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Nitrogen rate and plant density effects on yield and late-season leaf senescence of cotton raised on a saline field

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ABSTRACT

Plant density and nitrogen fertilization are two important practices for field-grown cotton (*Gossypium hirsutum* L.). The objective of this study was to investigate the effects of plant density and N fertilization rate, especially their interactions, on yield, yield components, late-season leaf senescence and *Cry1Ac* expression in Bt (*Bacillus thuringiensis*) cotton under salinity conditions. To achieve this goal, we conducted a three-year experiment with a high-yielding Bt cotton cultivar (SCRC 28) in a moderately saline (ECe = 11 dS/m) field, using a split-plot design in the Yellow River Delta of China. The main plots were assigned to low, medium and high plant densities (3.0, 5.25 and 7.5 plants/m^2), while low, moderate and high nitrogen rates (120, 225 and 300 kg N/ha) were assigned to the subplots. Biological yield, lint yield, yield components, harvest index, boll load, *Cry1Ac* expression and leaf senescence were significantly affected by plant density and N rate. Lint yield was also affected by plant density × N rate interaction. Increased plant density or N rate enhanced biological yield, but reduced harvest index. Considerably high lint yield (1604 kg/ha) was achieved only with a high dose of N fertilizer under low plant density, but comparable yields (1693 and 1643 kg/ha) were achieved with moderate and low N rate under medium and high plant density. Increased plant density and N rate reduced boll load, which had highly significant negative correlation with late-season leaf photosynthesis ($r = -0.928$) and significant correlation with *Cry1Ac* protein concentration ($r = -0.8131$). Leaf senescence was delayed by increasing plant density and N rate mainly due to reduced boll load and a combination of reduced boll load and nutritional effect. Medium plant density with moderate N rate or high plant density with low N rate would enhance cotton yield and moderate *Cry1Ac* expression at reduced cost in the Yellow River Delta of China and other areas with similar ecologies.

1. Introduction

With population growth and increasing demand for food in China, large areas of fertile land previously cultivated to cotton have been occupied by food crops like rice and maize (Zhao and Tisdell, 2009). Thus, cotton production has moved to marginal lands such as the salt-affected soils, especially the coastal saline soils (Wang et al., 2010; Eynard et al., 2005), since cotton is more salinity-tolerant than most food crops (Maas and Hoffman, 1977; Qadir and Shams, 1997). With 0.35 million ha of salt-affected land for cotton planting, the Yellow River Delta (close to the Bohai Sea) has become one of the largest cotton-producing regions in China (Dong et al., 2006a, 2008a). However, the increasing soil salinity due to high evapotranspiration and limited rainfall in spring and autumn, as well as low soil fertility and poor field management have imposed a great challenge to increasing cotton yield in the area (Dong et al., 2009, 2010a). Cotton yield can only be improved through proper management practices of which fertilizer levels and plant density are most important (Ali et al., 2007). Optimizing plant density and fertilizer rates may therefore be the key practices for improving cotton yield in salt-affected fields.

Nitrogen (N) is an essential macronutrient that is required most consistently and in larger amounts than other nutrients for cotton production (Hou et al., 2007). Nitrogen fertilization had significant impacts on plant growth, lint yields and fiber quality (Bondada et al., 1996; Boquet et al., 1993). It is an essential element for canopy area development and photosynthesis (Wullschleger and Oosterhuis, 1990). However, N management in cotton is particularly difficult due to problems with either excessive or inadequate rates, influence of other agronomic practices (e.g. plant density and chemical control) as well as aboitic stresses (e.g. drought and salinity) (Rinehardt et al., 2004). Deficient N levels from emergence to early blooming could lead to inadequate vegetative growth, resulting in decreased fruiting (Gardner and Tucker, 1967). Nitrogen

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deficiency throughout the growing season will lead to decreased boll production as a result of poor plant development and premature senescence (Zhang et al., 2011). In contrast, an over-dose of N will promote excessive vegetative development and delay maturity (Hodges, 2002). Thus, N nutrition, unequivocally, is one of the most pivotal facets of cotton production (Bondada and Oosterhuis, 2001). In recent times, increasing N fertilizer costs and increased focus on greenhouse gas emissions have prompted greater attention to the efficient use of N fertilizers (Rochester et al., 2007). These issues and the need to optimize fertilizer inputs to meet crop requirements have also increasingly been identified as priorities in feedback from cotton growers and consultants. In Australia, it was reported that an excess of about 50 kg N/ha was applied to cotton fields and 15–25% of the N fertilizer inputs may be safely reduced without yield reduction (Rochester et al., 2009). Our recent study (Dong et al., 2010b) also suggested that N fertilizer can be used at a moderately lower rate and more efficiently than have been traditionally used. However, the optimum N rate under salt-affected field conditions has not been determined, although it is understood that optimum N rates and use efficiency are affected by a number of factors like yield potential, soil fertility and field management (Chen et al., 2010; Dong et al., 2010b).

Another important factor influencing cotton yield and yield components is plant density. The effects of plant density on cotton yield have been considerably studied under non-saline (Bednarz et al., 2005) and saline conditions (Feinerman, 1983; Keren et al., 1983). Although final lint yield in cotton was relatively stable across a wide range of plant densities under non-saline conditions (Bednarz et al., 2000), it was significantly affected by plant density under saline conditions (Francois, 1982). Maximum lint yield under salinity stress can be achieved only at an optimum plant density (Feinerman, 1983). However, the optimum plant density depends not only upon salinity level, but also upon nitrogen rate and other agronomic practices (Zhang et al., 2011). Reports have indicated a significant interaction between plant density and N rate under non-saline conditions (Dong et al., 2010b), but information is lacking on the effects of this interaction on yield under saline conditions. Also, early leaf senescence in the late season had significant negative effects on cotton yield and yield components (Dong et al., 2006b). Early leaf senescence may arise from cotton's poor ability to take up nutrients from soil in the late season or from an imbalance between source and sink (Wright, 1999). A study of the effects of plant density and N fertilization on source–sink ratio in relation to early leaf senescence may help clarify the effects of plant density and N rate on yield and yield components.

Transgenic cotton expressing Bt (*Bacillus thuringiensis*) toxins is currently cultivated on a large commercial scale in many countries, including China. Although the *Cry1Ac* expression in Bt cotton is relatively robust (Rochester, 2006), and Bt cotton has so far been one of the most environment-friendly and effective methods of insect control (Kranthi et al., 2005), observations have shown no consistency in the efficacy of *Cry1Ac* transgenic plants against target insects over the entire growing season (Dong and Li, 2007). Decreased Bt cotton efficacy against target insect pests was mainly attributed to the reduction in Bt protein content (Benedict et al., 1996) either due to genetic background (Dong et al., 2007) or environmental stress like high temperature (Chen et al., 2005), salinity stress (Jiang et al., 2006), and nitrogen deficiency (Coviella et al., 2002). There are also reports on the improvement of *Cry1Ac* protein expression in Bt cotton through agronomic practices like high doses of N fertilizer (Pettigrew and Adamczyk, 2006) or removal of early fruiting forms (Zhang et al., 2009). However, it is not known if plant density, N rate or their interaction also affects *Cry1Ac* protein expression under saline field conditions.

Plant density or N fertilization greatly affects cotton plant growth and yield, and their single effects have been well documented (Bondada et al., 1996; Boquet et al., 1993; Bednarz et al., 2000, 2005). However, their interaction effects were seldom studied especially in salt-affected fields. It is not clear if and how plant density and N rate affect sink/source ratio in relation to leaf senescence. Our objectives were to investigate the effects of plant density and N fertilization rate on lint yield, yield components, harvest index, and boll load in relation to leaf senescence and *Cry1Ac* expression, and most importantly to determine optimal combinations of plant density and N rate for a moderately saline field. We hypothesized that a considerably low N rate could also produce a considerable high lint yield under increased plant density and that leaf photosynthesis and *Cry1Ac* protein concentration in the late season vary with plant density or N rate through manipulation of sink/source ratio (boll load).

2. Materials and methods

2.1. Experimental site and cultivar

The experiment was conducted on a saline field in Dongying district (118°40′E, 37°26′N), Shandong Province, in the Yellow River Delta of China, from 2008 to 2010. The experimental site has a temperate climate and is underlain with a saline water (ECe: 20–30 dS/m) table at a depth of 2.5 m. The soil is saline sandy loam (similar to Vinton sandy loam: sandy, mixed, thermic Typic Torrifluvent) with pH 8.26, ECe 10.9 dS/m, organic matter 10.7 g/kg, available N 54.2 mg/kg, available P 12.1 mg/kg and available K 156 mg/kg. The experiment was conducted in different parts of the same field for three years to avoid residual effects of N fertilization from the previous year's experiment.

SCRC 28, a high-yielding commercial transgenic cotton cultivar carrying *Cry1Ac* gene from *B. thuringiensis* var. *kurstaki* (Berliner) was used in the experiment. Acid-delinted seeds (percentage germination ≥80%) were treated with imidacloprid (Gaucho FS600, Bayer CropScience, Monheim, Germany) by the Luyi Cottonseed Company Ltd., Jinan, Shandong.

2.2. Experimental design

A split-plot design with three replications was used for the study each year. The main plots were assigned plant density (3.00, 5.25 and 7.5 plants/m^2, hereafter referred to as low, medium and high density), while nitrogen (in the form of urea) rates (120, 225 and 300 kg N/ha, hereafter referred to as low, moderate and high level) fertilization were assigned to the subplots. These three plant densities and N rates were selected because 5.25 plants/m^2 and 225 kg N/ha are the typical plant density and fertilization rate in the local area. Each subplot contained six rows of cotton, 10 m long with an inter-row spacing of 0.80 m.

Half of each N rate was applied basally before planting, and the other half was top-dressed at early flowering. All plots received a basal rate of 33 kg/ha P as calcium superphosphate (5.2% P) and 105 kg/ha K as potassium sulfate (41.5% K) based on local practice.

2.3. Field management

The soils were subjected to excess irrigation (2500 m^3/ha) to drench salt 25–30 days before sowing each year. Soils were then plowed and harrowed when their mellowness was considered physically acceptable. Cotton was sown on 24 April 2008, 19 April 2009 and 25 April 2010. By passing an animal-drawn plough at 80 cm row-distance, seeding furrows (35 mm deep and 50 mm wide) were directly formed. Using manual hill-drop planting method, six to eight seeds were dropped per hill into the prepared furrow at hill–hill distances within row of 40.0, 23.8 and