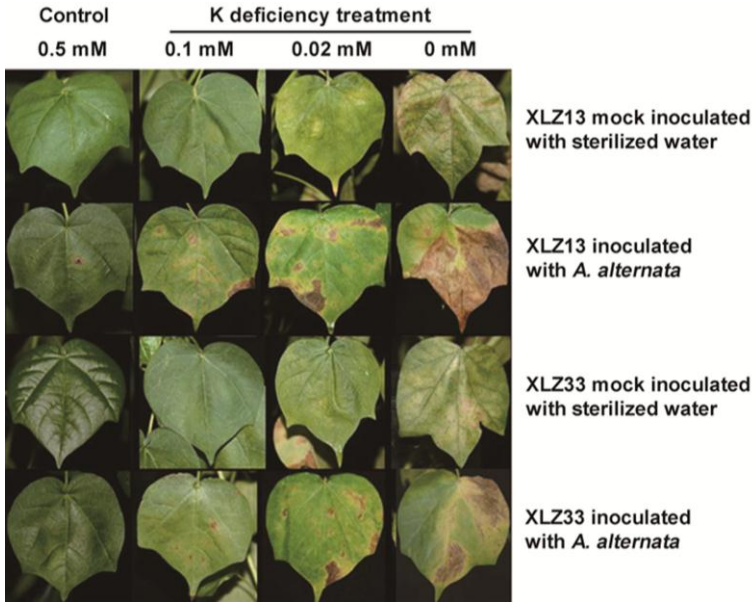

Fig 1. Appearance of Alternaria disease on leaves of cotton plants grown in adequate and potassium (K) deficient nutrient solutions. Cotton seedlings with four true leaves were treated with various K concentrations in nutrient solution for 10 days. The third and fourth true leaves from each treatment were then inoculated by lightly brushing 1.2×10^4 conidial/mL inoculum which is a suspension of *A. alternata* from isolate A1. Control inoculations were performed with sterilized water. Pictures were photographed on day 15 after inoculation.

decrease in cotton leaf chlorophyll content when compared to controls (Fig 4a, lines 2, 4), with the greatest decrease observed in the leaves treated with 0 mM K (Fig 4d, lines 2, 4). This decrease in chlorophyll caused by K deficiency was a time-dependent process, and extended if the cotton plants were maintained in K deficiency conditions for longer periods (Fig 4c-d, lines 2, 4). While an additional biotic stress (inoculation with *A. alternata*) was added to the K deficiency, the decrease in cotton chlorophyll content was more severe and sharp (Fig 4b-d, lines 1, 3) compared to the decrease caused by K deficiency alone (Fig 4b-d, lines 2, 4). These results indicate that prolonged K deficiency certainly caused cotton leaf senescence, which was accelerated when a secondary stress such as *A. alternata* infection occurred, resulting in Alternaria spot disease.

Changes in Fv/Fm ratio during Alternaria disease progression is promoted by K deficiency treatments

Fv/Fm value is the maximum quantum yield of the primary photochemical reactions in leaves adapted to dark (Lichtenthaler et al., 1998). As shown in Fig 5, changes in Fv/Fm values corresponded to changes in chlorophyll contents. K deficiency in leaves caused a significant decrease in Fv/Fm ratio (Fig 5c-d, lines 2, 4), indicating that K deficiency impaired the reaction centers of PSⅡ in energy utilization. When these leaves were further inoculated with *A. alternata* on day 10, a decrease in Fv/Fm ratio was observed on day 15 after inoculation, when compared to the non-inoculated leaves (Fig 5b-d, lines 1, 3). The Fv/Fm value in the control leaves of XLZ13 and XLZ33 cultivars were 0.394 and 0.455, while in leaves with 0 mM K deficiency and *A. alternata* infection were 0.052 and 0.146, which were lower than controls (Fig 5d). These results suggest that the fluorescent level is decreased to near-zero levels when coinciding with visual lesion development in the leaves that severely exposed to both stresses.

Discussion

Premature leaf senescence is one of the important barriers for cotton production. Therefore, the investigation on key factors causing cotton leaf senescence has been considerably emphasized in recent years (Dong et al., 2005a). Majority of studies on the senescence response of cotton leaves to abiotic and biotic stresses focused on a single stress applied to plants under controlled conditions. However, a number of different stresses factors can occur simultaneously in the field and cause a cumulative effect.

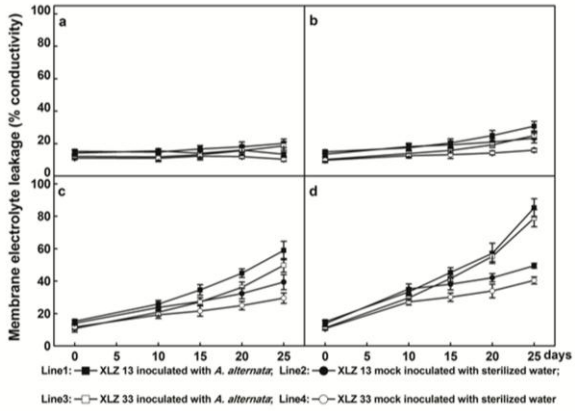

Fig 2. Changes in electrolyte leakage during Alternaria disease progression promoted by potassium (K) deficiency treatment. a: Control cotton plants sustained growing at 0.5 mM K supply in nutrient solution; b, c, d: Cotton plants treated with low K supply of 0.1 mM, 0.02 mM, 0 mM. Inoculation were performed on day 10 after K deficiency treatments with 1.2×10^4 conidial/mL inoculum which is a suspension of *A. alternata* from isolate A1, and then continued growing under various K supply solutions. All collected data (average values ± standard deviation (SD) with 6 replicates) are presented as relative values of membrane electrolyte leakage.

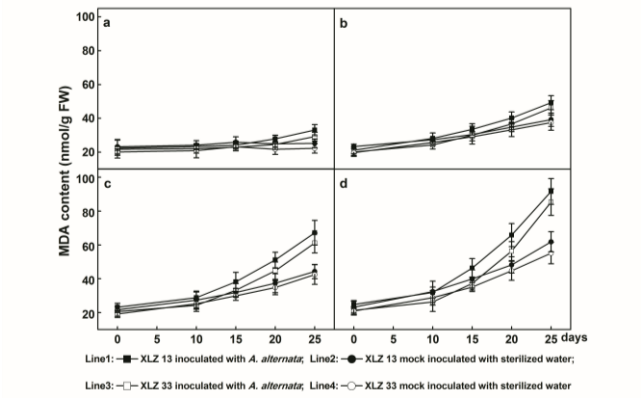

Fig 3. Changes in malondialdehyde (MDA) content during Alternaria disease progression promoted by potassium (K) deficiency treatment. a: Control cotton plants sustained growing at 0.5 mM K supply in nutrient solution; b, c, d: Cotton plants treated with low K supply of 0.1 mM, 0.02 mM, 0 mM. Inoculations were performed on day 10 after K deficiency treatments with 1.2×10^4 conidial/mL inoculum which is a suspension of *A. alternata* from isolate A1, and then continued growing under various K supply solutions. All collected data are presented as average values ± standard deviation (SD) with 6 replicates.

In this study, the additive effect of K deficiency and *A. alternata* infection on cotton leaf senescence was investigated. The presented data clearly shows that *A. alternata* infection was more pronounced in cotton leaves under K deficiency, causing severe leaf spot disease. The severity of infection was greatest under zero K supply, but decreased rapidly as K concentration increased to 0.5 mM. These results obtained under controlled laboratory conditions were consistent with those reported by Harris (1997) in the field, where occurrence of Alternaria disease was usually associated with low soil K. Similar results were obtained with *Helminthosporium cynodontis* on bermudagrass (Matocha and Smith, 1980), and onion infected with the downy mildew fungus *Peronospora destructor* (Develash and Sugha 1997). It is well known that K levels in plant nutrition impact a number of physiological, metabolic and hormonal processes that are crucial for plants susceptibility and sensitivity to pathogens. Although it is not known how K deficiency increases disease susceptibility in plants, nutritional deficiency has been reported to reduce host resistance by increasing cell permeability and reducing the ability of photosynthesis (Amtmann et al., 2008). This is shown in Fig 2, 5 in this study.

Chlorophyll loss is a widely used marker for the leaf senescence syndrome (Hörtensteiner and Matile, 2004) in addition to physiological indices such as impaired photosynthesis (Gergoff et al., 2010), increase in membrane electrolyte leakage and lipid peroxidation (Woo et al., 2004). In the present study, *A. alternata* inoculation of cotton grown under K deficiency appeared yellowish leaves (Fig 1) with an observable increase in membrane electrolyte leakage (Fig 2), MDA content (Fig 3), reduced chlorophyll content (Fig 4) and Fv/Fm ratio (Fig 5). Senescence in these leaves was accelerated than in the non-inoculated control leaves, suggesting that senescence syndrome is caused by the combination of two stress factors. It has been reported that pathogen proliferation after successful colonization has an influence on K uptake and partitioning (Walters and Bingham, 2007). Under field conditions, *Verticillium dahliae* infection also reduced the potassium content of cotton foliage (DeVay et al., 1997). Alternaria disease progression may affect on the K mobility and its uptake and assimilation, thereby increasing the severity of K deficiency, which in turn, can promote Alternaria disease. Thus, the two stress factors interact to accelerate senescence in cotton leaves. It is reasonable to presume that similar interaction would exist in this case.

In our previous field studies we found that XLZ13 was more resistant to cotton premature leaf senescence when compared to XLZ33 (Liu et al., 2010). In this study, we also observed differences between the cultivars in the development and severity of K deficiency and Alternaria disease symptoms. As shown in the results, differences in physiological indices were observed between these two cultivars. In XLZ33 leaves, there was a delay in the onset of Alternaria disease, relatively lower disease index and lower level of cell impairment, compared to XLZ13. It is yet to be determined if the cultivar that is resistant to premature senescence is also resistant to K deficiency.

This work clearly indicates the adverse effects of K deficiency, are beneficial to Alternaria disease pathogen infection, and at last the cotton leave senescence was accelerated. In this study, the mechanisms of interaction between K deficiency/Alternaria disease and leaf senescence was not addressed at the molecular level although, it is highly likely that changes in metabolic, hormonal and signaling pathways caused independently by K nutrition or Alternaria disease affect each other (Amtmann et al., 2008). Further molecular studies on K nutrition-disease interaction would open new avenues to alter fertilizer composition for field application and disease control, which should have a great impact in the reduction of premature senescence in cotton.

Understanding the effects of K deficiency and Alternaria disease in premature senescence of cotton leaves would greatly help in the management of cotton crops. Based on this, recommendations could be made to alleviate premature senescence in cotton. These include elimination of K

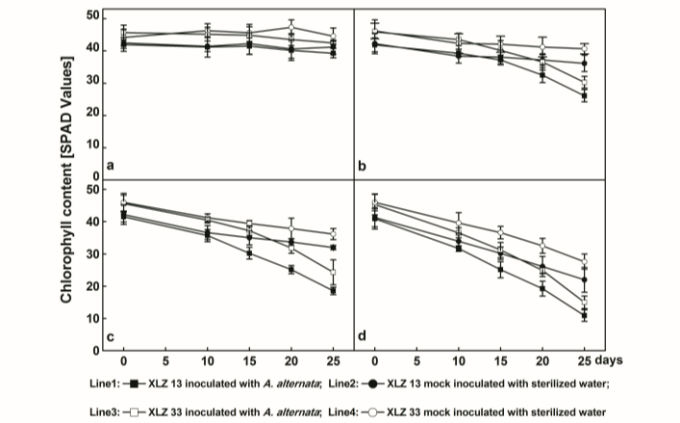

Fig 4. Changes in chlorophyll contents during Alternaria disease progression promoted by potassium (K) deficiency treatment. a: Control cotton plants sustained growing at 0.5 mM supply in nutrient solution; b, c, d: Cotton plants treated with low K supply of 0.1 mM, 0.02 mM, 0 mM. Inoculation were performed on day 10 after K deficiency treatments with 1.2×10^4 conidial/mL inoculum which is a suspension of *A. alternata* from isolate A1, and then continued growing under various K supply solutions, respectively. All data are presented as average values ± standard deviation (SD).

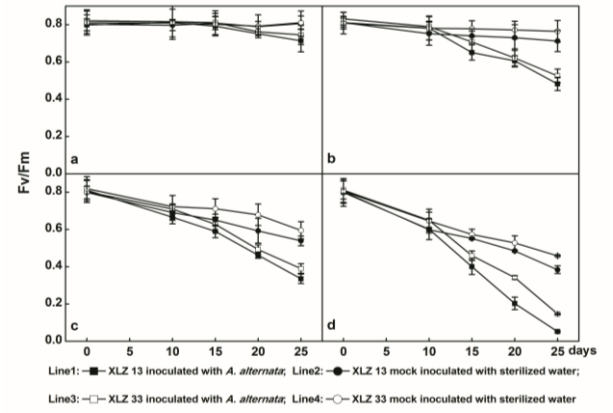

Fig 5. Changes in Fv/Fm ratio during Alternaria disease progression promoted by potassium (K) deficiency treatment. a: Control cotton plants sustained growing at 0.5 mM K supply in nutrient solution; b, c, d: Cotton plants treated with low K supply of 0.1 mM, 0.02 mM, 0 mM. Inoculations were performed on day 10 after K deficiency treatments with 1.2×10^4 conidial/mL inoculum which is a suspension of *A. alternata* from isolate A1, and then continued growing under various K supply solutions, respectively. All data are presented as average values ± standard deviation (SD).

deficiency by proper soil K fertilization and foliar application of K, prevention of cotton Alternaria disease occurrence and progression, and plantation of cultivars that are resistant to premature leaf senescence and less sensitive to low K levels. Although premature leaf senescence occurs in the late growth stage, our experiments revealed the relationship between K deficiency, *A. alternata* infection and cotton leaf senescence in the seedling stage. It remains to be seen if the recommended approaches are effective for controlling premature leaf senescence in cotton in the field.

Materials and methods

Plant and fungi

Cultivar XLZ33, resistant to premature leaf senescence and cultivar XLZ13, which is susceptible were provided by the Agricultural Scientific Research Institute of Xinjiang Production and Construction 7th Agricultural Division, Kuitun City, Xinjiang. Premature leaf senescence for two upland cotton (*Gossypium hirsutum* L.) cultivars were confirmed in our previous study (Liu et al., 2010). The experiment was performed in a growth chamber with 14h light/10h dark conditions at 30±2/22±2 °C, and 65-75% humidity with 400 μmol photons m^{-2} s^{-1} photosynthetically active radiation. Seeds were surface-sterilized for 2 min in 75% (v/v) ethanol and 10 min in 6% (v/v) sodium hypochloride solution, thoroughly washed in sterilized water, and germinated on two sheets of moist filter paper at 28℃ in the growth chamber. After emergence (4 d after germination), uniform seedlings were cultured hydroponically by transferring to plastic pots filled with 1.2 L of 1/2-strength modified Hoagland's solution. The constituents of the nutrient solution were (mM) 2.5 Ca $(NO_3)_2$, 1 $MgSO_4$, 0.5 $(NH_4)H_2PO_4$, 2×10^{-4} $CuSO_4$, 1×10^{-3} $ZnSO_4$, 0.1 Fe Na EDTA, 2×10^{-2} H_3BO_3, 5×10^{-6} $(NH_4)_6Mo_7O_{24}$ and 1×10^{-3} $MnSO_4$, 0.5 KCl, pH 6.5 adjusted with 1M NaOH. For each cultivar, there were forty pots with six plants each. All solutions were changed twice per week. De-ionized water was then added daily to replace the water loss by evapo-transpiration. Air was bubbled into the solution to provide O_2 and achieve nutrient homogeneity.

The *A. alternata* isolate A1 was isolated from cotton leaf spot disease lesions and maintained in our laboratory on potato-dextrose agar (PDA) slants at 4 ℃ (Li et al., 2011).

K deficiency treatments and A. alternata inoculation procedure

All treatments were performed, when plants reached the fourth true leaf stage. Treatments consisted of: (1) providing the complete nutrient solution described above, (2) nutrient solution with 0.1 mM KCl, (3) nutrient solution with 0.02 mM KCl, (4) nutrient solution with 0 mM KCl.

The inoculum of *A. alternata* isolate A1 was prepared as previously described (Bashan 1984). Fourteen-day-old cultures grown on PDA slants at 26 to 28℃ were scraped with a sterile inoculating loop, and then suspended in sterile deionized water. The suspension was further filtered through cheesecloth, diluted to the optimal concentration of 1.2×10^4 conidial/mL, and subsequently used for inoculation. Spore concentration was estimated under a microscope with a counting chamber (Büker type).

The third and fourth true leaves of 60 seedlings from various K treatments were inoculated with the freshly prepared conidial suspension on day 10 after K treatment, while control leaves were treated with sterilized water. Inoculation was accomplished by lightly brushing the leaves with a small brush containing spore suspension at a rate of 1 mL/leaf. Inoculated leaves were then covered in separate, loosely sealed, pre-wet polyethylene bags (Bashan et al., 1991). All treatments were randomly design with three replications.

Alternaria disease evaluation

After inoculation, each leaf was inspected daily for the appearance of spot disease symptoms.

For evaluation of Alternaria disease, infected leaves were photographed on day 15 after inoculation with a standard digital camera. The collected digital images were analyzed for the affected leaf area with the software Image-Pro Plus (Image-Pro Plus, Version 6.0, Media Cybernetics, L. P, Silver Spring, MD) (Fourie et al., 2009). The degree of Alternaria disease was divided into six grades with scores ranging from 0 to 5 based on the percentage of affected leaf area as previously described (Mehta 1998) with slight modifications: 0 = no disease; 1 = minute pinhead size spots, less than 1% leaf tissue diseased; 2 = small brown to dark brown necrotic lesions, 1 to 5% diseased; 3 = necrotic lesions coalescing, 5 to 10% diseased; 4 = necrosis lesions coalescing, 10 to 20% diseased; and 5 = lesions coalescing, >20% diseased, and / or with desiccated and abscised leaves. The disease index was calculated according to the following formula:
Disease index = [(∑ disease score × number of infected leaves for each score) / total inoculated leaves × highest score (5)] ×100.

Biochemical assays

All measurements were performed at room temperature of about 25 °C. The measured leaf areas were about 1-2 cm beyond the edge of the disease spot on the leaves.

Membrane electrolyte leakage determination

Membrane electrolyte leakage was determined by measuring electrolyte ion leaked from the leaves as described previously (Gómez et al., 2008). Briefly, 10-15 leaf discs excluding the main veins were washed and placed in 20 mL deionized water. Water conductivity was recorded at the beginning of the incubation (initial cond.) and after incubation for 3 hours with gentle shaking (cond. 3 h), at 25 °C. Then leaf discs were boiled for 5 min for the determination of maximum conductivity (max. cond.). Electrolyte leakage was calculated as: [(cond. 3 h − initial cond.) / (max. cond. − initial cond.)] × 100.

Malondialdehyde (MDA) measurements

Leaf segments were collected from an average of at least 10 leaves and immediately frozen in liquid nitrogen and stored at -80 °C until used for MDA measurements. The MDA content was determined according to methods primarily described by Peever and Higgins (Peever and Higgins, 1989), and modified by Lv et al (Lv et al., 2009). Cotton leaves were homogenized in 5 ml of 10% (w/v) trichloroacetic acid (TCA), and centrifuged at 12,000×g for 10 min. About 2 ml of clear supernatant was added to 4 ml of 0.6% (w/v) thiobarbituric acid (TBA, in 10% TCA) and the reaction mixture was incubated at 100 °C in a water bath for 15 min. The reaction was terminated at room temperature, and the absorbance of the supernatant at 450, 532 and 600nm was determined with a spectrometer. The concentration of MDA was calculated by the formula: C (μmol/L) = 6.45 (OD532 – OD600) - 0.56OD450. MDA content was further calculated and expressed in nmol/gFW.

Measurement of chlorophyll content

Relative chlorophyll content per unit leaf area was determined using a portable chlorophyll SPAD-502 (Minolta Crop, Osaka, Japan) which measures transmission of intact leaves at wavelengths of 650 and 940 nm, and the measuring area was 6 mm^2 (Humbeck and Krupinska, 2003). Calibrations show that relative SPAD values depend on chlorophyll content in a linear manner over a wide range. Each data point represents the mean value of ten independent measurements.

Measurements of photosystem II efficiency

Chlorophyll fluorescence measurements after adaptation to dark was carried out as described (Humbeck et al., 1996) using a chlorophyll fluorometer (Junior PAM, Walz, Effeltrich, Germany). Mean values of Fv/Fm ratio were based on ten independent measurements.

Experimental design and statistical analysis

Experiments were carried out in a completely randomized design with three replicates for each treatment. All experiments were repeated twice and data from the duplicate experiments were analyzed separately and in combination. Since results of the replicates analyzed separately and in combination were similar data from the two were combined (six replicates) and analyzed together using the SAS program (SAS Institute, Cary, NC, USA). For multiple comparisons, least significant differences (LSD) test was performed. Differences were considered significant at a probability level of $P<0.05$.

Conclusion

A. alternata infection was accelerated in cotton leaves exposed to K deficiency, causing severe leaf spot disease. Senescence was more acute in these leaves than the non-inoculated control leaves as measured by yellowing appearance, increase of membrane electrolyte leakage, MDA contents, decline in chlorophyll content and Fv/Fm ratio. This study clearly indicates that the Alternaria disease is the second main problem after K deficiency in predisposing of cotton leaves to premature senescence.

Acknowledgements

This work was supported by the National Science and Technology Supported Project (2012BAD19B05), and the Xinjiang Production and Construction Corps Fund Project (2011AB009, 2009JC004).

References

Amtmann A, Troufflard S, Armengaud P (2008) The effect of potassium nutrition on pest and disease resistance in plants. Physiol Plantarum 133: 682-691

Bashan Y (1984) Transmission of *Alternaria macrospora* in cotton seeds. J Phytopathol 110: 110-118

Bashan Y, Hernandez-Saavedra NY (1992) Alternaria-blight of cotton: Epidemiology and transmission. In: Chelkowski J, Visconti A (eds) Alternaria: biology, plant diseases and metabolites. Elsevier Scientific Publishers, Amsterdam, pp 233-266

Bashan Y, Levanony H, Or R (1991) Association between *Alternaria macrospora* and *Alternaria alternata*, casual agents of cotton leaf blight. Can J Bot 69: 2603-2607

Bashi E, Sachs Y, Rotem J (1983) Relationship between disease and yield in cotton fields affected by *Alternaria macrospora*. Phytoparasitica 11: 89-97

Bednarz CW, Oosterhuis DM, Evans RD (1998) Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. Environ Exp Bot 39: 131-139

Cassman KG, Kerby TA, Roberts BA, Bryant DC, Brouder SM (1989) Differential response of two cotton cultivars to fertilizer and soil potassium. Agron J 81: 870-876

Cassman KG, Kerby TA, Roberts BA, Bryant DC, Higashi SL (1990) Potassium nutrition effects on lint yield and fiber quality of acala cotton. Crop Sci 30: 672-677

Cope JT (1981) Effect of 50 years of fertilization with phosphorous and potassium on soil test levels and yields at six locations. Soil Sci Soc Am J 45: 342-347

Cotty PJ (1987) Evaluation of cotton cultivar susceptibility to Alternaria leaf spot. Plant Dis 71: 1082-1084

DeVay JE, Weir BL, Wakeman RJ, Stapleton JJ (1997) Effects of *Verticillium dahliae* infection of cotton plants (*Gossypium hirsutum*) on potassium levels in leaf petioles. Plant Dis 81: 1089-1092

Develash RK, Sugha SK (1997) Factors affecting development of downy mildew (*Peronospora destructor*) of onion (*Allium cepa*). Indian J Agr Sci 67: 71-74

Dong HZ, Li WJ, Tang W, Zhang DM (2005a) Research progress in physiological premature senescence in cotton. Cotton Sci 17: 56–60

Dong HZ, Tang W, Li ZH, Zhang DM, Li WJ (2005b) Morphological and physiological disorders of cotton resulting from potassium deficiency. Acta Bot Boreal-Occident Sin 25: 615-624

Dong HZ, Shu WH, Zhai XJ, Zhang BM, Liu F, Song GQ (2005c) The change and relationship of several endogenesis hormone in different organs of cotton plant. Acta Agr Nucl Sin 19: 62-67

Fourie PH, du Preez M, Brink JC, Schutte GC (2009) The effect of runoff on spray deposition and control of Alternaria brown spot of mandarins. Australas Plant Path 38: 173-182

Ganieva RA, Allahverdiyev SR, Guseinova NB, Kavakli HI, Nafisi S (1998) Effect of salt stress and synthetic hormone polystimuline K on the photosynthetic activity of cotton (*Gossypium hirsutum*). Turk J Bot 22: 217-221

Gergoff G, Chaves A, Bartoli CG (2010) Ethylene regulates ascorbic acid content during dark-induced leaf senescence. Plant Sci 178: 207-212

Gómez F, Fernández L, Gergoff G, Guiamet JJ, Chaves A, Bartoli CG (2008) Heat shock increases mitochondrial H_2O_2 production and extends postharvest life of spinach leaves. Postharvest Bio Tec 49: 229–234

Harris G (1997) Potassium deficiency in cotton linked to leaf spot disease. Better Crops 81: 10-11

Hillocks RJ, Chindoya R (1989) The relationship between *Alternaria* leaf spot and potassium deficiency causing premature defoliation of cotton. Plant Pathol 38: 502-508

Hörtensteiner S, Matile P (2004) How leaves turn yellow: catabolism of chlorophyll. In: Noodén LD (ed) Plant Cell Death Processes. Elsevier Academic Press, San Diego, pp 189-202

Humbeck K, Krupinska K (2003) The abundance of minor chlorophyll *a/b*-binding proteins CP29 and LHCI of barley (*Hordeum vulgare* L.) during leaf senescence is controlled by light. J Exp Bot 54: 375-383

Humbeck K, Quast S, Krupinska K (1996) Functional and molecular changes in the photosynthetic apparatus during senescence of flag leaves from field-grown barley plants. Plant Cell Environ 19: 337-344

Ishida Y, Kumashiro T (1988) Expression of tolerance to the host-specific toxin of *Alternaria alternata* (AT toxin) in cultured cells and isolated protoplasts of tobacco. Plant Dis 72: 892-895

Kakani VG, Reddy KR, Zhao D, Gao W (2004) Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. Physiol Plantarum 121: 250-257

Kerby TA, Adams F (1985) Potassium nutrition of cotton. In: Munson RD (Ed) Potassium in Agriculture. American Society Agronomy, Madison, WI, pp 843-860

Kramer GF, Norman HA, Krizek DT, Mirecki RM (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. Phytochemistry 30: 2101-2108

Li B, Wang Y, Zhang ZY, Wang BM, Eneji AE, Duan LS, Li ZH, Tian XL (2012) Cotton shoot plays a major role in mediating senescence induced by potassium deficiency. J Plant Physiol 169: 327-335

Li S, Zhang WW, Qi FJ, Si N, Jian GL (2011) Effect of environment conditions on conidia germination of cotton Alternaria leaf spot. Cotton Sci 23: 472-475

Lichtenthaler HK, Wenzel O, Buschmann C, Gitelson A (1998) Plant stress detection by reflection and fluorescence. Ann NY Acad Sci 851: 271-285

Liu L, Chen Y, Wang SZ, Ye YX, Li S, Guo TF, Ma YP, Li JS, Jian GL, Qi FJ (2010) Comparison and evaluation of cotton varieties resistant to premature aging. Plant Protect 37: 107-111

Lv SL, Lian LJ, Tao PL, Li ZX, Zhang KW, Zhang JR (2009) Overexpression of *Thellungiella halophila* H+-PPase (TsVP) in cotton enhances drought stress resistance of plants. Planta 229: 899-910

Marani A, Baker DN, Reddy VR, McKinion JM (1985) Effect of water stress on canopy senescence and carbon exchange rates in cotton. Crop Sci 25: 798-802

Matocha JE, Smith L (1980) Influence of potassium on *Helminthosporium* cynodontis and dry matter yields of coastal bermudagrass. Agron J 72: 565-567

Mehta YR (1998) Severe outbreak of Stemphylium leaf blight, a new disease of cotton in Brazil. Plant Dis 82: 333-336

Miller JW (1969) The effect of soil moisture and plant nutrition on the *Cercospora-Alternaria* leaf blight complex of cotton in Missouri. Phytopathology 59: 767-769

Padaganur GM, Hiremath RV, Basavaraj MK (1989) Estimation of yield loss due to Alternaria leaf spot and blight in cotton. J Indian Soc Cotton Improv 14: 144-145

Peever TL, Higgins VJ (1989) Electrolyte leakage, lipoxygenase, and lipid peroxidation induced in tomato leaf tissue by specific and nonspecific elicitors from *Cladosporium fulvum*. Plant Physiol 90: 867-875

Pettigrew WT (2003) Relationships between insufficient potassium and crop maturity in cotton. Agron J 95: 1323-1329

Pettigrew WT, Heitholt JJ, Meredith WR (1996) Genotypic interactions with potassium and nitrogen in cotton of varied maturity. Agron J 88: 89-93

Radin JW, Ackerson RC (1981) Water relations of cotton plants under nitrogen deficiency. Plant Physiol 67: 115-119

Sciumbato GL, Pinckard JA (1974) *Alternaria macrospora* leafspot of cotton in Louisiana in 1972. Plant Dis Rep 58: 201-208

Walters DR, Bingham IJ (2007) Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. Ann Appl Biol 151: 307-324

Watkins GM (1981) Alternaria leaf spot. In: Watkins GM (ed) Compendium of cotton diseases. American Phytopathological Society, pp 28

Woo HR, Kim JH, Nam HG, Lim PO (2004) The delayed leaf senescence mutants of *Arabidopsis*, *ore1*, *ore3* and *ore9* are tolerant to oxidative stress. Plant Cell Physiol 45: 923-932

Wright PR (1998) Research into early senescence syndrome in cotton. Better Crops Int 12: 14-16

Wright PR (1999) Premature senescence of cotton (*Gossypium hirsutum* L.) - predominantly a potassium disorder caused by an imbalance of source and sink. Plant Soil 211: 231-239

Yong JWH, Wong SC, Letham DS, Hocart CH, Farquhar GD (2000) Effects of elevated [CO_2] and nitrogen nutrition on cytokinins in the xylem sap and leaves of cotton. Plant Physiol 124: 767-779

Zhan MM, Wang N, Tian XL (2012) Review of genotypic differences and underlying mechanisms in potassium efficiency of Cotton (*Gossypium hirsutum*). Cotton Sci 24: 176-182

Zhao D, Oosterhuis DM, Bednarz CW (2001) Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastracture of cotton plants. Photosynthetica 39: 103-109

Zhao JQ, Li S, Jiang TF, Liu Z, Zhang WW, Jian GL, Qi FJ (2012) Chilling stress-the key predisposing factor for causing *Alternaria alternata* infection and leading to cotton (*Gossypium hirsutum* L.) leaf senescence. PloS ONE 7(4): e36126.