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EFFECTS OF 5-AZAC ON VERNALIZATION AND FLOWER-BALL DEVELOPMENT IN BROCCOLI (*BRASSICA OLERACEA* VAR. *ITALICA*)

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Abstract

The experiment was carried out with different application ways of 5-azaC on two varieties of broccoli 'Qing-feng 103' and 'Lv-xiu' in order to study the influences of DNA demethylation on vernalization, flower bud differentiation and flower-ball development in broccoli. The results showed that, the 5-azaC spraying could promote vernalization, advance flower-bud differentiation stage, rapid growth and initial harvesting period.

Introduction

Broccoli (*Brassica oleracea* var. *italica*), rich in thiocyanate and indoles compound, is the popularity vegetable in the world. Broccoli produces the flower-ball on the premise that they can induce vernalization. Moreover, studies in past 10 years have shown that plant vernalization correlated closely to the DNA methylation, vernalization promoted flowering through DNA methylation level falling.

5-azaC is the DNA demethylation reagent, which can reduce the DNA methylation level. Burn *et al*., (1993) found that 5-azaC could partialy replace low temperature to promote *Arabidopsis* flowering. Finnegan *et al*., (1998) found that plant could positive correlate flower time with methylation level is falling, and the depressed methylation level, during earlier plant flowering. Subsequent studies showed that effects of DNA demethylation and low temperature had additive property (Sheldon *et al*., 1999, Lizal *et at*., 2001, Horvath *et al*., 2003), and maybe it was because the very important gene or promoter of flowering demethylated that vernalization promoted plant flowering. Therefore, we tested premature broccoli hybrid named 'Qing-feng 103' and medmaturity broccoli hybrid named 'Lv-xiu' to study the effects of 5-azaC on vernalization, flower bud differentiation and flower-ball development by spraying or root-application of 5-azaC.

Materials and Methods

The experiments were performed with premature broccoli (*Brassica oleracea* var. *italica*) hybrid named 'Qing-feng 103' (provided by Hongkong Weiqin Germchit Company) and medmaturity broccoli hybrid named 'Lv-xiu' (provided by Korean Xingnong Germchit Joint-stock Corporation) in Horticulture Installation Engineering Center of Northeast Agricultural University from May to October in 2007. 5-azaC was provided by Harbin Saituo Biological Technology Corporation. Objects were sown on May 25, and transplanted in 12 cm×12 cm nutrition pot when seedlings grew to 1~2 euphyllas. Then seedlings were chose randomly to demethylate when 'Qing-feng 103'

seedlings grew to 3 euphyllas and 'Lv-xiu' seedlings grew to 5 euphyllas. For 5-azaC (azaC-y) spraying the consistency of 5-azaC was 10 mg·L^{-1}, and 1~2 drops of tween 20 were added in the solution so that it could be absorbed adequately by broccoli leaves. Here we sprayed 5-azaC continuously until the whole leaves were bedewed, and for 5-azaC root-applied treatment (azac-r), the consistency of 5-azaC was 0.2 mg·L^{-1}, and 50 ml of the solution was applied to each nutrition pot. The 5-azaC spraying and root-applied treatments were applied at 5-day intervals, and water spraying treatment (CK-W), water + tween 20 spraying treatment (CK-T) were set as the controls.

The study had two experiments. The one was growing tip croexamination experiment: We set 120 seedlings each treatment, and five seedlings were chose randomly each treatment for croexamination the day when they applied and then every three days later. The method in the paper of Peichong Guan & Chengyu Liang (1992) was referred to observe the vernalization and flower bud differentiation by OLYMPUS SZH-ILLD BO71 anatomical stereomicroscope. The other was flower-ball development observation experiment: 'Lv-xiu' broccoli was fixed planting on July 17, and 'Qing-feng 103' broccoli was fixed planting on July 27. The individual and row spacing was 70 cm×50 cm and plot area was 1 m×7 m, here we set 20 seedlings each treatment. Flower-ball developmental stage was observed during the vegetation period: Bud stage-30% plants had flower-balls with 1 cm diameter in plot area, rapid growing stage-30% plants had flower-balls with 7 cm diameter in plot area, and initial period of harvest-30% plants had flower-balls with 14 cm diameter in plot area. There were three replicates in each experiment, and the treatments were arranged randomly.

Result and analysis

Effects of 5-azaC on the growing tip in broccoli: Effects of 5-azaC on the growing tip in broccoli has been shown in Fig. 1. In stage of vegetative growth, volume and surface area of growing tip were small and trigonal leaf primordia were differentiated around chief axis of growing tip (QF-a, LX-a). In stage of vernalization end, growing tip bulged, broadened, flattened gradually, and its volume and surface area became so large that leaf primordia hardly wrapped it (QF-b, LX-b). In stage of primary furcation scape primordium differentiation, there were many markedly protuberances around growing tip (QF-c, LX-c). In stage of secondary and tertiary furcation scape primordium differentiation, the secondary furcation scape primordia began to be differentiated around the primary furcation scape primordia when the primary furcation scape primordia were differentiated continuously, and the tertiary furcation scape primordia began to be differentiated around the secondary furcation scape primordia when the primary and secondary furcation scape primordia were differentiated continuously (QF-d, LX-d). This result was the same to the one in the paper of Peichong Guan & Chengyu Liang (1992).

Effects of 5-azaC on vernalization and flower bud differentiation in broccoli: Vernalization end stage was the significant symbols that plant had completed vernalization and began to flower bud differentiate. Sprayed or root-applied 5-azaC could promote vernalization, especially advance flower-bud differentiation stage (Table 1). And the effect of 5-azaC spraying treatment was best.

Effects of 5-azaC on flower-ball developmental stage in broccoli: Bud stage, rapid growing stage and initial period of harvest of 5-azaC spraying or root-applied treatments were earlier than the water spraying control and water + tween spraying control Table 2. The effect of 5-azaC spraying treatment was best.

Table 1. Effects of 5-azaC on vernalization and flower bud differentiation in broccoli (Unit: d).

Treatments	Stage of vernalization end		Stage of primary furcation scape primordium differention		Stage of secondary and tertiary furcation scape primordium differention	
	'Qing-feng 103' broccoli	'Lv-xiu' broccoli	'Qing-feng 103' broccoli	'Lv-xiu' broccoli	'Qing-feng 103' broccoli	'Lv-xiu' broccoli
azaC-y	14.33 ± 0.42 c	25.13 ± 0.23 c	23.17 ± 0.21 d	35.17 ± 0.29 c	35.07 ± 0.03 c	46.87 ± 0.21 d
azaC-r	25.23 ± 0.21 b	36.17 ± 0.29 b	35.23 ± 0.40 c	45.13 ± 0.40 b	46.17 ± 0.57 b	59.03 ± 0.06 c
CK-W	27.20 ± 0.26 a	37.30 ± 0.26 a	38.23 ± 0.15 a	47.13 ± 0.12 a	50.23 ± 0.21 a	61.93 ± 0.12 a
CK-T	25.07 ± 0.29 b	37.27 ± 0.46 a	37.33 ± 0.021 b	47.13 ± 0.75 a	50.20 ± 0.26 a	61.13 ± 0.06 b

Table 2. Effects of 5-azaC on flower-ball developmental stage in broccoli (Unit: d).

Treatments	Bud stage		Rapid growing stage		Initial period of harvest	
	'Qing-feng 103' broccoli	'Lv-xiu' broccoli	'Qing-feng 103' broccoli	'Lv-xiu' broccoli	'Qing-feng 103' broccoli	'Lv-xiu' broccoli
azaC-y	45.37 ± 0.49 d	60.07 ± 0.11 c	52.07 ± 0.06 d	71.13 ± 0.15 d	60.17 ± 0.47 c	80.10 ± 0.10 d
azaC-r	55.20 ± 0.35 c	71.97 ± 0.15 b	61.17 ± 0.57 c	79.03 ± 0.55 c	71.27 ± 0.31 b	87.27 ± 0.25 c
CK-W	57.33 ± 0.42 a	74.17 ± 0.55 a	65.10 ± 0.17 a	83.27 ± 0.23 a	73.27 ± 0.21 a	92.17 ± 0.49 a
CK-T	56.37 ± 0.35 b	74.07 ± 0.70 a	62.93 ± 0.20 b	81.23 ± 0.21 b	73.13 ± 0.78 a	90.13 ± 0.15 b

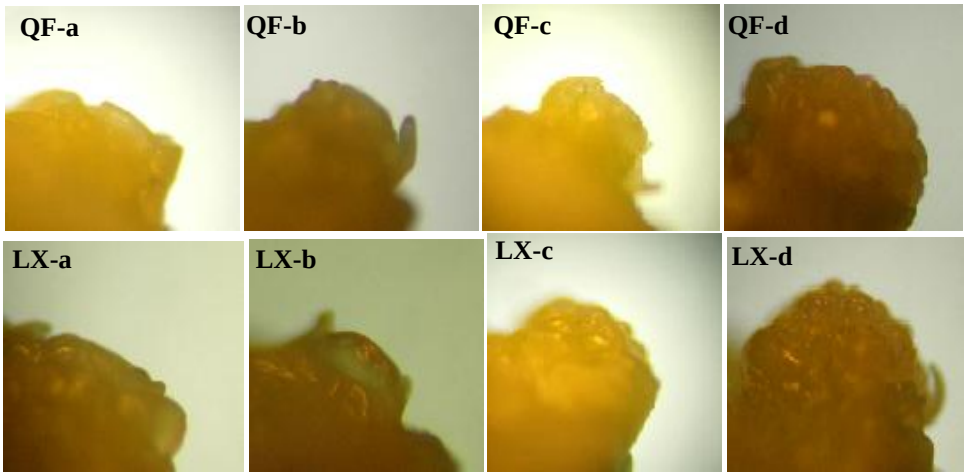

Fig. 1. Effects of 5-azaC on the growing tip in broccoli of shows 'Qing-feng 103' Broccoli; LX shows 'Lv-xiu' Broccoli.
a. Stage of vegetative growth, b. Stage of vernalization end (Critical stage of flower-bud differentiation), c. Stage of primary furcation scape primoridum differentiation, d. Stage of secondary and teriary furcation scape primordium differentiation

Discussion and Conclusion

Our experiment used prematurity broccoli hybrid named 'Qing-feng 103' and medmaturity broccoli hybrid named 'Lv-xiu' as the objects to study the effects of 5-azaC on the vernalization and flower-ball development by spraying or root-applied of 5-azaC, the result showed that, 5-azaC could promote vernalization, made broccoli entered into flower-bud differentiation process earlier on certain extent, and then advance bud stage, rapid growing stage and initial period harvest. The effect of 5-azaC spraying treatment was best.

The reasons why 5-azaC spraying treatment could promote vernalization, and then advance bud stage, rapid growing stage and initial period harvest might be that, the first was 5-azaC promoted vernalization end stage, the second was 5-azaC shortened flower-bud differentiation process, and the third was 5-azaC shortened flower-ball developmental stage process. That corresponded with our earlier research (Xinmei JIANG & Xihong Yu2006), which was that low temperature treatment of germinationg seeds could make broccoli enter into flower-bud differentiation process earlier and advance bud stage and initial period harvest.

Our experiment found that lead times for starting vernalization in different maturity broccoli hybrids were different; in comparison with prematurity broccoli hybrid, medmaturity broccoli hybrid needed more time to start vernalization. That might mean the earlier maturity was, the shorter time it needed to start vernalization, and medmaturity broccoli hybrid might need more energy to start vernalization.

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