Pak. J. Bot., 41(4): 1611-1618, 2009.

EFFECTS OF KANAMYCIN ON GROWTH AND DEVELOPMENT OF *ARABIDOPSIS THALIANA* SEEDLING, COTYLEDON AND LEAF

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Abstract

In this research, growth and development of *Arabidopsis thaliana* seedling cotyledon and leaf were distinctly influenced by kanamycin. Contrasted against the control, cotyledon on MS with kanamycin was very small and took on etiolation, etiolation of cotyledon was serious and some even died as cultured for 10 d. Along with culture time increasing, cells in the epidermis tissue of cotyledon on MS with kanamycin were irregularly arranged, the intercellular space in the mesophyll tissue was large, and ability of cell division in the meristematic zone of shoot tip gradually weakened. In addition, it was detected that effects of kanamycin on seedling cotyledon and shoot tip exhibited reversibility to a certain extent. Accordingly, it is guessed that kanamycin may affect growth of *Arabidopsis thaliana* seedling cotyledon and leaf by restraining protein synthesis, and then influence growth of *Arabidopsis thaliana* seedling.

Introduction

Leaf is the main organ of plant, in which photosynthesis is carried through and some materials required to growth of plant such as carbohydrate, fat and protein and so on are synthesized (Simpson & Lee, 1976; Zhang & Shangguan, 2006; Zeeman *et al*., 2007). Furthermore, leaf is the primary organ doing transpiration which not only promotes transport of mineral elements but also debases temperature of leaf surface to avoid strong solar burn (Liu & Teskey, 1995; Fleck *et al*., 1998; Sun *et al*., 2001; Gregg *et al*., 2006; Meharun-Nisa *et al*., 2009). In addition, leaf has many other functions such as absorbability, secretion and the like (Zheng & Feng, 2006; Sakcali *et al*., 2008). Leaf is commonly formed by apical growth, marginal growth and intercalary growth of leaf anlage upside, however growth and development of leaf are often influence by various environment factors (Zamin & Soaliha, 2006), such as kanamycin, hygromycin, geneticin and other antibiotics.

Kanamycin belongs to one kind of aminoglycoside antibiotic, makes green organs of plant etiolation by disturbing protein synthesis and then results in the death of plant (Nap *et al*., 1998; Lu, 2001; Wang *et al*., 2003; Chen *et al*., 2005). At present, *Arabidopsis thaliana* is the model plant preferred in genetics, molecular biology, biology of development, and in other research. In this article, effects of kanamycin on growth of *Arabidopsis thaliana* seedling cotyledon and leaf were studied in order to reveal the relation between kanamycin and development of plant seedling leaves.

Materials and Methods

Plant materials: Seeds of wild-type *Arabidopsis thaliana* (Colombia type) available in our laboratory were used.

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Culture of *Arabidopsis thaliana* seedling: Seeds of *Arabidopsis thaliana* were incubated in sterile water for 30 min., 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 thaliana* were sown on MS, and cultured at 22^{o}C/18^{o}C with a 16 h light and 8 h dark photoperiod.

Effects of kanamycin on growth of *Arabidopsis thaliana* seedling: Seeds of *Arabidopsis thaliana* were respectively sown on MS with different concentration of kanamycin, 0 mg/L, 10 mg/L, 30 mg/L, 50 mg/L, 70 mg/L or 90 mg/L, respectively, and were cultured at 22^{o}C/18^{o}C with a 16 h light and 8 h dark photoperiod. There are three replications in each group.

Effects of kanamycin on cotyledon and leaf of *Arabidopsis thaliana* seedling: Seeds of *Arabidopsis thaliana* were sown on MS or MS with 50 mg/L kanamycin and cultured at 22^{o}C/18^{o}C with a 16 h light and 8 h dark photoperiod. There are three replications in each group. Furthermore, seedlings cultured for 5 d on MS medium with 50 mg/L kanamycin were transferred on MS to be cultured for 2~5 d.

Histology analysis: Cotyledon and shoot tip of *Arabidopsis thaliana* seedlings cultured on MS or MS with 50 mg/L kanamycin for different days and those cultured restoratively for 2~5 d were fixed into 50% FAA solution, and then processed according to the following steps: dehydration with series of ethanol, transparence of xylene, immersion and embedment of paraffin wax. The paraffin-embedded tissue samples were sliced by microtome, and each sample was repeated three times and observed with Olympus photos microscope.

Results

Effects of kanamycin on growth of *Arabidopsis thaliana* seedling: When seeds of *Arabidopsis thaliana* sown on MS with different concentration of kanamycin, 0 mg/L, 10 mg/L, 30 mg/L, 50 mg/L, 70 mg/L, or 90 mg/L kanamycin were cultured for 2 d, some began to bourgeon and divorced from seed capsule. When cultured for 5 d, difference between seedlings on MS with kanamycin and the control was very evident, especially in the roots and color of cotyledons (Fig. 1a). At 7 d, lateral root of seedling on MS with kanamycin did not come into being, some seedlings took on etiolation and died, however main root of seedling on MS was very long and there were 1~2 lateral roots (Fig. 1b). At 10 d, two pairs of leaves and 2~3 lateral roots were found in seedling on MS, but the leaf was not found in seedling on MS with kanamycin making an exception of that on MS with 10 mg/L kanamycin; when concentration of kanamycin increased, main roots of seedlings were shorter and shorter, and lateral root was not formed too, etiolation degree of seedling was severer and severer, and the etiolation rate is respectively 0%, 10%, 60%, 78%, 97% or 100%, furthermore, death rate of brown was higher and higher, in turn 0%, 1 %, 5%, 7%, 11% or 18% (Fig. 1c). Accordingly, when the concentration of kanamycin added in MS is 70 mg/L or 90 mg/L, seedling almost exhibited etiolation, and the death rate exceeded 10%, however is 10 mg/L kanamycin, seedling only took on 10% etiolation rate and 1% death rate.

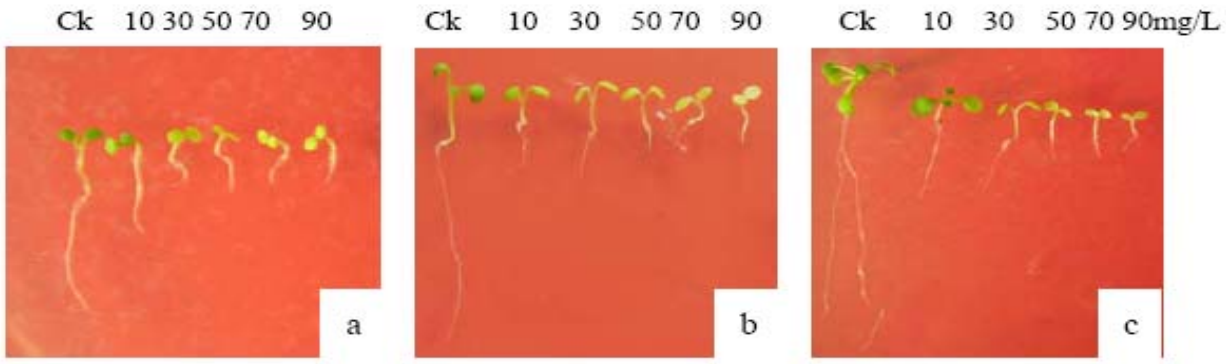

Fig. 1. Effects of kanamycin on growth of *Arabidopsis thaliana* seedling (a), (b) and (c) separately represents *Arabidopsis thaliana* seedling cultured on MS with 0 mg/L, 10 mg/L, 30 mg/L, 50 mg/L, 70 mg/L, or 90 mg/L kanamycin for 5 d, 7 d, 10 d.

Effects of kanamycin on cotyledon and leaf of *Arabidopsis thaliana* seedling: When seeds of *Arabidopsis thaliana* sown on MS with 0 mg/L kanamycin or 50 mg/L kanamycin were cultured for 3 d, 90% cotyledons on MS divorced from seed capsule, however there were 50% cotyledons on MS with kanamycin divorcing from seed capsule. It was found in Fig. 2, cotyledon went up all along during culture, but cotyledon on MS were relatively larger than those on MS with kanamycin and the difference was very obvious, otherwise difference between cotyledon on MS with kanamycin and that restoratively cultured was very unobvious.

As shown in Table 1, etiolation degree of cotyledons was severer and severer when culture time increased. At 7 d, 73% cotyledons exhibited etiolation and a few were dead, but cotyledon cultured restoratively for 2 d still took on Kelly. At 10 d, 78% cotyledon presented etiolation and 7% cotyledons were dead, yet 60% cotyledons restoratively cultured exhibited etiolation and without death. In addition, the first pair of leaves was found in seedling cultured for 7 d on MS, and the second pair of leaves was formed as cultured for 10 d on MS, whereas the leaf was not found in seedling on MS with kanamycin and seedling cultured restoratively.

Effects of kanamycin on structure of *Arabidopsis thaliana* cotyledon: In this study, it was found that cotyledon of *Arabidopsis thaliana* seedling consisted of cuticle, mesophyll and leaf veins (Fig. 3). The epidermis of cotyledon on MS was composed of flat cells which were tightly arranged 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 with a abundance of chloroplast, the major axes of cells was vertical to epidermis, and cells were closely arranged; the sponge tissue consisted of 3~4 layers cells with lesser chloroplasts, and the size and shape of cells exhibited rather atactic and arranged loosely, which could come into being large intercellular space to benefit photosynthesis and gas exchange (Fig. 3a).

However, compared with the control, along with culture time enhancing, the intercellular space in the epidermis of cotyledon on MS with 50 mg/L kanamycin gradually increased and the shape of cells slowly became atactic from flat; the arrangement of cells in stockade tissue took on looser and looser, and number of chloroplast also reduced; cells in the sponge tissue were loosely arranged and the intercellular space enlarged (Fig. 3b-d). Furthermore, as in Fig. 3 (f-g), structure of cotyledon cultured restoratively for 2~5 d exhibited changes, for example, the intercellular space and shape of cells in the epidermis gradually came back, and the arrangement of cells in stockade tissue and sponge tissue was rather regular and intense.

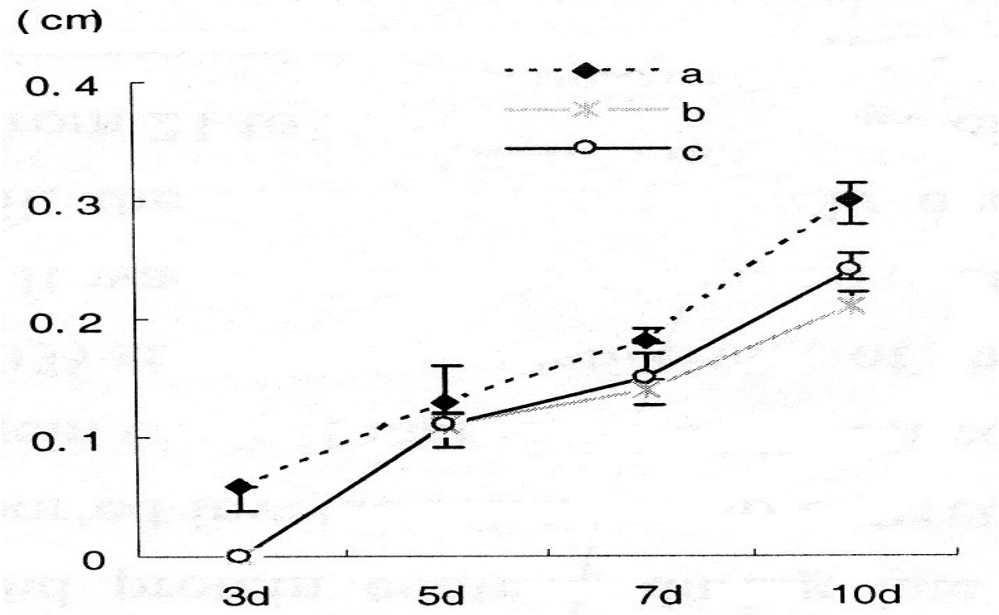

Fig. 2. Effects of kanamycin on cotyledon of *Arabidopsis thaliana* seedling. (a) and (b) represent the width of cotyledon on MS or MS with 50 mg/L kanamycin for 3 d, 5 d, 7 d and 10 d, respectively; (c) represents the width of cotyledon on MS with 50 mg/L kanamycin for 3~5 d, then transferred on MS and continued to be cultured for 2~5 d, respectively. Note: the width of cotyledon was formed at least three independent replicates, the error bars represent ses.

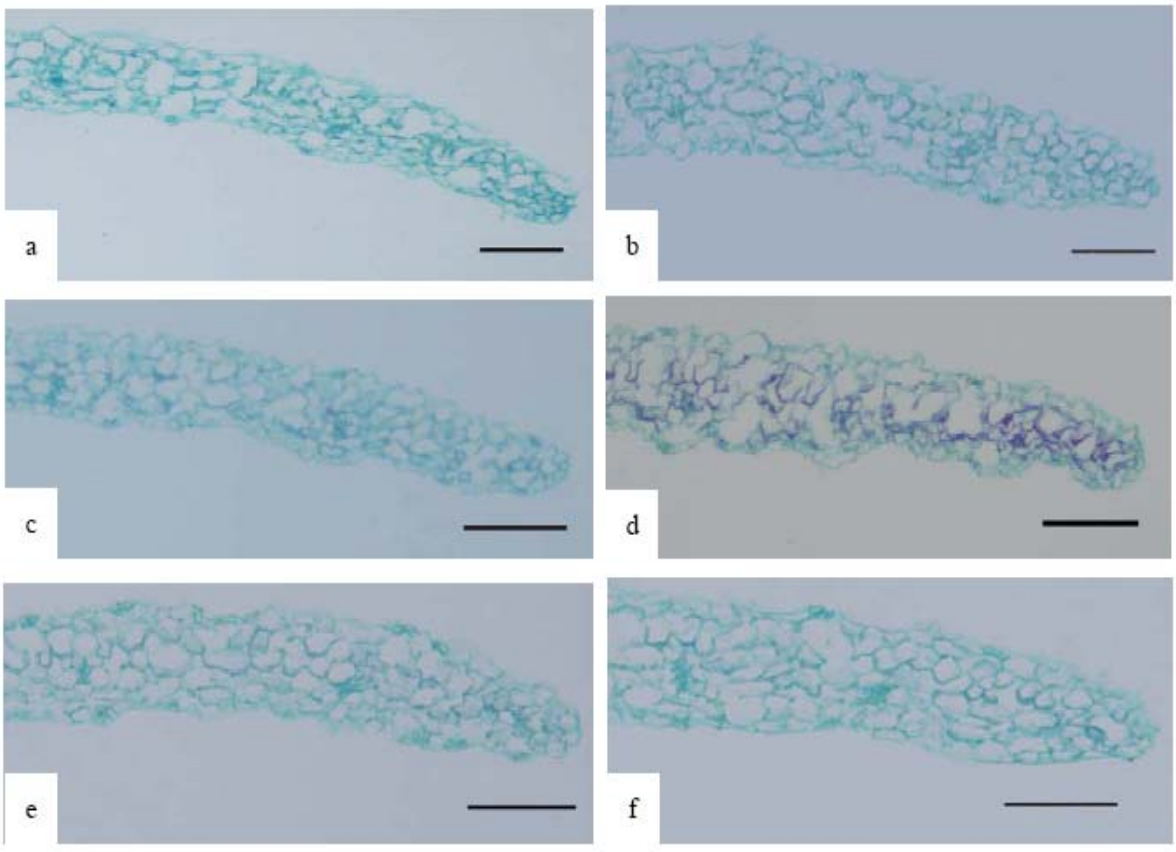

Fig. 3. Effects of kanamycin on structure of *Arabidopsis thaliana* seedling cotyledon. (a) The part transverse section of cotyledon from *Arabidopsis thaliana* seedling cultured on MS for 5 d; (b), (c) and (d) respectively represents the part transverse section of cotyledon from *Arabidopsis thaliana* seedling cultured on MS with 50 mg/L kanamycin for 5 d, 7 d and 10 d; (e) and (f) represent the part transverse section of cotyledon from *Arabidopsis thaliana* seedling on MS with 50 mg/L kanamycin for 5 d and then transferred on MS for 2 d, or 5 d respectively. The scales represent 100 μm.

Table 1. Effects of kanamycin on etiolation rate and death rate of *Arabidopsis thaliana* seedlings.

kanamycin (mg/L)	%	Cultured time			Cultured restoratively time	
		5d	7d	10	2d	5d
0	Etiolation rate	0	0	0		
	Death rate	0	0	0		
50	Etiolation rate	58	73	78		
	Death rate	0	5	7		
0	Etiolation rate				39	60
	Death rate				0	0

Effects of kanamycin on shoot tip of *Arabidopsis thaliana* seedling: Shoot tip of plant was commonly made up of meristematic zone, elongation zone and maturation zone. In this study, cells in the surface of meristematic zone from shoot tip of *Arabidopsis thaliana* seedling on MS were regularly arranged, and the leaf primordium was formed, one pair, one pair, two pairs, three pairs, respectively (Fig. 4a, c, e, g). As compared with the control, cells in the surface of shoot tip meristematic zone from seedling on MS with 50 mg/L kanamycin were quite regular, but their division ability was weaker and weaker during continuous culture. Furthermore, although one pair of leaf primordium was formed in seedling for 5 d on MS with 50 mg/L kanamycin, other leaf primordium was not found along with culture time increasing (Fig. 4b, d, f, h).

In addition, when seedling on MS with 50 mg/L kanamycin was cultured restoratively for 2~5 d, cells in shoot tip meristematic zone exhibited regular arrangement, their division ability enhanced in contrast with that of seedling all along cultured on MS with kanamycin, and two pairs of leaf primordium were found in seedling cultured restoratively for 2 d and grew up in seedling cultured restoratively for 5 d (Fig. 4i, j).

Discussion

It is well known that kanamycin is a sort of aminoglycoside antibiotic, could combine with ribosome 30S subunit in the chloroplast and mitochondrial and interfere protein synthesis, finally render etiolation and death of plant (Nap *et al*., 1998; Lu, 2001; Yang *et al*., 2002; Wang *et al*., 2003; Chen *et al*., 2005; Rafia & Sehrish, 2008). In this article, effects of kanamycin on growth and development of *Arabidopsis thaliana* leaves were studied. Leaf is the main organ of plant carrying through photosynthesis, by which plant would synthesize enough nutriment to satisfy growth and development (Muraoka *et al*., 2003; Zhang & Shangguan, 2006; Rivas *et al*., 2007; Franco *et al*., 2007). In this study, etiolation degree of *Arabidopsis thaliana* seedling was severer and severer, and death rate of brown was higher and higher along with concentration of kanamycin increasing. The leaf was formed in control, yet was not found in seedling on MS with kanamycin with an exception of 10 mg/L kanamycin and seedling cultured restoratively for 2~5 d. It is presumed that kanamycin might not only restrain growth of *Arabidopsis thaliana* seedling cotyledon, but also influence formation and growth of leaf. In addition, etiolation of seedlings cultured restoratively for 2~5 d was not serious and there was absent of brown death, which indicates although effect of kanamycin on etiolation of seedlings was very great, this effect took on reversibility to a certainty.

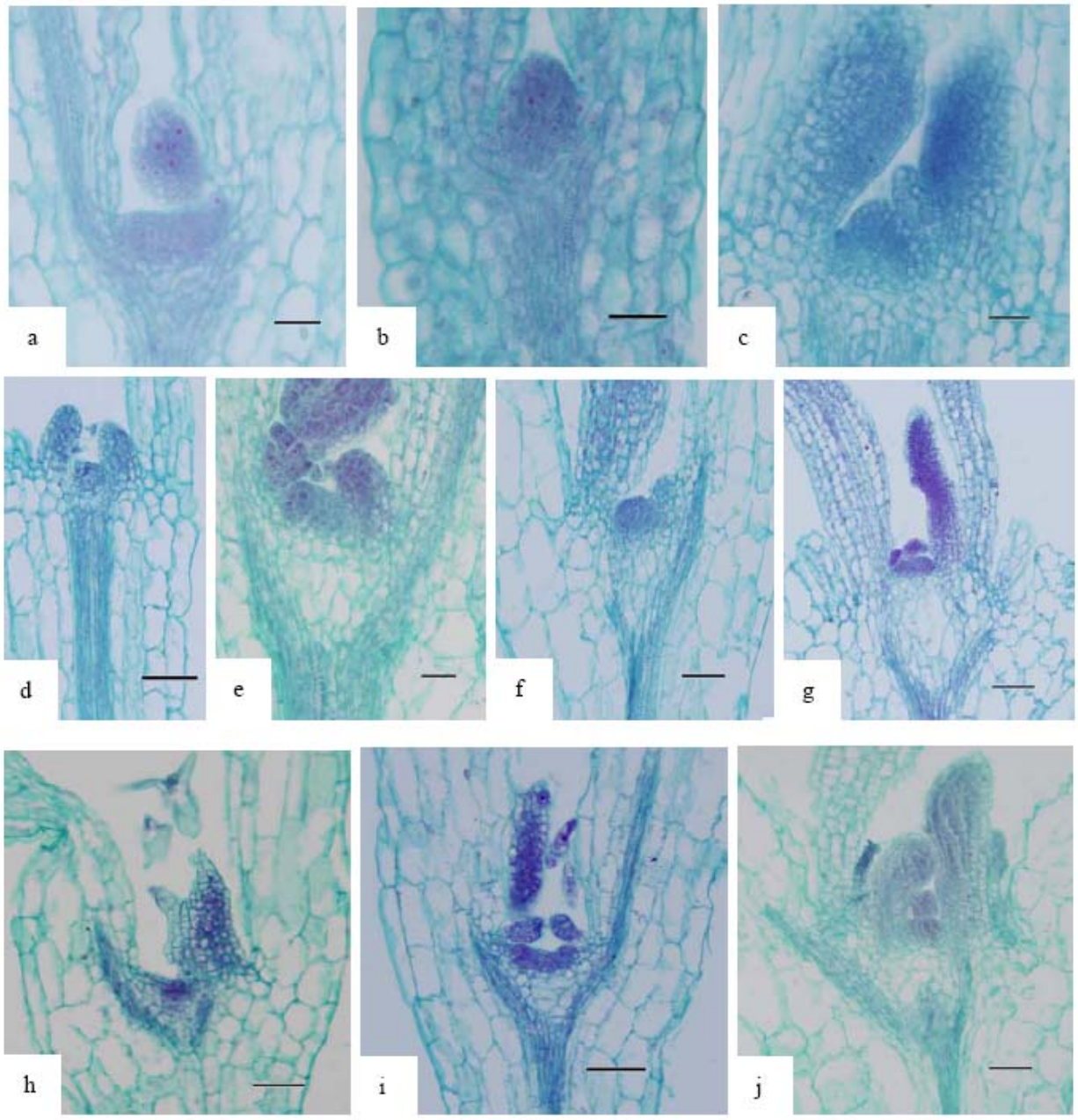

Fig. 4. Effects of kanamycin on shoot tip of *Arabidopsis thaliana* seedling. (a), (c), (e) and (g) respectively represents the vertical section of shoot tip from *Arabidopsis thaliana* seedling cultured on MS for 2 d, 5 d, 7 d, 10 d; (b), (d), (f) and (h) respectively represents the vertical section of shoot tip from *Arabidopsis thaliana* seedling cultured on MS with 50 mg/L kanamycin for 2 d, 5 d, 7 d and 10 d; (i) and (j) represents the vertical section of shoot tip from *Arabidopsis thaliana* seedling restoratively cultured on MS for 2 d, or 5 d, respectively. The scales of (a), (b), (c), (d) and (e) represent 20 μm, the scales of (f), (g), (h) and (i) represent 50 μm.

In order to further study functional mechanism of kanamycin, structure of *Arabidopsis thaliana* seedling cotyledon and shoot tip was studied by paraffine slice up technology. In comparison with the control, along with culture time increasing, the intercellular space in the epidermis of cotyledon on MS with kanamycin gradually enlarged, shape of cells slowly turned into atactic, and arrangement of cells in mesophyll tissue were looser and looser, which all were harmful to natural photosynthesis (Mitchell *et al*., 1991; Pettigrew *et al*., 2000; Stessman *et al*., 2002; Adamchuk, 2004). It was found

that photosynthesis efficiency would be very high and growth of plant was quick when concentration of mesophyll cell was very large (Geng *et al*., 2002), which was also confirmed by Ueno *et al*., (2006). Moreover, cell division in the meristematic zone of shoot tip on MS with kanamycin appeared weaker and weaker along with culture time increasing, only one pair of leaf primordium was formed and was not developed into leaf. Contrast with that of seedling all along cultured on MS with kanamycin, the intercellular space and shape of cells in the epidermis of cotyledon cultured restoratively for 2~5 d gradually came back, and the arrangement of cells in mesophyll tissue was rather regular and intense. Besides, cells in shoot tip meristematic zone exhibited regular arrangement, their division ability enhanced, and two pairs of leaf primordium were found. Therefore, effects of kanamycin on structure of mesophyll tissue and shoot tip of seedling also have some reversibility. In addition, number of leaf primordium in seedling cultured restoratively increased, but the leaf was not formed, possibly the restorative time was so short that seedling could not produce enough nutrition to satisfy its growth and then make growth of plant delay. Accordingly, kanamycin might indirectly influence photosynthesis of plant, which could not synthesize enough photosynthetic production to satisfy growth of cotyledon and leaf, then further delay the process of growth and development of plant, and finally even result in death of plant.

Acknowledgment

This study was supported by Science Fund of Henan (2008A180015, 2008B180007, 08N042, 082300430270, and 082102120019), and funds of Henan Normal University (06114 and 2007034), P. R. China.

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(Received for publication 7 June 2008)