Morphophysiological Changes of Cotton under Saline Stress in the Presence of *Fusarium oxysporum* sp. *vasinfectum*

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ABSTRACT

Fusarium wilt in cotton causes the greatest damage on sandy soil, acid, potassium deficient and saline soils. Because Fusarium wilt is more virulent under saline stress, the morphophysiological variations predisposing cotton plants under saline stress to the disease were evaluated. Seed of two cotton (Gossypium hirsutum L.) cultivars, Coker 304 (susceptible to wilt) and Acala SJ-2 (wilt resistant but susceptible in saline soils) were cultivated in hydroponic culture with non-saline nutrient solution (control group) or a range of nutrient solutions containing only FOV or only NaCl to EC 20mS/cm, or NaCl plus FOV. After 61 days, two transverse sections were cut from each plant of the four groups, one from the collar and one from the stem. From each sample, two characteristic were determined in the SEM, the average diameter of the xylem vessels (random selection, enlargement x156) and the total number of vessels per unit area. The experimental data (LSD test $p<0.05$) showed that the saline groups of plants had a lower diameter of the xylem vessels but a larger number of vessels per unit area. Both the greater number of vessels and the reduced lymph flow through these narrower vessels seem to be a precondition for cotton plants to be more susceptible to wilt.

Introduction

Fusarium oxysporum Schlecht f. sp. *vasinfectum* (Atk.) Snyder et Hansen (FOV), the causal agent of wilt on cotton (*Gossypium hirsutum* L.) belongs to Deuteromycotina. The fungus has a limited ability to survive as a saprophyte in the soil, but which can survive for long periods away from cotton plants in its chlamydospore stage, or by invading the root apparatus of weeds and other non-host plants (Hillocks, 1992).

FOV infection starts from infected seed or from the germination of the chlamydospores. When the pathogen has penetrated into the plants, it moves to vessel tissue and produces conidia that are transported to the apex of the plant in the xylem flow. When environmental conditions are optimal (Temp. 25-32° C; r.h. 80%) symptoms appear on the seedlings in 10-15 days: rapid wilting, chlorosis, and the fall of cotyledons. Adult plants show chlorosis, necrosis and complete wilting of the foliage, leading to plant death in the most severe cases, though some plants may reach the stage of boll formation. When symptoms become visible externally, there is marked browning of the vessels caused by the oxydation of phenols and by FOV producing fusaric acid (Hillocks, 1992).

The ability of cotton to grow in marginal soils (low potassium, acidic, sandy or saline) makes it a common crop in such soils. At least 10 % of all cultivated soils are already saline (Szabolcs, 1993), and a third of the land used world-wide for irrigated cultivation is at risk of becoming so (Epstein *et al.*, 1980) because of the use of saline water for irrigation and the inappropriate use of fertilizers. Wilt symptoms appear even in some resistant cotton cultivars when saline water is used for irrigation, as has occurred in parts of Angola and Nigeria (Ragazzi, pers. com.); that FOV biometry changes and that the pathogenicity becomes more aggressive when the fungus is grown in an enriched Na^+ ion environment (in the form of sodium chloride or sodium sulphate) (Ragazzi *et al.*, 1994; Ragazzi *et al.*, 1996). Furthermore, chlamydospore viability is enhanced in a NaCl-rich environment (Ragazzi and Vecchio, 1992), it is not surprising to find that cotton wilt incidence becomes higher and more damaging in saline soils.

To gain a better understanding of the mechanisms that increase cotton susceptibility to FOV in a saline environment, two cotton cultivars, Coker 304 (susceptible) and Acala SJ-2 (resistant) were grown with saline and non-saline watering and with and without FOV inoculation. The morphological variations induced in the cultivars by these four different growing environments were determined by examining stem and collar portions of the cotton plants by scanning electron microscopy (SEM).

Material and Methods

Eighty plants of cotton (*Gossypium hirsutum* L.), cv. Coker 304 and 80 plants of the cv. Acala SJ-2 were used for the tests. Coker 304 is susceptible to FOV and Acala is normally resistant but becomes susceptible in a saline environment. Plants were pre-germinated in the wet chamber for one week at 25°C, then grown in semi-hydroponic culture in a controlled-environment chamber with a day/night cycle of 13/11 h, temp 28/20°C, r.h. 70 %. All the plants were watered three times daily for 20 min. with a basic nutrient solution

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consisting of nutriflora, calcium nitrate and ferrous sulphate. After 18 days, half the plants from each cultivar were inoculated with a 10_6/ml conidial suspension of FOV isolate 141146 that was microinjected into wounds in the collar. Between 21 and 27 days after germination these two groups (inoculated - uninoculated) were further divided: half the plants in each group continued to receive the basic nutrient solution and the remaining plants were switched to a basic solution plus added levels of NaCl that gradually brought the solution up to EC 20 mS/cm (12g/l). The four groups per cultivar were thus: 1. non-saline nutrient solution, uninoculated (control); 2, saline solution, uninoculated; 3. non-saline solution plus FOV inoculation; 4. saline solution plus FOV inoculation. At 61 days from the start of germination, three plants from each cultivar in each treatment group were randomly selected from each group for examination by SEM. Transverse sections were cut from the stem and collar. These transverse sections were embedded in FAA and dehydrated in ethyl alcohol at increasing concentrations. For each treatment group and section type, the diameters of 100 randomly selected xylem vessels a total of 1600 measurements observable at 156 magnification, were determined by SEM,. The experiment was replicated three times. The total number of xylem vessels for each group and section type was counted, this measurement was also replicated three times.

Statistical analysis of the data was with the LSD test, at $P \leq 0.05$.

Results

The average diameter of the xylem vessels in each of the four groups from each cultivar is depicted graphically with stem and collar sections shown separately (Figure 1). The saline and saline/inoculated groups of both cultivars generally had the smallest diameters in both stems and collars. In the stems of Coker 304 plants inoculated with FOV but grown in non-saline conditions, vessel diameter was greater than in the control plants, but in the collars of the same plant-group vessel diameter was smaller than in the control plants. In the stems of Acala SJ-2, the diameter of xylem vessels was greater in the controls than in the other three groups, which were very similar, but in the collars of this cultivar, the lowest average diameter was found in the saline-enriched group.

The average number of xylem vessels per mm_2 was greater in the saline groups than in the control group of both cultivars (Figure 2). In the non-saline, FOV-inoculated group, the average number of xylem vessels per mm_2 was less than in the saline-only group, but it was greater than in the control (uninoculated) group. The saline+ FOV-inoculated groups of both cultivars had a greater number of xylem vessels than their respective control groups at both stem and collar levels. Moreover, the number of xylem vessels in the saline/inoculated groups was fairly similar to that of the saline/uninoculated group, except for Coker 304. stems, in which it was much higher. In the Coker 304, the average number of vessels per mm_2 seemed to be directly correlated both with salinity and with FOV presence, so that the greatest number of vessels was found in the saline/inoculated group, but in Acala SJ-2 the number of vessels was greatest in the two saline groups, with or without FOV inoculation.

Discussion and Conclusions

The morphological data indicate that NaCl reduced average vessel diameter but increased the number of vessels. The reduction in vessel diameter most probably affected the characteristics of the conveying vascular tissue and increased the resistance to raw lymph flow, as stated by Poiseuille´s law. However, it has been found that plants with such a narrowing of vessel diameter actually have a greater yield in drier conditions (Richards, 1987): though the efficiency of lymph translocation is reduced it is at the same time rendered more secure. A saline environment lowers the osmotic potential and creates a kind of physiological drought within the plant, This favours the growth of plants with smaller xylem vessels, as occurred in this experiment.

The increase in the number of vessels is more difficult to explain, not least because the research literature on this topic is scant. The increase may be an attempt by the plant to compensate for the narrowing of the vessels due to saline stress by providing more vessels and thus ensuring that the total amount of lymph remains the same despite the reduction in capacity of individual vessels.

Since FOV is a vascular pathogen, its translocation within a plant is directly promoted by the increase in the number of vessels. FOV infection may be also favoured indirectly by the partial stress conditions resulting from the narrowing of xylem vessels, but this disadvantage is at least in part outweighed by the greater number of vessels, as stated. It may be these changes explain the greater susceptibility of cotton plants when grown in a saline environment.

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Figure 1. Average diameter (µm) of 100 xylem vessels in the stems and collars of Coker 304 and Acala SJ-2 cotton cultivars grown with and without FOV inoculation and with and without saline watering.

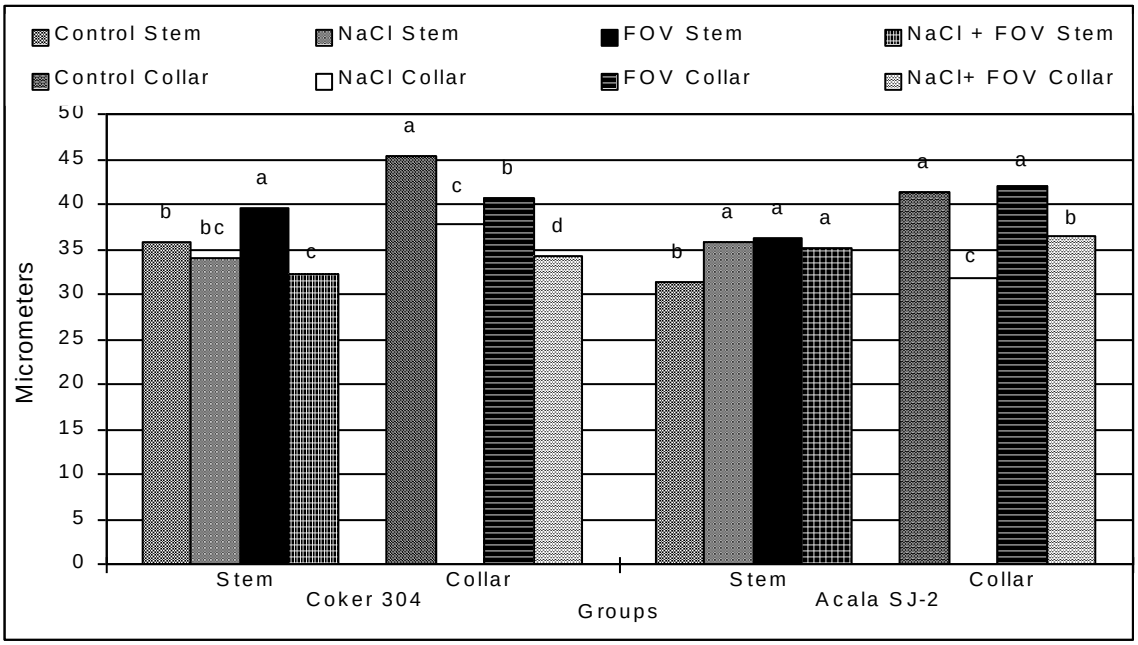
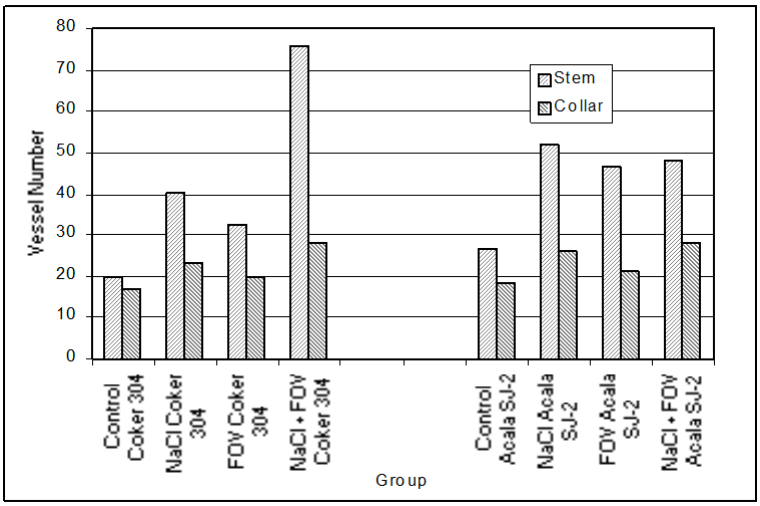


Figure 2. Average number of xylem vessels per mm2 in the stems and collars of Coker 304 and Acala SJ-2 cotton cultivars grown with and without FOV inoculation and with



and without saline watering.