The effects of different selective agents on seed germinating and callus forming in different cultivars in *Brassica napus*[*]

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Abstract

This experiment has been done to compare different effects of kanamycin, phosphiothricin and hygromycin on germinative seeds and callus of *Brassica napus ca* Ningyou 12, Ningyou 14 and Ningyou 16. Results showed the sensitivity of *B. napus* to those three selective agents was different during geminating stage. The seeds were less sensitive to kanamycin, while more sensitive to phosphiothricin. In addition, the sensitivities of those three cultivars to the same selective agent showed little difference. During the callus-inducing stage, formation of callus was inhibited when phosphiothricin was used as the selective agent. The optimal screening concentrations of Kan, PPT and Hyg, which were used to screen seeds and callus, have been obtained.

Key words: *Brassica napus,* selective, kanamycin, hygromycin, phosphiothricin, influence

Introduction

Rapeseed (*Brassica napus* L.) is an important oil and vegetable crop, so it is significant to improve its yield, quality and resistance to stress by genetic engineering methods. Transgenic rapeseed is a novel cultivar obtained through tissue culture after inserting foreign target-genes, which encoding new traits into recipient rapeseed cells by biotic, physical or chemical method. In the process of producing transgenic rapeseed, it is fast, effective and convenient to screen transgenic plants using selective marking gene. But the result of selection will be different if we use different selective agents. To investigate effects of selective agents on our screen, we did this experiment and found out the most optimal agents and concentrations to screen our materials. We also offer a reference to selection of optimal selective agent and screening concentration for other transformation experiments in future.

Materials and methods

Materials: Ningyou12, Ningyou14 and Ningyou16 were from Institute of Agriculture Science of Jiangsu province. Murashige and Skoog (MS) medium is used for root growth and 1/2 MS medium is used for callus induction. The callus induction medium consisted of half-strength MS medium, which supplemented with 0.2mg/L BAP (6-benzylaminopurine), 0.1mg/L a-naphthaleneacetic acid (NAA) and 5mg/L silver nitrate (Y Wang et al., 2004). The master liquor of selective agents is kanamycin (50mg/mL), hygromycin (5mg/mL) and phosphiothricin (2mg/mL).

Antibiotic selection of seeds: The root growth medium contains kanamycin (0, 50, 100, 150, 200, 250mg/L), hygromycin (0, 25, 50, 100mg/L) and phosphiothricin (0, 20, 40, 60, 80, 100mg/L) respectively after building MS salts in different cultural vessels. Since we had seeds obtained from three cultivals as material, three groups of experiments were prepared, each of which contained 14 cultural vessels (for 0mg/L only one vessel). Health and well-stacked seeds of Ningyou12, 14 and 16 cultivars were selected. Seeds were surface-sterilized for 5 min with 10% sodium hypochlorite with 0.1% Tween added as a surfactant. The sterilization was followed by a 1-min rinse with 95% ethanol. The seeds were then washed thoroughly with sterile distilled water and germinated on MS basal medium with 20g/L sucrose, solidified with 2 g/l Gel rite (Cardoza et al., 2003). The sterilized seeds were sowed in the root growth culture. The cultures were grown in the culture chamber at 24±1°C under a 16/8-h (light/dark) photoperiod with light provided by an equal mixture of warm-white fluorescent for 3 days after 2day's culture without light. Four days later, seedlings were taken out to investigate their differences. 20 shoots were taken as samples and the lengths of axial roots and hypocotyls were measured.

Antibiotic selection of callus: Seeds of Ningyou 12, 14 and 16 cultivars were sterilized and sowed on the MS salts to germinate. Cotyledonary petioles were sliced from 5- to 7-day-old seedlings as explants. Then those explants were cultured in the callus inducing medium, which were dispensed into Petri plates and supplemented with kanamycin (0, 5, 10, 15, 20, 25, 30mg/L), hygromycin (0, 5, 10, 15mg/L) and phosphiothricin (0, 5, 10, 15mg/L) respectively at 24±1°C, 16/8-h (light/dark) photoperiod .The rates of callus-formation were obtained 9 days later.

Results

The influence of kanamycin on seeds germination

The results showed appropriate concentration of kanamycin could promote the extension of hypocotyls. And seedling

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growth was depressed at high concentration of kanamycin (Fig. 1). In addition, effects of high concentration of kanamycin on different cultivars were different The hypocotyls of Ningyou 12 and 14 elongated at the concentrations of kanamycin ranged 0-50mg/L and 0-100mg/L respectively, while the length of hypocotyls of Ningyou 16 had little change at the concentration ranged from 0mg/L to 150mg/L. When the concentration exceeded the range above, the elongation of hypocotyls was inhibited obviously. Kanamycin restrained the elongation of main roots of these three cultivars.

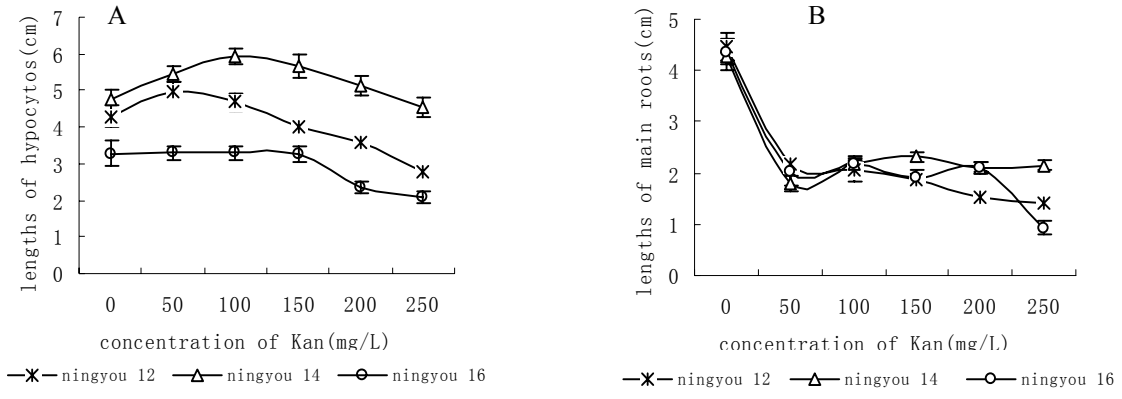

Fig. 1 The effects of kanamycin on the germination of seeds from different cultivars (A) The effect of kanamycin on elongation of hypocotyls (B) The effect of kanamycin on elongation of main root. Each spot in the curve was the average of measured data of 20 samples. The bar on the spot represents standard deviation

The influence of phosphiothricin on seeds germination

There was obviously inhibitory action when phosphiothricin was used as selective agent. 20mg/L of phosphiothricin could inhibit the elongation of hypocotyls of all three cultivars (Fig.2) The lengths of main roots of these three cultivars were negatively correlated with the concentration of phosphiothricin.

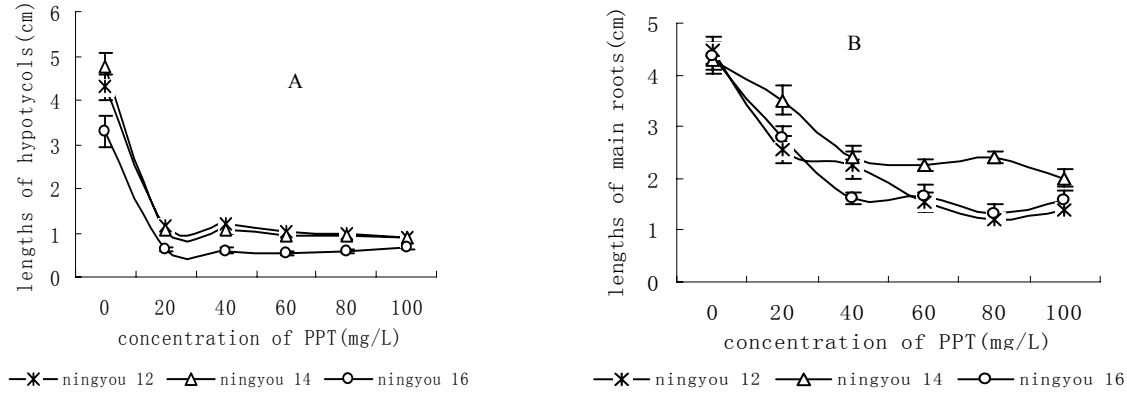

Fig.2 The effect of phosphiothricin on the germination of seeds from different cultivars (A) The effect of phosphiothricin on elongation of hypocotyls; (B) The effect of phosphiothricin on elongation of main roots. Each spot in the curve was the average of measured data of 20 samples. The bar on the spot represents standard deviation.

The influence of hygromycin on seeds germination

The relationships of hygromycin and elongation of hypocotyls and main roots were tended to resemble linear correlation in all cultivars (Fig.3). The lengths of both hypocotyls and main roots trended to shorten as long as the concentration of hygromycin increased. The inhibition of hygromycin on the germination of rapeseeds was not as fierce as that of phosphiothricin, but fiercer than that of kanamycin.

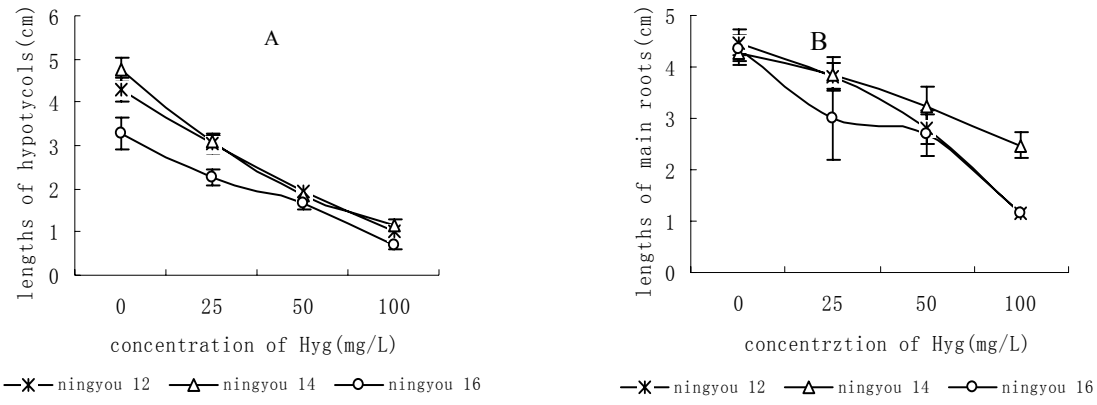

Fig. 3 The effects of hygromycin on the germination of seeds from different cultivars (A) The effect of hygromycin on the growth of hypocotyls (B) The effect of hygromycin on the growth of main roots. Each spot in the curve was the average of measured data of 20 samples. The bar on the spot represents standard deviation.

The effects of different selective agents on the callus formations of different cultivars

Nine days later after cotyledonary petioles sliced from different cultivars were transferred into the callus induction medium supplemented with different kind of selective agents, the numbers of callus of three cultivars were counted respectively and their percentages were obtained (Table 1). Obviously, all these three cultivars seemed to be recipient to kanamycin while this was added into the callus induction medium. To screen Ningyou 12, Ningyou 14 and Ningyou 16 effectively, 10mg/L, 20mg/L and 15mg/L of kanamycin could be used. In table 1, the most optimal concentrations of phosphiothricin to screen callus of Ningyou 12, Ningyou 14 and Nningyou 16 were obtained, which were 15mg/L, 10mg/L and 5mg/L respectively.

Table.1 Percentage of callus formation of cotyledonary petioles sliced from different cultivars with different selective agent used

Concentration ($Mg \cdot L^{-1}$)	Ningyou 12			Ningyou 14			Ningyou 16		
	Kan	PPT	Hyg	Kan	PPT	Hyg	Kan	PPT	Hyg
0	71.4	71.4	71.4	97.0	97.0	97.0	72.2	72.2	72.2
5	26.7	58.1	44.1	25.9	25.8	51.6	41.0	0	58.8
10	0	3.1	36.4	15.4	0	24.2	29.0	0	32.0
15	0	0	28.6	4	0	20.1	0	0	8.0
20	0	-	-	0	-	-	0	-	-
25	0	-	-	0	-	-	0	-	-
30	0	-	-	0	-	-	0	-	-

Discussions

It has been reported that different selective agents resulted in different effects (Wang et al). Different selective agents should be chosen according to different cultivars. To screen resistant shoots, the suitable concentration of antibiotics should be determined first, for the reason that incorrect concentration would result in failures. “Escape” would appear if the concentration was too low, while the green shoots would die or turn into white shoots if it is too high. Beside of the concentration of the selective agents, some other factors also contributed to the improvement of high frequency plant regeneration of the explants of cotyledonary petioles, such as physiological status of explants, AS, bacterium concentration, immerged time and so on. (Y Wang et al., 2006)

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