

EFFECTS OF ANTIBIOTICS ON ROOT OF *ARABIDOPSIS THALIANA*

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Abstract: In this article, it was found that growth and development of *Arabidopsis thaliana* seedling root were evidently affected by kanamycin and Hygromycin. Compared to the controls, main root of *Arabidopsis thaliana* seedling on MS with kanamycin or Hygromycin was very short, lateral root was not formed. In addition, cells in the meristematic zone of root tip exhibited abnormal array, weak division ability and large intercellular space. Therefore, it is presumed that kanamycin and Hygromycin might influence growth of main root and formation of lateral root of *Arabidopsis* seedlings by restraining synthesis of some proteins.

INTRODUCTION

Roots have great many functions, such as support and fixation, uptake, transmitting and storage, synthesis, secretion, and so on (Fitter 1996, Nasholm and Persson 2001, Karthikeyan and Kulakow 2003, Kirk and Kronzucker 2005). Therefore, development of roots directly affects growth of plants and is closely relevant with agricultural production. The main root is developed from radice, subsequently, some lateral roots and ramifications would gradually come into being at the topmost direction of the main root, and then enlarge the absorption scope of the plant. Thus, the developed root system benefit to the growth and development of plant (Yang et al. 2000, Wei et al. 2003, Burgess and Bleby 2006). In addition, the formation and growth of main and lateral roots are closely linked to the growth environment (Fitter et al. 1991, 1992; Lopez-Bucio et al. 2003, Malamy 2005, Okushima et al. 2007).

Arabidopsis thaliana is one model plant preferred in genetics, molecular biology, biology of development, and other research. Thus, researchers could rapidly and effectively examine various hypotheses and build reference system to push forward progress in the study of plant by *Arabidopsis thaliana*. In this article, effects of antibiotics on growth and development of *Arabidopsis thaliana* seedling root were studied in order to reveal function mechanism of kanamycin and Hygromycin on growth and development of plant seedling root.

MATERIALS AND METHODS

Plant materials

Seeds of *Arabidopsis thaliana* (Colombia) are kept in our laboratory.

Methods

Effects of antibiotics on growth of *Arabidopsis thaliana* seedling

Seeds of *Arabidopsis thaliana* (Colombia) were incubated in sterile water for 30min, surface-sterilized with 75% ethanol for 30s, and then sterilized with 5% sodium hypochlorite for 10min, and washed several times. Subsequently, seeds of *Arabidopsis thaliana* were respectively sown on MS with different concentration of kanamycin (0mg/L, 10mg/L,

30mg/L, 50mg/L, 70mg/L or 90mg/L) or Hygromycin (0µg/mL, 10µg/mL, 20µg/mL, 30µg/mL and 50µg/mL), and were cultured at 22/18°C with a 16h light and 8h dark photoperiod. There are three replications in each group.

Effects of antibiotics on root of *Arabidopsis thaliana* seedling

In order to study the effects of antibiotics on root and root tip of *Arabidopsis thaliana* seedling, seeds were sown on MS, MS with 50mg/L kanamycin or 30µg/mL Hygromycin, and then cultured at 22°C/18°C with a 16h light and 8h dark. There were three replications in each group. Moreover, *Arabidopsis* seedlings cultured for 5d on MS with 50mg/L kanamycin or 30µg/mL Hygromycin were transferred and cultured on MS in order to study the growth of *Arabidopsis* seedling roots.

Histology analysis

Root tip of *Arabidopsis thaliana* seedling cultured on MS, MS with 50mg/L kanamycin or 30µg/mL Hygromycin for different days was fixated into 50% FAA solution, and then processed according to the following steps: dehydration by series of ethanol, transparency with xylene, immersion and embedment in paraffin wax. The paraffin-embedded tissue samples were sliced by microtome with slices of 8µm. Every material was repeated three times and observed with Olympus microscope.

RESULTS AND DISCUSSIONS

Effects of antibiotics on growth of *Arabidopsis thaliana* seedling

When seeds of *Arabidopsis thaliana* sown on MS with different concentration of kanamycin or Hygromycin were cultured for 2d, some began to bourgeon. The difference between seedlings on MS with kanamycin or Hygromycin and the control was very evident as cultured for 5d, especially in root and color of cotyledon (Fig.1, a and d), cotyledons of seedlings on MS with Hygromycin were very small, the length of main roots decreased consistently with Hygromycin level. At 7d, lateral root of seedling on MS with kanamycin or Hygromycin did not come into being, and some seedlings took on etiolation and died, however root of seedling on MS was very long and there are 1~2 lateral roots (Fig.1, b and e). At 10d, 2~3 lateral roots were found in seedling on MS, however, when concentration

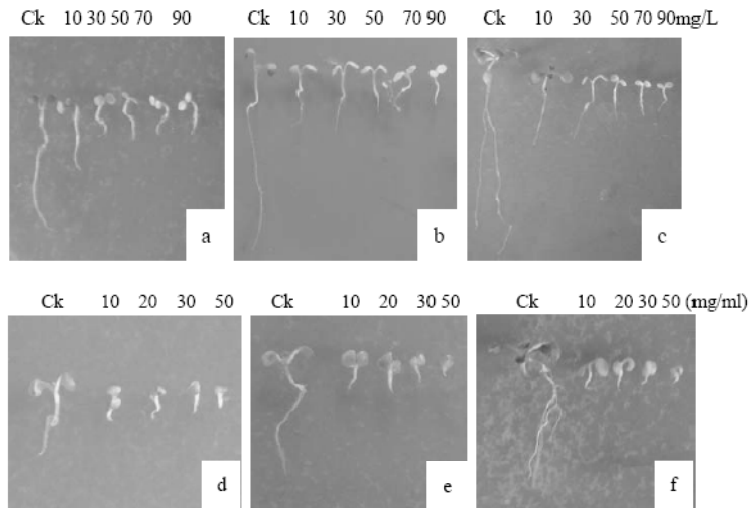


Fig. 1 Effects of antibiotics on growth of *Arabidopsis thaliana* seedling

(a), (b) and (c) separately represents *Arabidopsis thaliana* seedling cultured on MS with 0mg/L, 10mg/L, 30 mg/L, 50 mg/L, 70 mg/L, or 90 mg/L Kna for 5d, 7d, or 10d, respectively. (d), (e) and (f) separately represents *Arabidopsis thaliana* seedling cultured on MS with 0 μ g/ml, 10 μ g/ml, 20 μ g/ml, 30 μ g/ml, 50 μ g/ml Hygromycin, respectively for 5d, 7d, or 10d.

of kanamycin or Hygromycin increased, main roots of seedlings were shorter and shorter, and lateral root was not formed too (Fig.1, c and f). In addition, etiolation degree of seedling on MS kanamycin was severer and severer, death rate of brown was higher and higher.

Effects of antibiotics on root of *Arabidopsis thaliana* seedling

Root is important to absorb and exploit soil nutrients, and influenced by various factors during its formation and growth, such as genetic characteristics, environmental factors, and so on (Schiefelbein and Benfey 1991, Fitter 1991, Robinson 1994, Lopez-Bucio 2003, Malamy 2005, Okushima 2007). In this research, growth of seedling root was severely influenced by kanamycin and Hygromycin. As shown in Fig. 2, along with culture time increasing, main root of seedling on MS was longer and longer, whereas changes in main root of seedling on MS with kanamycin or Hygromycin was much less, especially the main root of seedling on MS with Hygromycin hardly grew. Furthermore, when seedling cultured for 5d on MS with kanamycin or Hygromycin was transferred on MS to be cultured 2~5d, their main roots hardly changed. Thus, it is indicated that elongation of main root were restrained by kanamycin and Hygromycin at the initial stage of seedlings growing, and inhibition of kanamycin and Hygromycin gradually enhances along with

culture time increasing, which was hardly reversible. In addition, effect of kanamycin or Hygromycin on lateral root of seedling was very obvious. As cultured on MS for 7d, lateral root of seedling was found, at 10d, the number of lateral root was 2~3, but lateral root was not discovered in seedlings on MS with kanamycin or Hygromycin during the whole experiment. Thereby, inhibition in the formation of lateral root due to kanamycin or Hygromycin was

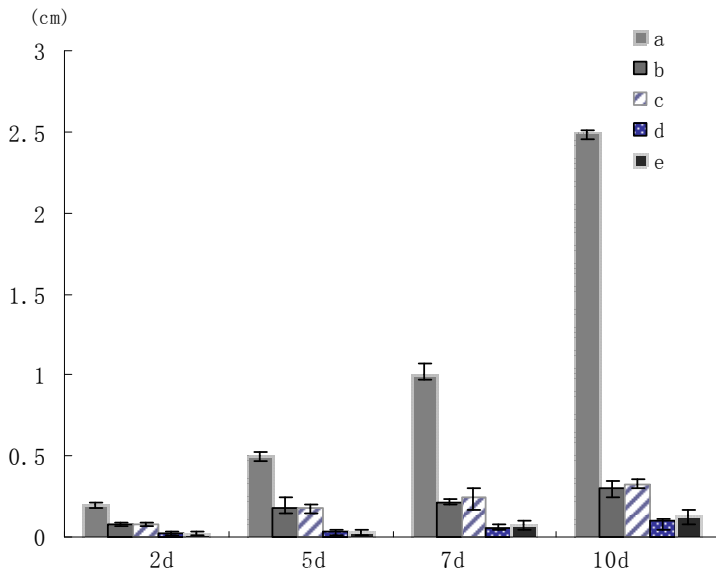


Fig. 2 Effects of antibiotics on elongation of *Arabidopsis thaliana* seedling main root

(a), (b), (d) represents respectively the length of main root on MS, MS with 50mg/L Kna or 30µg/mL Hygromycin for 2d, 5d, 7d and 10d; (c) and (e) represents the length of main root on MS with 50mg/L Kna or 30µg/mL Hygromycin for 5d, and then transferred on MS and continued to be cultured for 2~5d, respectively. Note: the length of main root was formed at least three independent replicates, the error bars represent ses.

remarkable and differed from their effects on the main root, and it is inferred that the mechanisms of kanamycin or Hygromycin influencing elongation of main root and formation of lateral root may be different.

Effects of antibiotics on root tip of *Arabidopsis thaliana*

Elongation of root is accomplished by the primary growth of meristematic zone in root tip. In this article, structure of meristematic zone in the root tip was studied to understand effects of kanamycin and Hygromycin on main root of *Arabidopsis thaliana* seedling. As shown in Fig.3,

in root tip of seedling on MS, the root cap exhibited intact cap structure, the meristematic zone looked like taper, in which the stratification characteristics in the array and division activity of promeristem was found, division of cells in primary meristem gradually weakened, the outermost layer of primary meristem was procuticle, and its center was columelliform procambium and looked like canister (Fig.3, a).

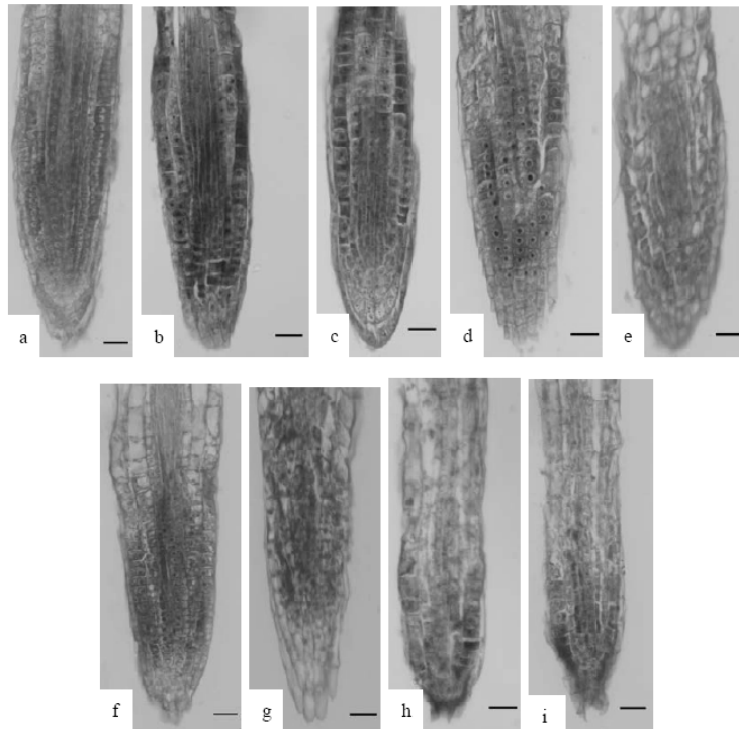


Fig. 3 Effects of antibiotics on structure of *Arabidopsis thaliana* seedling root tip

(a) represents the part vertical section of root tip from *Arabidopsis thaliana* seedling cultured on MS for 2d; (b), (c), (d) and (e) respectively represents the part vertical section of root tip from *Arabidopsis thaliana* seedling cultured on MS with 50mg/L Kna for 2d, 5d, 7d and 10d. (f), (g), (h), and (i) respectively represent the part vertical section of root tip from *Arabidopsis* seedling cultured on MS medium with 30 µg/ml Hygromycin for 2d, 5d, 7d, and 9d. The scales represent 20µm.

Otherwise, in root tip of seedling on MS with 50mg/L kanamycin, the root cap took on intact cap structure, the meristematic zone looked like taper too, but the intercellular space in meristematic zone became wide and the whole meristematic zone domain became small along

with culture time increasing (Fig. 3, b-e). In addition, the cap structure in root tip of seedling cultured for 2d on MS with 30µg/mL Hygromycin was also intact (Fig. 3, f), but the cap structure was unobvious, the outer cells of the root cap being almost desquamated and shown anomalous array along with culture time increasing. Moreover, the obvious changes were found in the meristematic zone of the root tip as the culture time increased, the meristematic zone hardly looked like taper and only a small part was concealed by the intact root cap. The cells in meristematic zone took on an anomalous array and obvious differentiation phenomenon. Their division activity had no stratification characteristics and division ability weakened, and the intercellular space was very large. On the other hand, the whole meristematic zone was diminished, transited evidently to the elongation zone and exhibited atrophy (Fig. 3, g-i). Accordingly, it is indicated that effect of kanamycin on root tip of *Arabidopsis thaliana* seedling was lower than that of Hygromycin. Furthermore, these results imply that kanamycin or Hygromycin might choke back division of cells, accelerate differentiation of cells in meristematic zone of root tip, and then restrain the elongation of *Arabidopsis* seedling main root. It is well known that kanamycin and Hygromycin belongs to aminoglycoside antibiotic, could destroy function of ribosome disturb protein synthesis, and then affect the growth and development of plant (Napj Bijvoet 1992, Yang 2002, Chen 2005; Gritz *et al.*, 1983; Santerre *et al.*, 1984; Cullen *et al.*, 1987). In this article, etiolation degree of *Arabidopsis thaliana* seedlings were severer and severer, death rate of brown was higher and higher along with concentration of kanamycin or Hygromycin increasing. Accordingly, it is presumed that kanamycin or Hygromycin might not only restrain growth and development of *Arabidopsis thaliana* seedling leaf, but also gravely influence photosynthesis, which would not synthesize enough nutriment to satisfy growth of *Arabidopsis thaliana* seedlings and then influence growth of its root.

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