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Histopathological Response of Giant Cell Induced by Root-knot Nematode, Meloidogyne javanica, in Tomato Roots under Potassium Stress

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Abstract: Comparison of histopathological response and quantitative measurement of giant cell (GC) induced by Meloidogyne javanica in tomato root were studied under potassium-deficient (0.2 mmol/L K^+) and replete conditions (control, 6.0 mmol/L K^+). K^+-deficient stress did not impede the formation and maintenance of GC. The mean number of GC per feeding site as well as the mean diameter of GC did not differ between the treatments. However, the thickness of cell wall including components resulted from the accumulated polysaccharide and the length of cell-wall ingrowth increased 5 - 25 d after inoculation in K^+-deficient as compared with K^+-replete conditions. An increase of cell-wall ingrowth suggested a kind of compensational response to the potassium stress.

Key words: cell-wall ingrowth; giant cell; histopathological responses; macro-nutrient stress; Meloidogyne javanica

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Giant cells (GC) induced by root-knot nematodes (Meloidogyne spp.) in the pericycle and vascular parenchyma of host root tissues are highly specialized cells which function as transfer cell and provide nutrients to support the growth and reproduction of nematodes[1]. It is well established that K^+ deficiency leads to growth arrest, impairs nitrogen and sugar balance due to inhibition of protein synthesis, photosynthesis, and long distance transport[2]. However, knowledge on the formation and function of GC under K^+-deficient condition is scarce. Investigations of the relationship between nematode infection and uptake of K^+ by plants have produced contradictory results[3,4]. So, study of the development of GC under stress might help understand the influence of macro-nutrition on the relationship between host and root-knot nematode. The main purpose of this work was to compare the changes in GC induced by M. javanica under different K^+ levels.

1 Materials and Methods

M. javanica nematodes were extracted from

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infested roots of Achyrauthes aspera from campus of Xiamen University. The nematodes were reared on compatible tomatoes by inoculating freshly hatched second-stage juveniles (J2) from a single egg mass in earthen pots containing autoclaved sandy soil. Seedlings of tomato, Lycopersicon esculentum cv. z-5, were initially formed through germination of seeds in fresh sand and later transferred individually to 20-cm-diam. clay pots filled with autoclaved fresh coarse sand; each plant was inoculated with 2 000 freshly hatched J2 after three weeks. Plants were fertilized with modified Hoagland nutrient solution. Nutrient solution low in K^+ was used for the germination and the initial growth of seedlings as the K^+-deficient solution treated group. Histopathological response of GCs were assessed by comparing their development under K^+-deficient (0.2 mmol/L K^+) with those on K^+-replete (control, 6.0 mmol/L K^+) conditions.

There were ten replicated pots for both K^+-replete and -deficient solutions. Root tissues were sampled at 2, 5, 10, 15 and 25 d after inoculation; at each sampling day, two root systems were randomly selected from each treatment. The experiment was repeated one time and all plants were kept outdoors during the experiment from May to July in 2005.

For histopathological study, five root segments with galls were collected per plant, a vertical section was examined for one segment, and the others were examined on cross section of each root segment at its point of maximum root gall expansion. The root segments were fixed in formaldehyde chromo-acetic solution (FAA) for at least 48 h, dehydrated in a tertiary butyl alcohol series (40%, 70%, 85%, 90%, and 100%), and embedded in 58 ℃ melting point paraffin wax. Serial sections were cut on a rotary microtome at 10 μm. The periodic acid-Schiff extraction technique (i.e. PAS stained) was used for staining. The sections were examined with an Olympus BX41 light microscope equipped with an Olympus DD50 camera.

Quantitative measurements were only performed on the cross sections of galls collected at 15 d after inoculation. Microscopic measurements were taken at point of maximum root gall expansion with a micrometer installed in optical microscope. The following parameters of GC were determined: (i) number of GC per feeding site; (ii) maximum diameter of GC; (iii) maximum thickness of cell wall (primary wall plus deposited saccharide, excluding the cell-wall ingrowth); and (iv) maximum length of cell-wall ingrowth. Quantitative data were subjected to analysis of mean comparison by SPSS 13.0 software at $\alpha = 0.05$.

2 Results

Typical arrested growth of lateral roots was observed in tomatoes grown on K^+-deficient medium. However, the root system supported the growth of tomatoes during the experiments; root galls induced by M. javanica occurred in all tomatoes. Histological observation of galling tissues showed that the mean number of GC per feeding site as well as the mean diameter of GC made no difference ($P = 0.678$ and 0.799, respectively) between treatments. A highly thicker cell wall ($P < 0.01$) and longer cell-wall ingrowth ($P < 0.01$) occurred under potassium stress as compared with the control.

Although there were no differences in some quantitative parameters, GC exhibited different patterns of development under K^+-deficient stress and K^+-replete conditions. Under K^+-replete condition, GCs were induced at 2 d after inoculation (Fig. 1-A). Despite neither the cell wall thickening nor the GC hyperplasia happened as compared with the surrounding vascular cells at this point of time, high density of cytoplasm and a few nuclei in GC indicated that those vascular cells adjacent to the head of nematode had re-differentiated into GC. Observations taken from 5 d to 25 d showed the classic hallmarks of GC, including increase of nucleus number, systematical thickening of cell wall, and higher density of granular cytoplasm (Fig. 1-B). However, the typical cell-wall ingrowth of GC was not obvious (Fig. 1-B, C), especially during the formation of GC (within 10 d).

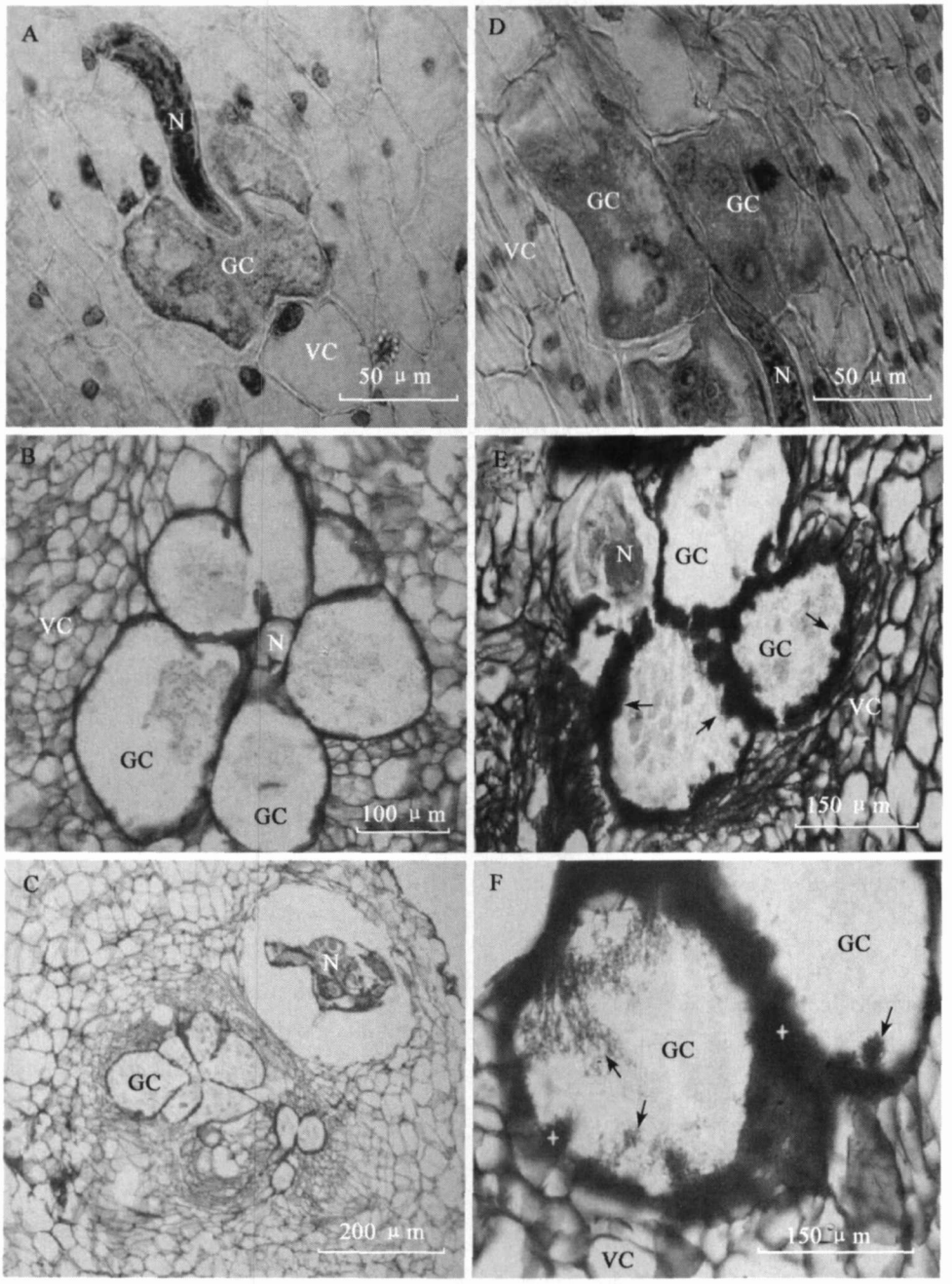

Fig. 1 Profile of giant cells induced by Meloidogyne javanica in tomato roots under the K^+-replete (A - C) and K^+-deficient (D - F) conditions

GC = giant cell; N = M. javanica; VC = vascular cylinder cell

A: Longitudinal section of a gall at 2 d after inoculation, showing high density of granular cytoplasm in GC when compared with surrounding vascular tissue cells; but hyperplasia of GC hasn't occurred yet; B: Transverse section of GC at 10 d after inoculation showed the thickened cell wall and granular starch in cytoplasm, but cell-wall ingrowth was indistinguishable; C: Transverse section of a gall at 25 d after inoculation, showing GC and the mature female nematode; D: Longitudinal section of a gall at 2 d after inoculation, showing the similarity of GC as compared with the corresponding period of GC under control; E: Transverse section of the developed GC at 10 d after inoculation. The cell wall were irregularly thickened, both cell wall and cell-wall ingrowths (arrow) were darkly stained; F: Transverse section of GC at 25 d after inoculation. The fibrous polysaccharide (arrow) built up in cytoplasm.

In contrast to the control, a similar view of GC came about in K^+-starved condition at 2 d after inoculation (Fig. 1-D). Subsequently, the cell-wall ingrowth in GC sampled from 10 d to 25 d under stress condition exhibited an obvious increase both in number and size (Fig. 1-E), indicating much more sugar deposited on the cell wall; all these changes resulted in even more deeper red color and thicker cell wall as compared with control of the corresponding period. As a result, the thickness of cell wall was five to seven fold and two to four point five folds thicker than that of the surrounding vascular cells and GC in the control separately. The most particular feature of GC was the fibrillar polysaccharide built up in cytoplasm (Fig. 1-F).

PAS stained technique showed that the thickened cell wall and cell-wall ingrowth were mainly resulted from accumulation of polysaccharide, whereas, sugar accumulate was uneven. Cell-wall ingrowth started from a spot in cell wall (Fig. 2-F, cross star), then it built up gradually in cytoplasm.

3 Discussion

The potassium transported in plants correlate with potassium channel and high-affinity transporter. The mechanism of high-affinity potassium absorption play an important role in uptaking K^+ from the soil at the K^+ level in the soil within 0.001 - 0.2 mmol/L. Considering no differences in some quantitative parameters of GC under K^+-deficient and replete conditions in present study, it was easily to comprehend that K^+-deficient condition did not obstruct inducement of GC and probably did not impact the GC acting as a permanent source of nutrient for M. javanica. But, cell wall of GC responded differently under K^+-deficient stress, suggesting a certain kind of nutrient carrier, such as K^+ transporter, existed in the plasma membrane of GC.

Giant cells, metabolically active and typical transfer cells, allow them to produce large amounts of proteins and funnel a great lot soluble sugar. Previous studies have reported the phenomena of cell-wall ingrowth[5] or cell-wall labyrinth in GC induced by Meloidogyne species. It is speculated that numerous projections facilitate the import of photosynthates, minerals and other metabolites. Although potassium starvation weakens many enzyme actions of plant, interferes with their carbohydrate synthesis and disturbs sugar translocation, the results in this study suggested that increasing of cell-wall ingrowth was conceivably a kind of compensational response to the nutrient stress; it was believed that the aggregated polysaccharide might be helpful in keeping the osmotic potential unchanged, in return, improving the loading capacity for sugar; whereas, function of thickened cell wall is still unclear. PAS demonstrated that the different color of cell wall and cell-wall ingrowth excluded the possibility that these protuberances are invaginated (or extension of) cell wall. We know that such a claim would require a more careful electron microscopy project using a rapid fixation method that better preserves the cellular integrity to exclude the fixation artifacts. If so, the histopathological differences in GC should be attributable mainly to the K^+ stress.

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