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EFFECTS OF HYGROMYCIN ON GROWTH AND DEVELOPMENT OF *ARABIDOPSIS* SEEDLING ROOTS

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Abstract

In this study, it was found that growth and development of *Arabidopsis* seedling main root and lateral root were affected by Hygromycin. Compared to the controls, main roots of *Arabidopsis* seedlings cultured on MS medium with Hygromycin were very short, and lateral roots were not formed. In addition, cells in the meristematic zone of root tip exhibited abnormal array, weak division ability, evident differentiation and large intercellular space. Accordingly, it is presumed that 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 & Kulakow, 2003; Kirk & 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 & 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).

In this research, the sensitivity of *Arabidopsis* seedlings to Hygromycin, effects of Hygromycin on growth of *Arabidopsis* seedling roots and the structure of root tip, and so on, were studied in order to study how far Hygromycin affects the growth and development of plant seedling roots.

Materials and Methods

Plant materials: The seeds of wild-type *Arabidopsis* (Colombia type) were available in our laboratory.

Culture of *Arabidopsis* seedlings: Seeds of *Arabidopsis* were incubated in sterile water for 30min, surface-sterilized with 75% ethanol for 30 seconds, and then sterilized with 5% sodium hypochlorite for 10 min, and washed several times. Subsequently, the seeds of *Arabidopsis* were sown on MS medium, and then cultured at 22°C/18°C with a 16 h light and 8 h dark photoperiod.

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Screening of Hygromycin concentration: The seeds of *Arabidopsis* were respectively sown on MS medium with different concentrations of Hygromycin, 0 (the control group), 10, 20, 30 and 50 µg/ml Hygromycin, and cultured at 22°C/18°C with a 16 h light and 8 h dark. In addition, there were three replications in each group.

Effects of Hygromycin on the roots of *Arabidopsis* seedlings: The seeds of *Arabidopsis* were sown on MS medium, or MS medium with 30 µg/ml Hygromycin, and then cultured at 22°C/18°C with a 16 h light and 8 h dark. There were three replications in each group. Moreover, *Arabidopsis* seedlings cultured for 7 d on MS medium with 30 µg/ml Hygromycin were transferred and cultured on MS medium in order to study the growth of *Arabidopsis* seedling roots.

Histology analysis: The root tip of *Arabidopsis* seedlings cultured for different days on MS medium or MS medium with 30 µg/ml Hygromycin was fixed into 50% FAA solution, and then processed according to the following steps: dehydration by a series of ethanol, transparence with xylene, immersion and embedment in paraffin wax. The paraffin-embedded tissue samples were sliced by microtome with slices of 8 µm. Every material was repeated three times and observed with Olympus microscope.

Results

Screening of Hygromycin concentration: When the seeds of *Arabidopsis* sown on MS medium with different concentration of Hygromycin were cultured for 2 d, then began to bud, but only *Arabidopsis* seedlings in the control group had root hairs (Fig. 1, a). When cultured for 5 d, some cotyledons of *Arabidopsis* seedlings departed from seed capsule, and the significant difference was found between *Arabidopsis* seedlings cultured on MS medium with Hygromycin and without Hygromycin (Fig. 1, b). Compared to the controls, cotyledons of *Arabidopsis* seedlings on MS medium with Hygromycin were very small, in addition, the length of main roots decreased consistently with Hygromycin level. At 12 d, the main roots of *Arabidopsis* seedlings on MS medium with Hygromycin hardly changed, root hairs were less and only found in the *Arabidopsis* seedlings cultured on MS medium with 10 µg/ml or 20 µg/ml Hygromycin, respectively. However, lateral roots were not formed in *Arabidopsis* seedlings cultured on MS medium with Hygromycin (Fig. 1, c). Consequently, the optimal concentration of *Arabidopsis* seedlings resisting Hygromycin is approximately 30 µg/ml Hygromycin according to the growth of *Arabidopsis* seedlings.

Effects of Hygromycin on the elongation of main root: Seeds of *Arabidopsis* were sown and cultured on MS medium or MS medium with 30 µg/ml Hygromycin. As shown in Fig. 2, the roots of *Arabidopsis* seedlings on MS medium were long, and greatly went up along with culture time, but the length of *Arabidopsis* seedling main root on MS medium with 30 µg/ml Hygromycin inconspicuously changed. Otherwise, when *Arabidopsis* seedlings cultured for 7 d on MS medium with 30 µg/ml Hygromycin were transferred on MS medium and continued to be cultured for 2~5 d, the main roots were found to be relatively long, but did not evidently increase. Besides, three kinds of *Arabidopsis* seedlings could be clearly distinguished according to the shape and length of roots, but the difference of roots on MS medium with Hygromycin and those cultured restoratively was unobvious.

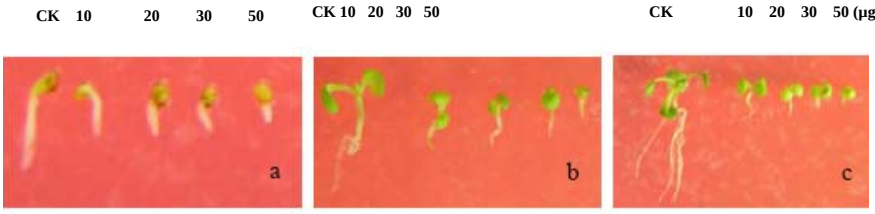

Fig.1 Effects of Hygromycin on the growth of *Arabidopsis* seedlings
(a). *Arabidopsis* seedlings cultured for 2 d on MS medium with 0 μg/ml, 10 μg/ml, 20 μg/ml, 30 μg/ml, 50 μg/ml Hygromycin, respectively; (b). *Arabidopsis* seedlings cultured for 5 d on MS medium with 0 μg/ml, 10 μg/ml, 20 μg/ml, 30 μg/ml, 50 μg/ml Hygromycin, respectively; (c). *Arabidopsis* seedlings cultured for 12 d on MS medium with 0 μg/ml, 10 μg/ml, 20 μg/ml, 30 μg/ml, 50 μg/ml Hygromycin, respectively.

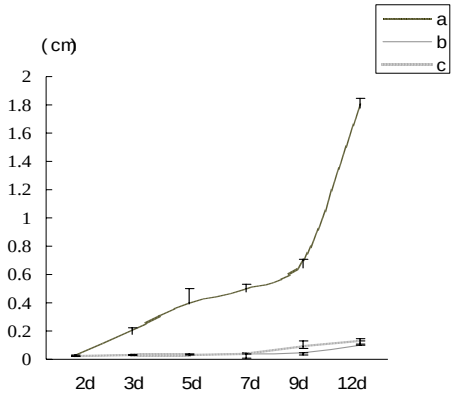

Fig.2 Effects of Hygromycin on the elongation of *Arabidopsis* seedling main root
(a). The length of main root on MS medium for 2 d, 3 d, 5 d, 7 d, 9 d and 12 d, respectively; (b). The length of main root on MS medium with 30 μg/ml Hygromycin for 2 d, 3 d, 5 d, 7 d, 9 d and 12 d, respectively; (c). The length of main root on MS medium with 30 μg/ml Hygromycin for 2 d, 3 d, 5 d, 7 d, and then transferred on MS medium and continued to be cultured for 9 d and 12 d, respectively. Note: the length of main root was formed at least three independent replicates, the error bars represent ses.

Furthermore, when cultured for 2 d, the seeds of *Arabidopsis* sown on MS medium bud and produced root hairs. Some seeds sown on MS medium with Hygromycin also bud, but the root hairs were not found even when cultured for 15 d. Whereas, when *Arabidopsis* seedlings were cultured restoratively for 2 d, the root hairs were formed. Thus, it is indicated that the elongation of *Arabidopsis* seedling main root were restrained by Hygromycin at the initial stage of seedlings growing, and the inhibition of Hygromycin to seedling root gradually enhances along with culture time increasing, which was hardly reversible.

Effects of Hygromycin on the ultrastructure of root tip: Elongation of root is accomplished by the primary growth of meristematic zone in root tip. In this article, the structure of meristematic zone in the root tip was studied to understand the effects of Hygromycin on elongation of *Arabidopsis* seedling main root. As shown in Fig. 3(a), in

the root tip of *Arabidopsis* seedling cultured on MS medium, the root cap exhibited intact cap structure, the meristematic zone looked like taper, and the stratification characteristics were found in the array and showed division activity of promeristem. Besides, the division ability of cells in primary meristem was found to gradually weaken; the outermost layer of primary meristem was procuticle covering outside of the meristematic zone; and the center of primary meristem was columelliform procambium like canister.

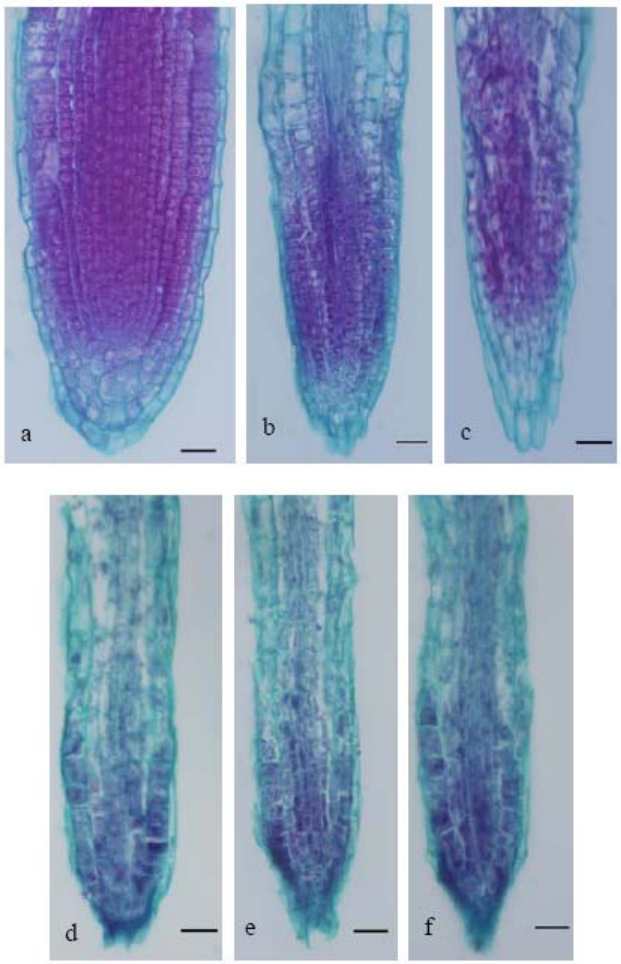

Fig.3 Effects of Hygromycin on the ultrastructure of *Arabidopsis* seedling root tip
(a). The part vertical section of root tip from *Arabidopsis* seedling cultured on MS medium for 2 d; (b), (c), (d), (e) and (f) respectively represent the part vertical section of root tip from *Arabidopsis* seedling cultured on MS medium with 30 μg/ml Hygromycin for 2 d, 5 d, 7 d, 9 d and 12 d. The scales represent 20 μm.

In addition, the cap structure in the root tip of *Arabidopsis* seedling growing on MS medium with 30 µg/ml Hygromycin cultured for 2 d was also intact (Fig. 3, b), but the cap structure was unconspicuous, the outer cells of the root cap being almost desquamated and shown anomalistic 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 anomalistic 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, c-f),

Effects of Hygromycin on the formation of lateral root: Seeds of *Arabidopsis* were sown and cultured on MS medium with 0 µg/ml (control group), 10 µg/ml, 20 µg/ml, 30 µg/ml or 50 µg/ml Hygromycin, respectively, and it was found that the lateral root of *Arabidopsis* seedling changed obviously. When *Arabidopsis* seedling were cultured for 7d on MS medium, the lateral root was found, as cultured for 15d, the number of lateral roots was 3~4. But the lateral roots were not discovered in *Arabidopsis* seedling on MS medium with Hygromycin during the whole experiment. Thereby, inhibition in the formation of *Arabidopsis* seedling lateral root due to Hygromycin was remarkable and differed from the effects of Hygromycin on the main root of *Arabidopsis* seedling, and it is inferred that the formation period of main root and lateral root of *Arabidopsis* seedling, or the mechanisms of Hygromycin influencing elongation of main root and formation of lateral root may be different.

Discussion

An important component of plant is root, through which plant could absorb water and mineral nutrition from soil. At the same time, root also has other functions, such as support, transporting, storage, synthesis, secretion, and so on (Keleimo, 1989; Nasholm & Persson, 2001; Wei *et al*., 2003; Kirk & Kronzucker, 2005). As the model plant of biology research, *Arabidopsis* could promptly make researchers testify various hypotheses. In this article, effects of Hygromycin on the root of *Arabidopsis* seedlings were studied in order to explore the effects and functional mechanism of Hygromycin on growth and development of seedling root.

The formation and growth of root are affected by both genetic characteristics and environmental factors (Schiefelbein & Benfey, 1991; Robinson, 1994). It was indicated in this experiment that the elongation of *Arabidopsis* seedling main root was restrained by Hygromycin. It is well known that elongation of root is accomplished by the primary growth of root tip and mainly relative to the meristematic zone of root tip, in which a number of functions of root, such as absorbance, storage, synthesis, secretion and so on, are principally carried through (Yang *et al*., 2000). In this research, it was discovered that, compared with control groups, there were obvious changes in the meristematic zone of *Arabidopsis* seedling root tip along with culture time increasing. The meristematic zone diminished, transited evidently to the elongation zone and exhibited atrophy, in which the cells were abnormally arrayed and took on obvious differentiation phenomenon, and the intercellular space enlarged. Furthermore, the cap structure of root cap was unconspicuous, its outer cells almost desquamated and shown anomalistic array. Accordingly, these results imply that 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.

The lateral root is also crucial in root system and plays a key role in absorbing nutrients, anchoraging plantlet and establishing rhizosphere intergrowth system (Sanchez-Calderon *et al*., 2005). The lateral root results from primordium of lateral root, and is formed by cells with dedifferentiation ability in the pericycle of main root or apical region of endodermis (Blakely *et al*., 1982; Fahn 1990; Casero *et al*., 1995; Laskowski *et al*., 1995; Beeckman *et al*., 2001). In addition to genetic factors, the formation and development of lateral root are also influenced by environmental factors (Lopez-Bucio *et al*., 2003; Malamy, 2005). For example, the numbers of lateral roots in many mutants with defects of auxin synthesization or signal transduction decrease (Casimiro *et al*., 2003), and the interdiction of transporting auxin from overground part to root segment also restrains origination of lateral root (Reed *et al*., 1998). Besides, it is found that the auxin transported from overground part of *Arabidopsis* seedling to root segment is very necessary for the elongation of lateral root to break through mother root cuticle (Bhalerao *et al*., 2002; Ljung *et al*., 2002; Okushima *et al*., 2007). In this research, the lateral root was not discovered in *Arabidopsis* seedling cultured on MS medium with Hygromycin. Moreover, Hygromycin could destroy the function of ribosome in various cells by competing the binding site between ribosomes in chloroplast and mitochondria and the elongation factors EF-2, disturb protein synthesis, and then affect the growth and development of plant (Gritz *et al*., 1983; Santerre *et al*., 1984; Cullen *et al*., 1987; Rosa *et al*., 1991). Accordingly, it is guessed that Hygromycin might restrain auxin synthesis or block off the transportation of auxin from overground part to root segment, and then influence the formation of *Arabidopsis* seedling lateral root.

Over all, root is important to absorb and exploit soil nutrients, and influenced by various factors during its formation and growth. In this research, it was found that the cells in the meristematic zone of *Arabidopsis* seedling root tip cultured on MS medium with Hygromycin appeared a series of changes, such as feeble division ability, evident differentiation, abnormal array, and large intercellular space, which could restrain elongation of main root and formation of lateral root of *Arabidopsis* seedling. Taken together, all the results of this research suggest that effects of Hygromycin on root of *Arabidopsis* seedling are very remarkable, but the inhibitory mechanism needs to be studied further.

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