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Full Length Research Paper

The influences of Hygromycin B on growth of *Arabidopsis thaliana* cotyledon and leaf

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In this article, growth and development of *Arabidopsis thaliana* seedling cotyledon and leaf were evidently affected by Hygromycin B. As compared to the control, cotyledon of seedling on Murashige and Skoog (MS) with Hygromycin B was very small and its leaf was not formed. Along with increase in culture time, cells in the mesophyll tissue of cotyledon were loose arranged and the intercellular space was large. In addition, cells in the meristematic zone of shoot tip exhibited abnormal array and even atrophy. Thereby, it is conferred that Hygromycin B might influence growth of cotyledon and leaf, and accordingly affected growth and development of *A. thaliana* seedling.

Key words: Hygromycin B, *Arabidopsis thaliana*, cotyledon, leaf.

INTRODUCTION

Leaf is the main organ of plant, in which photosynthesis takes place. Some materials required for growth and development of plant, such as carbohydrate, fat and protein and so on, are synthesized (Simpson and Lee, 1976; Zhang and Shangguan, 2006; Zeeman et al., 2007). Furthermore, leaf is also the primary apparatus doing transpiration, which not only promotes transport of mineral elements but also debases temperature of leaf surface to avoid strong solar burn (Liu and Teskey, 1995; Fleck et al., 1998; Sun et al., 2001; Gregg et al., 2006; Franco et al., 2007). In addition, leaf has many other functions such as absorbability, secretion, etc.

Hygromycin B could restrain growth of plant by disturbing protein synthesis, and can help to screen cell, tissue and regeneration system transformed with extrinsic gene. Also, it is used abroad as an antibiotic in plant gene engineering. In this study, effects of Hygromycin B on growth of cotyledon and leaf were researched in order to reveal the relationship between Hygromycin B and growth of plant seedling cotyledon and leaf.

MATERIALS AND METHODS

Seeds of *Arabidopsis thaliana* (Colombia type) were incubated in sterile water for 30 min, surface-sterilized with 75% ethanol for 30 s, and then sterilized with 5% sodium hypochlorite for 10 min, and washed several times. Subsequently, seeds of *A. thaliana* were sown on MS culture medium, and then cultured at 22/18°C with a 16 h light and 8 h dark photoperiod.

Effects of Hygromycin B on cotyledon and leaf

Seeds of *A. thaliana* were respectively sown and cultured on MS medium with different concentration of Hygromycin B, such as 0 µg/ml (control group), 10, 20, 30 or 50 µg/ml Hygromycin B. In addition, there were three replications in each group. Moreover, after *A. thaliana* seedlings were cultured for 7 days on MS with 30 µg/ml Hygromycin B, they were transferred and cultured on MS.

Histology analysis

Cotyledon and shoot tip of *A. thaliana* seedling cultured on MS or MS with 30 µg/ml Hygromycin B were fixated into 50% FAA solution, and then processed according to the following steps: dehydration by series of ethanol, transparence of xylene, immersion and embedment in paraffin wax. Subsequently, the tissue samples were sliced with microtome, each sample was repeated three times and observed with Olympus photos microscope.

RESULTS

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When seeds of *A. thaliana* sown on MS with different

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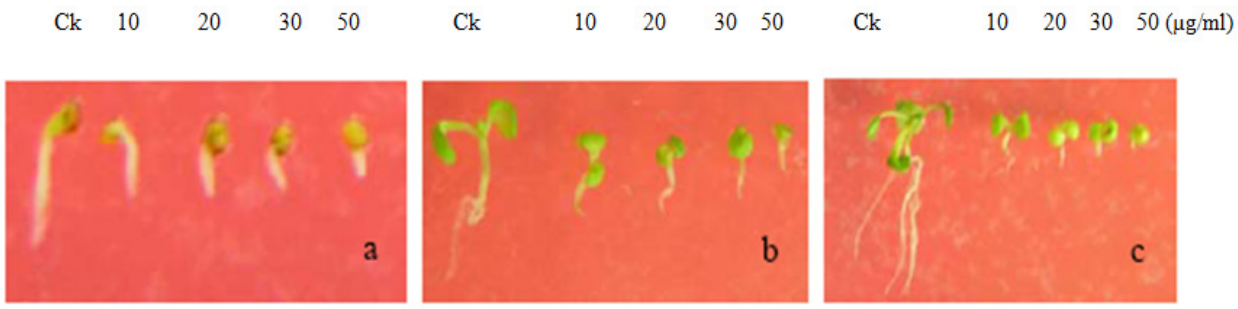

Figure 1. Effects of Hygromycin B on growth of *A. thaliana* seedlings. (a) *Arabidopsis* seedlings cultured for 2 days on MS medium with 0, 10, 20, 30 and 50 μg/ml Hygromycin B, respectively; (b) *Arabidopsis* seedlings cultured for 5 d on MS medium with 0, 10, 20, 30 and 50 μg/ml Hygromycin B, respectively;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

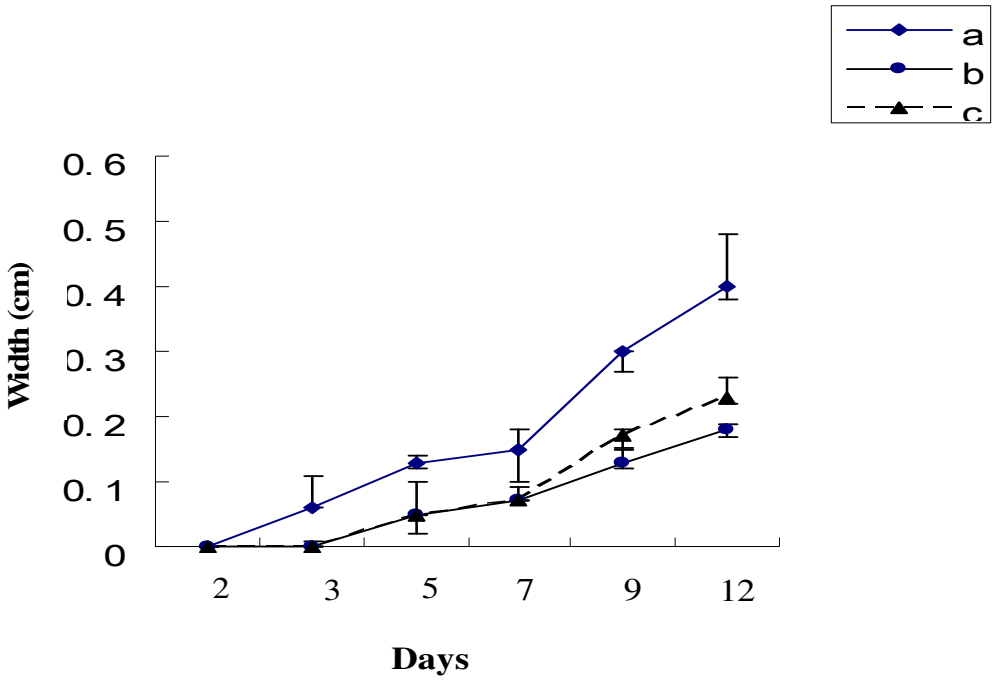

Figure 2. Effects of Hygromycin B on cotyledon of *A. thaliana* seedling. (a) The width of cotyledon on MS for 2, 3, 5, 7, 9 and 12 days, respectively; (b) The width of cotyledon on MS with 30μg/ml Hygromycin B for 2d, 3d, 5d, 7d, 9d and 12d, respectively; (c). The width of cotyledon on MS with 30 μg/ml Hygromycin B for 7 day, and then transferred to MS and continued to be cultured for 2 and 5 days, respectively. The width represents transverse diameter of cotyledon and was formed with at least three independent replicates, the error bars represent standard errors.

concentration of Hygromycin B: 0, 10, 20, 30 or 50 μg/ml were cultured for 2 days, they began to bud, but only seedlings in the control group had root hairs (Figure 1a). When cultured for 5 days, some cotyledons were separated from seed capsule, and significant difference was found between seedlings on MS with Hygromycin B and without Hygromycin B (Figure 1b). At 12 days, two pairs of leaves were found in control, but leaf was not found in seedling of MS with Hygromycin B (Figure 1c), and some cotyledons only spread on MS with 0, 10 or 20 μg/ml Hygromycin B.

In addition, seeds of *A. thaliana* were sown and cultured respectively on MS or MS with 30 μg/ml Hygromycin B. In culture of 2 days on MS medium, cotyledons were found in 40% seedlings, at 3 days, 90% cotyledons were separated from seed capsule, and a majority of cotyledons spread. But on MS with Hygromycin B, cotyledons were only found in 7% seedlings and had curliness. As shown in Figure 2, cotyledon of seedlings increased when culture time was

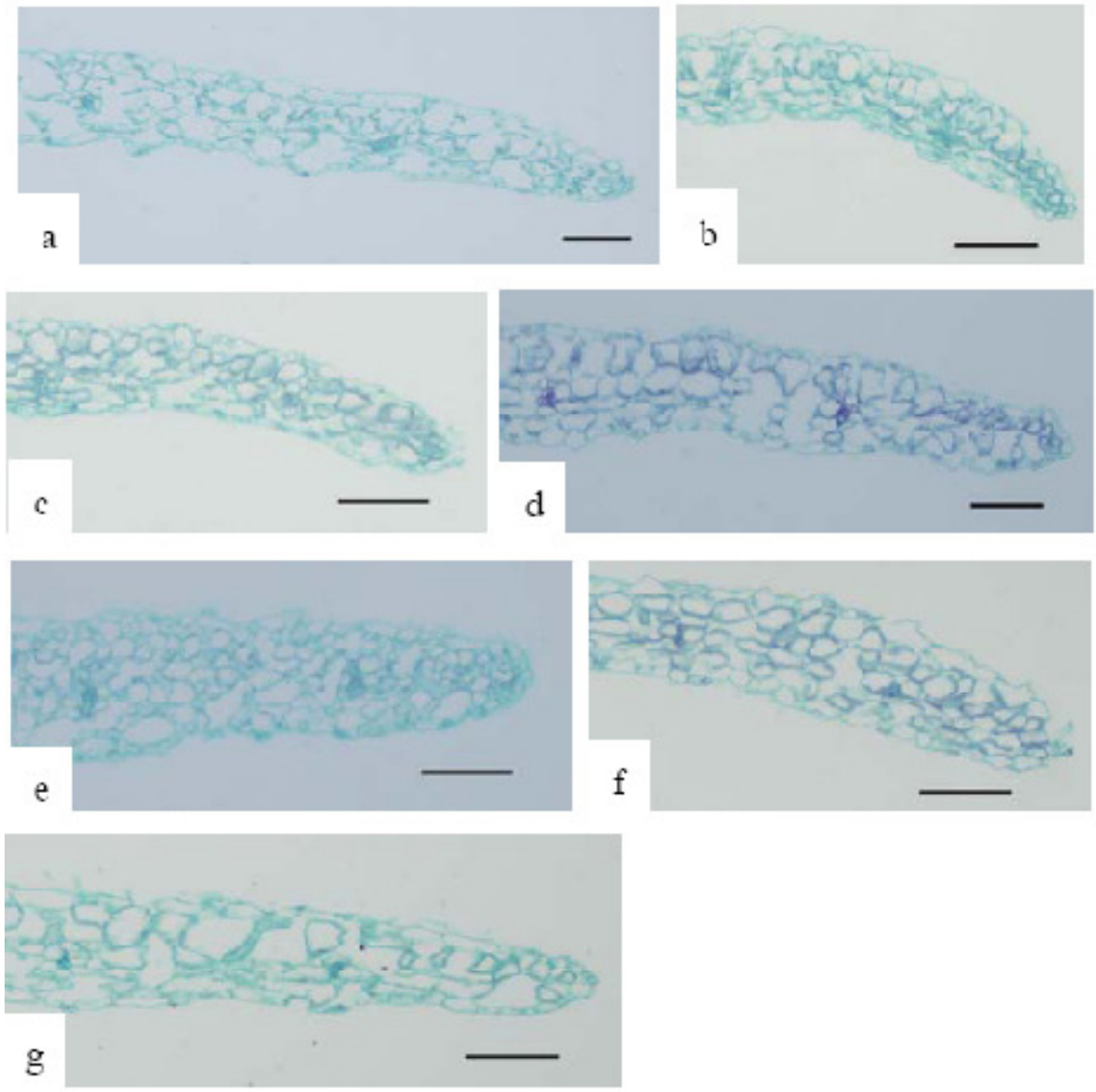

Figure 3. Effects of Hygromycin B on structure of *A. thaliana* seedling cotyledon. (a) The part transverse section of cotyledon on MS for 5 days; (b), (c), (d) and (e), respectively represent the part transverse section of cotyledon on MS with 30 µg/ml Hygromycin B for 5, 7, 9 and 12 days; (f) and (g) represents the part transverse section of cotyledon restoratively cultured on MS for 2 or 5 days, respectively. The scales represent 100 µm.

increased. However, cotyledons on MS were relatively larger than those on MS with Hygromycin B, and the difference was very obvious. However, difference between cotyledons on MS with Hygromycin B and those restoratively cultured was very unobvious. Furthermore, for culture of 12 days, some cotyledons on MS with Hygromycin B exhibited Kelly, 4% of them were brown and even died. As cultured restoratively for 5 days, cotyledons were still green. At 15 days, 90% cotyledons were blasted and died, but 40% cotyledons cultured restoratively, scorched and 1% of them were brown and died.

Effects of Hygromycin B on structure of cotyledon

As shown in Figure 3, cotyledon of *A. thaliana* seedling was composed of cuticle, mesophyll and leaf veins (Figure 3). In the control group, the upper and lower epidermis of cotyledon consisted of flat cells which were joined intensely to each other, and cells in the upper epidermis were larger than those in the lower epidermis; the stockade tissue was made up of columnar cells having large chloroplast, the major axes of cells was vertical to epidermis, and cells were closely arranged; the sponge tissue consisted of 3 to 4 layers of cells arranged

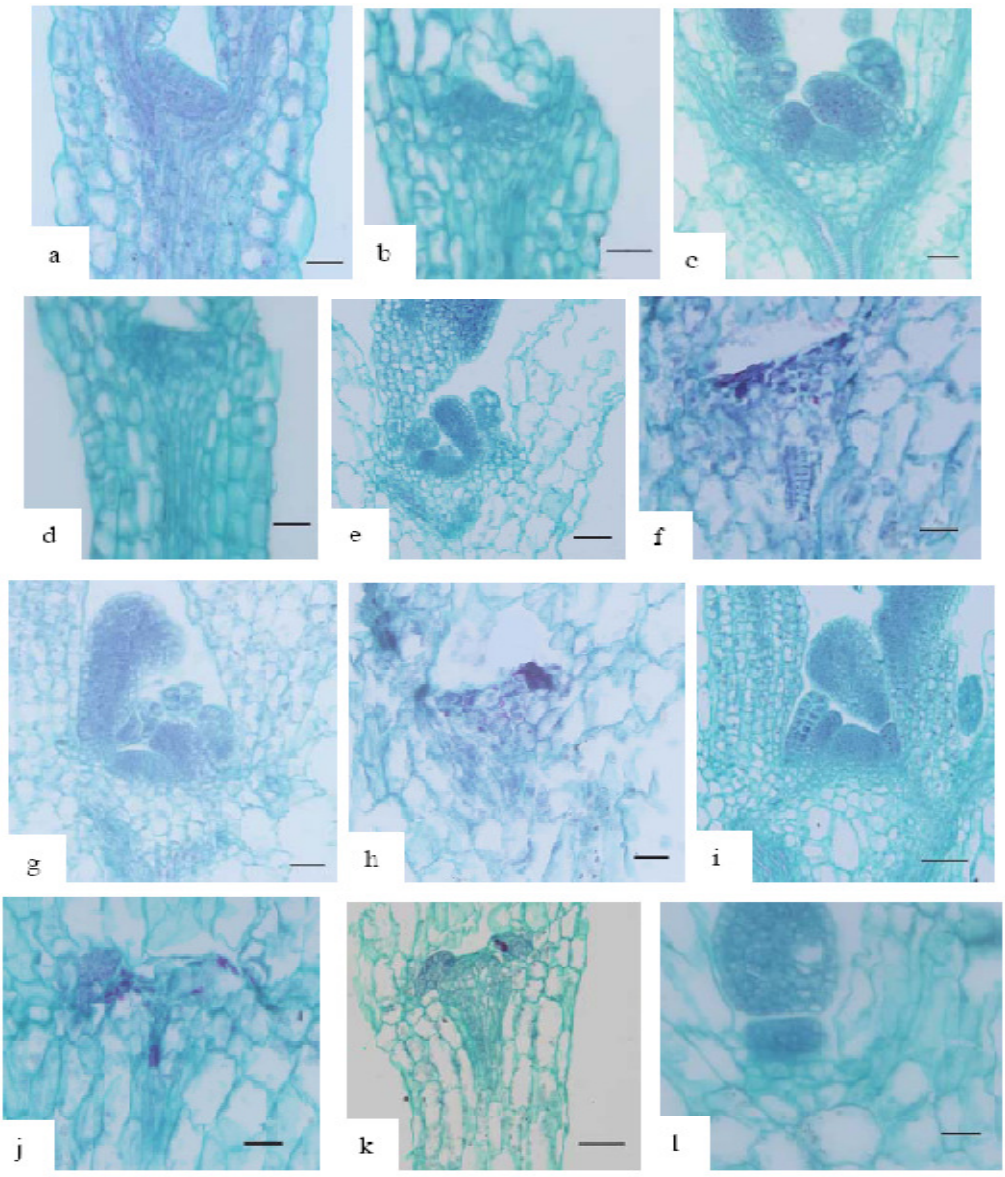

Figure 4. Effects of Hygromycin B on structure of *A. thaliana* seedling shoot tip. (a), (c), (e), (e), (g) and (i), respectively represents vertical section of shoot tip cultured on MS for 2, 5, 7, 9 and 12 days; (b), (d), (f), (h) and (J), respectively represents vertical section of shoot tip cultured on MS with 30 µg/ml Hygromycin B for 2, 5, 7, 9 and 12 days; (k) and (l) represents vertical section of shoot tip restoratively cultured on MS for 2 or 5 days, respectively. The scales represent 20 µm.

loosely, in which chloroplasts decreased obviously and the intercellular space was very larger (Figure 3a). Along with culture time increase, the intercellular space in the upper and lower epidermis of cotyledon on MS with Hygromycin B enlarged, and arrangement of cells was not intense, which gravely affected their safeguard function. The cells in stockade tissue were loosely arranged, in which chloroplasts decreased obviously, and cells in the sponge tissue were not intensely arranged and the intercellular space enlarged (Figure 3b to d). Furthermore, the structure of the cotyledon, cultured restoratively, hardly changed (Figure 3f to g).

Effects of Hygromycin B on shoot tip

Shoot tip of plant was formed by kinetic leaf buds, and generally comprised meristematic zone, elongation zone and maturation zone. As shown in Figure 4a, c, e, g and i, several layers of cells in the surface of shoot tip meristematic zone were regularly arranged and exhibited anticlinal division. Along with culture time increase, cells in the shoot tip meristematic zone of seedling on MS with 30 µg/ml Hygromycin B were more irregular and even exhibited atrophy (Figure 4, b, d, f, h and j). However, when seedling on MS with 30 µg/ml Hygromycin B was

cultured restoratively for 5 days, cells in shoot tip meristematic zone exhibited regular arrangement (Figure 4l), and for those cultured restoratively for 2 days, cells in meristematic zone had little regulation (Figure 4k).

In addition, it was found in Figure 4a, c, e, g and i, that leaf primordium of seedling on MS was formed (one, two, three pairs, respectively). But leaf primordium was not found in seedling on MS with Hygromycin B or cultured restoratively for 2 to 5 days.

DISCUSSION

Large leaf surface of plant could form bigger photosynthetic area to improve photosynthetic rate, and then promote growth of plant (Muraoka et al., 2003; Rivas et al., 2007). In this article, in comparison with the control, cotyledon of *A. thaliana* seedling on MS with Hygromycin B hardly grew and curved, which might not form effective photosynthetic area that influences photosynthesis, and hardly composes sufficient nutrition to benefit growth, and then makes *A. thaliana* seedling smaller. Furthermore, leaf of *A. thaliana* seedling on MS with Hygromycin B was not formed.

In order to further study functional mechanism of Hygromycin B on cotyledon and leaf, structure of cotyledon and shoot tip were studied by paraffine slice up technology. In comparison with the control group, the intercellular space in the upper epidermis and lower epidermis of cotyledon on MS with Hygromycin B enlarged along with culture time increase, which had greatly affected safeguard function and was harmful to natural photosynthesis (Simpson and Lee, 1976; Mitchell et al., 1991; Dai et al., 1995; Pettigrew et al., 2000; Stessman et al., 2002; Adamchuk, 2004). It was found that when concentration of mesophyll cell was very high, photosynthesis efficiency was very high, and then growth rate of plant was quick (Geng et al., 2002), which was also confirmed by Ueno et al. (2006). Furthermore, in this research, cells in the meristematic zone of shoot tip on MS with Hygromycin B appeared with atrophy, abnormal array, and leaf primordium was not found. It is indicated that shoot tip of *A. thaliana* seedling on MS with Hygromycin B was atrophied, which made leaf not to be formed. Hygromycin B could destroy the functions of ribosome in various cells by competing with the binding sites between ribosome and elongation factor EF-2, restrain protein synthesis, and then make sensitivity tissue brown and die (Gritz and Davies, 1983; Santerre et al., 1984; Cullen et al., 1987).

Thereby, it is conferred that Hygromycin B might restrain some protein synthesis of *A. thaliana* seedling, or make mesophyll intercellular space of cotyledon evidently enlarge, then affect photosynthetic velocity and photosynthesis of *A. thaliana* seedling. Accordingly, Hygromycin B could indirectly affect photosynthesis of *A. thaliana* seedling, and then photosynthesis production could not fully restore the growth of leaf and shoot tip, finally it restrained growth and development of cotyledon and leaf.

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