EFFECTS OF KANAMYCIN ON GROWTH AND DEVELOPMENT OF *ARABIDOPSIS THALIANA* SEEDLING AND ITS ROOT

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Abstract: In this article, it was found that growth and development of main root and lateral root of *Arabidopsis thaliana* seedling were evidently affected by kanamycin, and etiolation of *Arabidopsis thaliana* seedling was very serious. Compared to the controls, main root of *Arabidopsis thaliana* seedling on MS with kanamycin was very short, lateral root was not formed, and meristematic zone of root tip diminished and exhibited large intercellular space. Furthermore, effect of kanamycin on root tip of *Arabidopsis thaliana* seedling exhibited reversible, but the reversibility in elongation of main root was not obvious. Accordingly, it is presumed that kanamycin might have effects on photosynthesis of *Arabidopsis thaliana* seedling and supply of nutriment by influencing growth of seedling leaves, and then restrain growth and development of its main root and lateral root.

INTRODUCTION

Along with development of biological technology, plant genetics translation gradually becomes one popular topic in the research of application biology, such as plant disease-resistant, anti-insect, anti-weed killer, and so on. At present, some antibiotics are mainly used in plant genetics translation, such as kanamycin, hygromycin, and the like. Furthermore, kanamycin is widely applied in plant genetic engineering, which is one kind of aminoglycoside antibiotic disturbing protein synthesis, makes green organs of plant etiolation and then results in death of plant (Napj Bijvoet 1998, Lu 2001, Wang 2003, Chen 2005).

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 kanamycin on growth and development of *Arabidopsis thaliana* seedling, its main root and lateral root, and so on, were studied in order to reveal effect mechanism of kanamycin on growth and development of plant seedling and its root.

MATERIALS AND METHODS

Plant materials

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

Methods

Culture of Arabidopsis thaliana seedling

After seeds of *Arabidopsis thaliana* were surface-sterilized with 70% ethanol and sterilized with 5% sodium hypochlorite, sown on MS, and then cultured at 22/18° with a 16h light and 8h dark photoperiod.

Effects of kanamycin on growth of Arabidopsis thaliana seedling

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

Effects of kanamycin on root of Arabidopsis thaliana seedling

Seeds of *Arabidopsis thaliana* were sown on MS, or MS with 50mg/L kanamycin and cultured at 22/18°with a 16h light and 8h dark photoperiod. There are three replications in each group. Furthermore, *Arabidopsis thaliana* seedlings cultured for 5d on MS with 50mg/L kanamycin were transferred on MS to be cultured.

Histology analysis

Root tip of *Arabidopsis thaliana* seedling cultured on MS or MS with 50mg/L kanamycin for different days was fixated into 50% FAA solution, and then processed according to the following steps: dehydration by series of ethanol, transparence of xylene, immersion and embedment of paraffin wax. The paraffin-embedded tissue samples were sliced by microtome, and there were three replications in every material.

RESULTS AND DISCUSSIONS

Effects of kanamycin on growth of *Arabidopsis thaliana* seedling

When seeds of *Arabidopsis thaliana* sown on MS with different concentration of kanamycin were cultured for 2d, some began to bourgeon and divorced from seed capsule. As shown in table 1, when cultured for 5d, difference between seedling on MS with kanamycin and the control was very evident, especially in root and color of cotyledon (Fig.1, a). At 7d, lateral root of seedling on MS with kanamycin did not come into being and some seedlings took on etiolation and died, however root of seedling on MS was very long and had 1~2 lateral roots (Fig.1, b). At 10d, 2~3 lateral roots were found in seedling on MS, however, when concentration of kanamycin increased, main roots of seedlings were shorter and shorter, and lateral root was not formed too. In addition, etiolation degree of seedling was severer and severer, death rate of brown was higher and higher (Fig.1, c).

Table 1 Effects of Kna on etiolation rate and death rate of *Arabidopsis thaliana* seedling

		Kna（mg/L）					
Culture time	%	CK	10	30	50	70	90
5d	Etiolatio rate	0	0	35	58	90	96
	Death rate	0	0	0	0	0	0
7d	Etiolatio rate	0	5	51	73	93	98
	Death rate	0	0.5	1	5	9	14
10d	Etiolatio rate	0	10	60	78	97	100
	Death rate	0	1	5	7	11	18

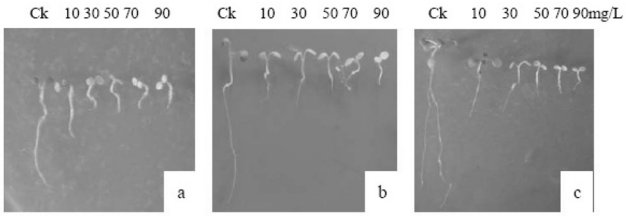

Fig.1 Effects of Kna on growth of *Arabidopsis thaliana* seedling

(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, 10d, respectively.

Effects of kanamycin on root of *Arabidopsis thaliana* seedling

Seeds of *Arabidopsis thaliana* were sown and cultured on MS or MS with 50mg/L kanamycin, respectively, it was found that growth of root was severely influenced by kanamycin. 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 was much less. Furthermore, when seedling cultured for 7d on MS with kanamycin was transferred on MS to be

cultured 2~5d, their main roots hardly changed. Furthermore, effect of kanamycin 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 them cultured restoratively for 2~5d.

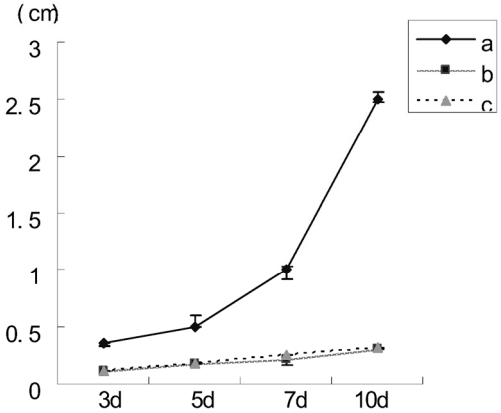

Fig.2 Effects of Kna on elongation of *Arabidopsis thaliana* seedling main root

(a), (b) represents respectively the length of main root on MS or MS with 50mg/L Kna for 3d, 5d, 7d and 10d; (c) represents the length of main root on MS with 50mg/L Kna for 3~5d, 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.

Effects of kanamycin on root tip of *Arabidopsis thaliana*

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). Otherwise, in root tip of seedling on MS with 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). Whereas, the intercellular space in meristematic zone of seedlings cultured restoratively for 2~5d gradually became small and the whole meristematic zone domain gradually increased (Fig. 3, f-g). Therefore, it is indicated that effect of kanamycin on root tip of *Arabidopsis thaliana* seedling was evidently reversible, but main root of *Arabidopsis thaliana* seedling elongated indistinctively.

Fig.3 Effects of Kna 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) and (g) represents the part vertical section of root tip from *Arabidopsis thaliana* seedling on MS with 50mg/L Kna for 5d and then transferred on MS for 2d, or 5d respectively. The scales represent 20 μm.

One important tissue of plant is root which has many roles such as sustentation, transporting, storage, synthesis, secretion, and so on (Fitter 1996, Nasholm 2001, Karthikeyan 2003, Kirk 2005), furthermore, growth and development of root has closing correlation with environment (Fitter 1991, Robinson 1994, Lopez-Bucio 2003, Malamy 2005, Okushima 2007). In this article, root of *Arabidopsis thaliana* seedlings on MS with kanamycin was shorter and shorter along with culture time increasing, lateral root was not formed, and changes were hardly found in root of *Arabidopsis thaliana* seedling cultured restoratively, which suggested that elongation of main root and formation of lateral root of *Arabidopsis thaliana* seedlings were restrained by kanamycin, and the reversibility of inhibition was very small. But, effects of kanamycin on formation of *Arabidopsis thaliana* seedling lateral root attained to the most remarkable level, which was different from its effects on elongation of *Arabidopsis thaliana* seedling main root. Therefore, it is guessed that the formation phage of main root and lateral root was different or effect mechanism of kanamycin on main root and lateral root of *Arabidopsis thaliana* seedling was diverse. It was further found in *Arabidopsis thaliana* seedlings on MS with kanamycin,

compared with control group, although the root cap exhibited intact cap structure and the meristematic zone looks like taper too, the intercellular space in meristematic zone enlarged and the whole meristematic zone domain became small. In addition, when seedlings were restoratively cultured for 2~5d, the intercellular space in meristematic zone gradually became small and the whole meristematic zone domain were gradually normal. It is indicated that effect of kanamycin on root tip of *Arabidopsis thaliana* seedlings was evidently reversible, but main root of *Arabidopsis thaliana* seedlings elongated indistinctively.

It is well known that kanamycin belongs to aminoglycoside antibiotic, could combine with ribosome 30S subunit in the chloroplast and mitochondrial, disturb protein synthesis, and then result in etiolation and death of plant (Napj Bijvoet 1998, Yang 2002, Chen 2005). 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 increasing. It was indicated that growth of *Arabidopsis thaliana* seedlings were inhibited by kanamycin, and the reversibility of inhibition was very small. Leaf is the main organ in photosynthesis of plant and has many other functions, such as transpiration, absorbability, secretion and the like (Simpson 1976, Liu 1995, Fleck 1998, Sun 2001, Zhang 2006, Franco 2007). Accordingly, it is presumed that kanamycin 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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