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MERISTEM DEVELOPMENT AND ITS RELATION TO ENDOGENOUS GA_3 AND IAA CONTENTS DURING FLORAL BUD DIFFERENTIATION IN BROCCOLI

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ABSTRACT

The effects of three temperature treatments on morphological changes in the apical meristem and contents of GA_3 and IAA in leaves during floral bud differentiation in early maturing cultivar of broccoli were studied. Plants went through every stage of flower-bud differentiation at day/night temperatures of 17.3±1/9.3±1°C. At 21.3±1/13.3±1°C, floral bud development ceased after primary axillary scape primordium differentiation and apical meristem entered a reversion stage. The apical meristem remained in the vegetative growth phase in plants growing at 25.3±1/17.3±1°C. Leaf GA_3 contents started to increase while IAA contents started to decrease when plants entered the flower bud initiation stage. GA_3 content was high and IAA content was low during all stages of axillary scape primordium differentiation.

Key words: Meristem development, Broccoli, Apical meristem, GA_3, IAA

INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica*) is commercially important vegetable in which commercial product is dependent on flower bud differentiation. There are three broccoli cultivars, namely early-maturing, mid-maturing, and late-maturing. In early-maturing cultivars, early inflorescence development occurs if seedlings are transplanted in early spring when the temperature remains in the range from 10 to 17°C. Therefore, research on flower bud differentiation in early-maturing broccoli is of great importance for commercial production. In this study, the effects of three temperature treatments on morphological changes in the apical meristem, and on GA_3 and IAA contents in leaves were investigated in an early-maturing broccoli cultivar.

MATERIALS AND METHODS

The experiments were carried out at the Horticultural Experiment Station and the Plant Physiological-biology Laboratory of Agriculture, Northeast Agriculture University, Harbin, China.

Seeds of *Brassica oleracea* L. var. *italica* 'Qing-Feng Broccoli 103' were sown in a greenhouse on February 11, 2008. Seedlings with one or two leaves were transplanted

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into culture pans. The seedlings were grown under a 25 ± 0.5°C/17 ± 0.5°C day/night temperature regime with 540 µmol/m^2/s. illumination intensity during the day. On April 12, 2008, uniform seedlings with five leaves were placed in a growth cabinet with 72 µmol/m^2/s. illumination intensity and 14 hours photoperiod for 30 d. Three treatments differing in temperature were applied, *viz*. T1, T2 and T3 under day/night temperature of 17.3/9.3, 21.3/13.3 and 25.3/17.3°C ranged, respectively.

For each treatment there were three sets of replicates, and each set comprised of 30 plants. Flower bud differentiation in each treatment was assessed with an Olympus SZH-ILLD BO71 dissecting microscope. Plants were examined every three days from the day (0d) when the plants were placed in the growth cabinet. In addition, for scanning electron microscope observations, the apical meristem was fixed in a buffered 3% glutaraldehyde fixative (Karnovsky 1965), washed in Hanks solution and post-fixed in 1% osmium tetraoxide (Dalton 1955). After dehydration with graded propanol solutions the specimens were dried by the critical point method (Anderson 1951, Cohen *et al.* 1968) and then coated with coal and gold. Successive series of pictures of the apical meristems were produced by using a scanning electron microscope Hitachi S-570. Extraction, purification, and determination of GA_3 and IAA were performed according to Luo *et al*. (1990). The leaves were taken every 3 days from the beginning when the plants were placed in the growth cabinet. Lyophilized leaves were grounded in liquid nitrogen and extracted with 60 ml of 80% methanol/g of fresh weight. Extraction was carried out by stirring overnight at 4□ in the dark and filtered to remove insoluble residues. The filtrate was reduced to an aqueous phase of 20 ml in vacuum evaporator, alkalified with 0.2 M Na_2HPO_4 to pH 8.0 and extracted three times with an equal volume of acetidin/ligarine (v/v). The aqueous phases were acidified with 0.2 M citric acid to pH 2.8 and extracted three times with an equal volume of acetidin, then the organic phases were evaporated to dryness and the residue was dissolved in 5 ml mixture of acetonitrile : methanol : 60% hawkinsin (5:50:45) and injected on to a HPLC (USA Water HPLC System) with a flow rate of 0.8 ml/min.

RESULTS AND DISCUSSION

Morphological changes in the apical meristem before and after flower-bud differentiation: The morphological changes in the apical meristem mark the turning point when the plants switch to reproductive growth from vegetative growth. Flower-bud differentiation in broccoli requires exposure to a period of low temperature. Five developmental stages were distinguished in the apical meristem, namely vegetative growth, flower-bud initiation, primary, secondary and tertiary axillary scape primordium differentiation, and reversion stage (Table 1, Fig. 1). The apical meristem of plants in the T3 treatment remained in the vegetative growth phase throughout the experiment and did

not exhibit any morphological change (Fig. 1, $T3_{-a-1}$, $T3_{-a-2}$, and $T3_{-a-3}$). The volume and surface area of the meristem were small. Some triangular phyllopodia were spirally differentiated surrounding the apical meristem.

Plants in the T2 treatment could undergo flower-bud differentiation, but could not complete the entire differentiation processes. Initiation of the flower-bud differentiation and the primary axillary scape primordium differentiation stages was a week later than in T1 plants. Thus the flower-bud initiation stage was initiated after 18 days (Fig. $T2_{-b}$) and the primary axillary scape primordium differentiation stage after 24 days in growth cabinet (Fig. 1 $T2_{-c}$). After this, the apical meristem stopped growing and entered into reversion stage, in which the volume and superficial area were greater than those of the apical meristem in the vegetative growth phase, and the apical meristem had the appearance of a steamed bun. This period was considered to be a stage of reversion of the apical meristem (Fig. 1 $T2_{-e}$).

Plants in the T1 treatment were passed through each stage of flower-bud differentiation. After incubation for 12 days, the plant entered the flower-bud initiation stage, with the apical meristem becoming gradually flattened and broader, its volume and surface area increased, and a protuberance was apparent inside the phyllopodia (Fig.1, $T1_{-b}$). After 18 days, the meristem entered the stage of primary axillary scape primordium differentiation; meanwhile, there were some protuberances all around the apical meristem (Fig.1, $T1_{-c}$). After 27 days plants entered the stage of secondary and tertiary axillary scape primordium differentiation.

Table 1. Effects of three temperature treatments on the stage of flower-bud differentiation in broccoli. Data are the number of days at each stage after broccoli plants were placed in a growth cabinet.

Treatments	Flower bud differentiation stage		
	Flower bud initiation	Primary axillary scape primordium differentiation	Secondary and tertiary axillary scape primordium differentiation
T1	12	18	27
T2	18	24	-
T3	-	-	-

The plant stopped flower-bud differentiation after entering the primary axillary scape primordium differentiation stage, and did not enter into the secondary and tertiary axillary scape primordium differentiation stage in the T2 treatment. Furthermore, the apical meristem remained similar in form to that at the flower-bud initiation stage (Fig. 1 $T2_{-e}$). A possible reason for this was that, firstly, the accumulation of carbohydrate was inadequate during flower-bud differentiation. A lot of energy and nutrients are required during flower-bud differentiation, but in this study the broccoli palnts were treated in growth cabinet which illumination intensity was only 72 $\mu mol/m^2/s$.

The authors think, respiration in the T2 treatment exceeded that in T1 while nutritional accumulation was lower than in T1. The primary axillary scape primordium had started to degrade because of inadequate nutrition.

Secondly, exposure to low temperature is required when the plants entered the flower-bud differentiation stage. According to Fujime and Okuda (Fujime *et al*. 1996), flower bud differentiation would stop, and even revert to the phyllopodium stage, if a high temperature of about 25°C was encountered after differentiation in cauliflower and broccoli. The results obtained during this study revealed that, if there was not enough low temperature, the plant could not finish all the stages of flower bud differentiation.

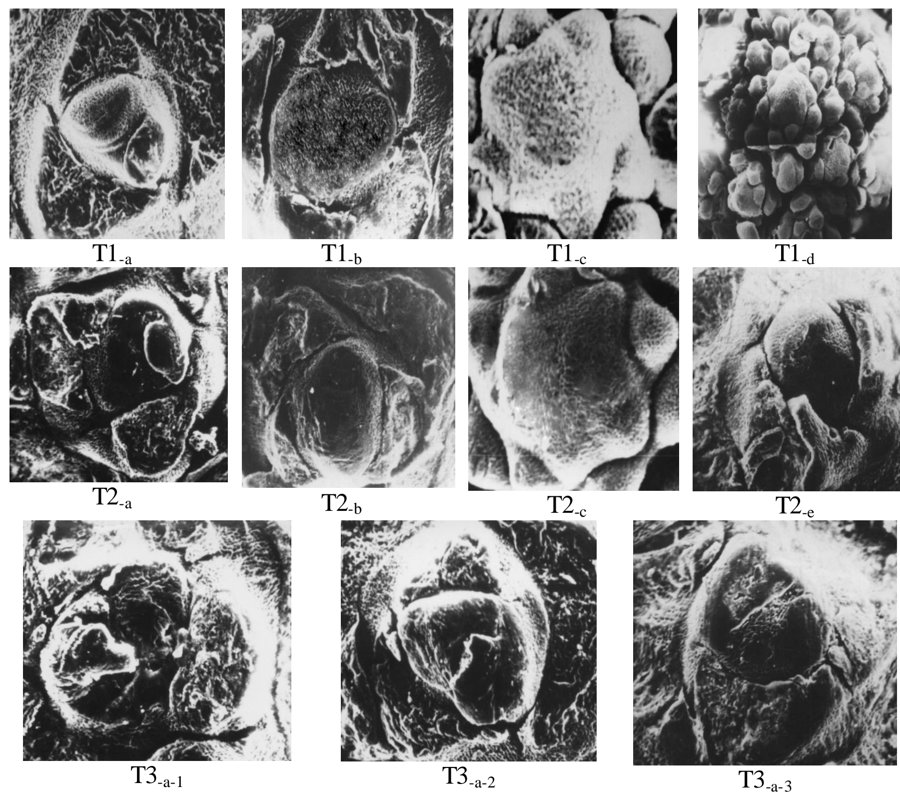

Fig. 1. Effects of three temperature treatments on the apical meristem in broccoli, a. vegetative growth (× 200), b. flower bud initiation (× 50), c. primary axillary scape primordium differentiation (× 150), d. secondary and tertiary axillary scape primordium differentiation (× 80), e. special stage of flower-bud differentiation (Reversion to vegetative growth) (× 120).

The GA_3 and IAA contents exhibited little change when the apical meristem was in the vegetative growth phase (T3 treatment). Following exposure to low temperature in the T1 and T2 treatments, both the GA_3 and IAA contents decreased gradually. GA_3 contents decreased to minimum values at the flower bud initiation stage, whereas IAA

contents continued to decrease after initiation of flowering, and subsequently the levels of both growth regulators increased. Each stage of inflorescence primordium differentiation was indicated to coincide with changes in the leaf GA_3 and IAA contents. Gibberellins may have promoted the elongation and expansion of cells during growth. GA_3 contents decreased when growth entered the flower-bud initiation stage. This finding is identical to those of Huang and Guan (1993) and Li *et al.* (2002), who investigated inflorescence differentiation in *Brassica parachinensis*. Maximum GA_3 contents were recorded at the primary axillary scape primordium differentiation and secondary and tertiary axillary scape primordium differentiation stages, which agrees with our previous results (2004) on the stimulatory effects of low-temperature treatment of germinating seeds on flower-bud differentiation in broccoli. This indicated that each stage of axillary scape primordium differentiation needs a high GA_3 content.

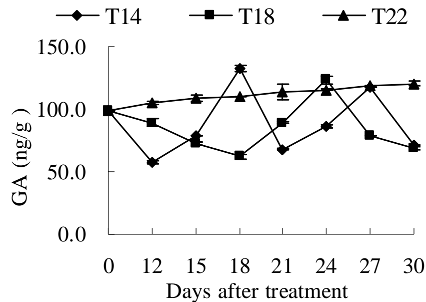

Fig. 2. Changes in GA_3 content in leaves during flower-bud differentiation in broccoli under three temperature treatments

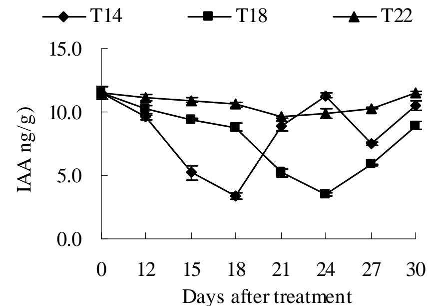

Fig. 3. Changes in IAA content in leaves during flower-bud differentiation in broccoli under three temperature treatments

The GA_3 content was high whereas the IAA content was low during all stages of axillary scape primordium differentiation in broccoli. The lower the temperature, the shorter the time required for vernalization. Plants in lower temperature were able to go through each stage of flower-bud differentiation.

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