Flowering Response of *Brassicoraphanus* (*Brassica oleracea* × *Raphanus sativus*) and Hakuran (*B.* × *napus*) in Relation to Endogenous Gibberellins

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Summary

Flowering response in intergeneric hybrid *Brassicoraphanus* (*Brassica oleracea* × *Raphanus sativus*, $2n=36$) and interspecific hybrid Hakuran (*B.* × *napus*, $2n=38$) was compared to their parental species with respect to their endogenous gibberellin (GA) contents. Flowering response of both hybrids was similar to that of their seed vernalization type parental species, e.g., *R. sativus* and *B. campestris*, respectively, but sensitivity of progenies to vernalization treatment was lowered and still unstable. Endogenous GA increased in hybrids and parental species after seed vernalization, but the GA content in vernalized hybrid seedlings was not intermediate compared to levels in the vernalized parental species. Increase in the endogenous GA in the vernalized plants was independent of the flowering response to vernalization.

Introduction

Since Cruciferae encompasses different groups having different chromosome numbers, breeding of new types of the plants useful for various purposes has been emphasized (17, 21) and proved to be possible through artificial interspecific and intergeneric hybridization (10, 11, 18, 23). *Brassicoraphanus* ($2n=36$), which originated from the progeny of *Brassica oleracea* × *Raphanus sativus*, is an amphidiploid (5). It possesses stable and strong resistance to clubroot disease and the resistance is dominant. Because of this breeding potential, *Brassicoraphanus* is considered to be a useful bridge plant for breeding clubroot resistant cruciferous plants (28). Hakuran (*B.* × *napus*, $2n=38$) is another amphidiploid derived from the progeny of *Brassica oleracea* × *B. campestris*. It is a new type heading vegetable and a promising bridge plant for introducing disease resistance from *B. oleracea* to *B. campestris* and exchanging valuable characters between *B. oleracea*, *B. campestris* and other *Brassica* species (18). However, the flowering behavior and its inheritance of these hybrid plants have been poorly understood. The purpose of the present study was to examine the effects of vernalization treatment on *Brassicoraphanus* and Haku-

ran in relation to the changes in endogenous gibberellins (GA) in comparison with those of their parental species. This may be one approach to study the role of GA in the flowering phenomenon.

Materials and Methods

Plant materials and growing conditions

Intergeneric hybrid *Brassicoraphanus*, strain 'K-11', interspecific hybrid Hakuran cv. Shōren and their parental species, *Brassica oleracea* var. *capitata* cvs. Tenkou and Miikechusei, *Brassica campestris* pekinensis group cvs. Kenshin and Tamanishiki and *Raphanus sativus* cv. Nanpou-daikon were used in the experiment.

Seed vernalization : Seeds were germinated on moistened filter paper in petri dishes at 25°C for 48 hr. After germination, they were vernalized at $5^{\circ}\pm 1^{\circ}\text{C}$ for 15 or 25 days. Nonvernalized seeds (control) were soaked in petri dishes at 25°C for 48 hr just prior to the end of the vernalization to provide seedlings of approximately the same size as the vernalized ones. Both treated and control populations of seedlings were planted in plastic containers filled with vermiculite on June 2, 1987, and grown in a glasshouse until July 2, 1987.

Green plant vernalization : Nonvernalized seedlings were transplanted in plastic pots (9.5 × 15 cm) filled with a mixture of vermiculite and soil (1 : 1

v/v) and grown at 20° and 25°C in the Biotron Institute, Kyushu University on November 5, 1987, under natural light conditions. Sixty days after sowing, a subsample of 6 plants was transferred to a cold glasshouse to be vernalized for 40 days by natural low temperature. After vernalization, plants were returned to 20° or 25°C and grown until March 31, 1988. Another subsample of 6 nonvernalized control plants were kept growing at 20° or 25°C.

During the seed and green plant vernalization treatments, plants were fertilized with a 0.1% solution of a compound fertilizer, OK-F-1 (Otsuka Chemical Co., Ltd.), as required. Dates of flower bud appearance and anthesis were recorded. The shoot apices of plants which had not yet reached the visible stage of flower bud appearance were examined under a dissecting microscope.

Seeds for plants destined for endogenous gibberellin assay were sown on March 1, 1988, after a 30-day vernalization treatment; then were grown in a glasshouse. The cultural methods were the same as those described above.

Fifteen- or 20-gram fresh weight samples (whole plants except roots and yellow or senescing leaves) were harvested 5 and 15 days after planting for the extraction of endogenous gibberellins. All samples were kept at -40°C in a deep freezer until analyzed.

Extraction, separation, purification and bioassay of endogenous gibberellins

Extraction and separation procedures were the same as those described before (15). After obtaining the acidic fraction, the ethyl acetate was removed under reduced pressure and the residue which was dissolved in a solution of acetone-methanol (1:1, v/v) was passed through a Sephadex LH-20 column (1.5×35 cm). Elution of all GA-like substances was achieved with a solution of 0.5–1.2 column volume proportion. The acetone-methanol eluate was evaporated and the residue was dissolved in 5% methanol to purify the extract further with Sep-Pak C₁₈, as described earlier (15).

After sterilization and rinsing, seeds of dwarf rice (*Oryza sativa* L. cv. Tan-ginbōzu) were treated with a solution of 20 mg/liter Uniconazol-P (S-07, Sumitomo Chemical Co., Ltd.), an inhibitor of GA biosynthesis (13), for 24 hr at 25°C in the dark. The treatment was to increase the sensitiv-

ity of dwarf rice seedlings to GA (19). After rinsing, bioassays were conducted in the same manner as described previously (20). The amounts of endogenous GA-like substances (sum of the activities at R_f 0.2 to 0.4 and 0.6 to 0.8 on the chromatograms) were expressed as GA₃ equivalents calculated using a standard curve obtained from the response of the rice seedlings to GA₃.

Results

Seed vernalization: After a 25-day vernalization treatment, 'Tenkou' (*Brassica oleracea* var. *capitata*) and all the control plants did not form any flower bud during the period of the experiment. Flower bud formation was 100% in 'K-11' (*Brassicoraphanus*), 'Kenshin' and 'Tamanishiki' (*Brassica campestris* *pekinensis* group) and 'Nanpou-daikon' (*Raphanus sativus*), whereas it was 10% in 'Shōren' (Hakuran) (Table 1). Flowering rate was 100% in 'Kenshin' and 'Nanpou-daikon' and 30% in 'K-11', but no flowering occurred in 'Tamanishiki' and 'Shōren'. After a 10-day vernalization treatment, although 'Nanpou-daikon' and 'Kenshin' formed flower buds, only the plants of the former reached anthesis.

The days from sowing vernalized seeds to the appearance of flower buds and first bloom in both intergeneric and interspecific hybrid plants corresponded closely to those of their seed vernalization-type parental species (Table 2).

Green plant vernalization: Flower buds were observed in all the vernalized plants (Table 3). All the plants flowered except 'Miikechusei' cabbage which showed less sensitivity to vernalization treatment. Only 67% of the 'Miikechusei' plants flowered at 20°C; none flowered at 25°C. None of the control intergeneric and interspecific hybrid plants or their parental species flowered.

After green plant vernalization treatment, the days to appearance of flower buds and the first anthesis in both hybrid plants closely approximated those of their seed vernalization-type parental species (Table 4).

The amount of endogenous GA-like substances was higher in 5-day old seedlings resulting from vernalized seeds than in those obtained from nonvernalized ones (Table 5). In vernalized 'Shōren' and 'Nanpou-daikon', the large increase in the GA was proportionate to the flowering response obtained by vernalization. Although seed vernaliza-

Table 1. Effects of seed vernalization treatment on percentage of flower bud appearance and flowering.

Cultivar or strain	Percentage of flower bud appearance			Percentage of flowering		
	C	V10	V25	C	V10	V25
K-11	0	0	100	0	0	30
Shōren	0	0	10	0	0	0
Nanpou-daikon	0	100	100	0	100	100
Kenshin	0	100	100	0	0	100
Tamanishiki	0	0	100	0	0	0
Tenkou	0	0	0	0	0	0

C, nonvernalization; V10, 10-day vernalization; V25, 25-day vernalization.

Table 2. Effects of seed vernalization treatment on days to flower bud appearance and flowering after sowing.

Cultivar or strain	Days to flower bud appearance after sowing			Days to flowering after sowing		
	C	V10	V25	C	V10	V25
K-11	—	—	21.4 ± 6.1	—	—	29.3 ± 0.6
Shōren	—	—	25.0 ± 0.0	—	—	—
Nanpou-daikon	—	17.1 ± 1.8	12.5 ± 0.7	—	27.5 ± 1.7	23.1 ± 2.0
Kenshin	—	25.6 ± 2.8	16.3 ± 2.6	—	—	26.8 ± 1.9
Tamanishiki	—	—	27.4 ± 1.7	—	—	—
Tenkou	—	—	—	—	—	—

C, nonvernalization; V10, 10-day vernalization; V25, 25-day vernalization.

Table 3. Effects of green plant vernalization treatment and growth temperature (20° or 25°C) on percentage of flower bud appearance and flowering.

Cultivar or strain	Percentage of flower bud appearance				Percentage of flowering			
	20°C		25°C		20°C		25°C	
	V	C	V	C	V	C	V	C
K-11	100	0	100	0	100	0	100	0
Shōren	100	0	100	0	100	0	100	0
Nanpou-daikon	100	0	100	0	100	0	100	0
Tamanishiki	100	0	100	0	100	0	100	0
Miikechusei	100	0	100	0	67	0	0	0

20° and 25°C, growth temperature; V, vernalization; C, non-vernalization control.

Table 4. Effects of green plant vernalization and growth temperature (20° or 25°C) on days to flower bud appearance and to flowering.

Cultivar or strain	Days to flower bud appearance				Days to flowering			
	20°C		25°C		20°C		25°C	
	V	C	V	C	V	C	V	C
K-11	106.2±1.1	—	105.2±1.9	—	130.2±6.8	—	126.7±7.6	—
Shören	113.2±1.5	—	108.0±2.7	—	132.0±1.3	—	134.0±5.2	—
Nanpou-daikon	104.7±0.5	—	104.0±1.4	—	117.2±1.1	—	115.3±1.8	—
Tamanishiki	116.7±2.7	—	115.8±4.0	—	142.3±2.4	—	136.8±2.3	—
Miikechusei	113.0±1.0	—	112.8±0.7	—	134.3±1.3	—	—	—

20° and 25°C, growth temperature; V, vernalization; C, non-vernalization; —, no flower bud appearance.

Table 5. Effect of seed vernalization on endogenous gibberellin content.

Cultivar or strain	Vernalization	Days to flower bud appearance	Percentage of flower bud appearance	Total number of leaves	Total endogenous GA(µg/kg. FW)	
					G5	G15
K-11	Yes	31.7±3.0	60	11.0±0.6	0.5	0.3
	No	—	—	7.9±0.6	0.3	0.3
Shören	Yes	30.9±4.1	88	12.1±2.3	2.6	1.8
	No	—	—	9.0±1.2	1.3	1.2
Nanpou-daikon	Yes	19.8±1.0	100	8.1±0.6	1.7	0.4
	No	—	—	7.6±0.7	0.4	0.4
Kenshin	Yes	20.6±1.6	100	8.2±0.4	0.3	0.9
	No	—	—	8.5±1.0	0.2	0.9
Miikechusei	Yes	—	—	8.3±0.7	0.8	0.4
	No	—	—	8.2±0.4	0.4	0.5

G5 and G15, 5 and 15 days after planting, respectively; —, no flower bud appearance.

tion was effective for flowering in 'K-11' and 'Kenshin', it increased GA level slightly. Seed vernalization increased GA levels in 'Miikechusei' but had no effect on flowering. The GA level in intergeneric hybrid 'K-11' was lower than that in its parental species, radish and cabbage, whereas it was higher in interspecific hybrid 'Shören' than in its parental species, Chinese cabbage and cabbage.

Discussion

There are two types of vernalization in Cruciferae crops: seed vernalization and green plant vernalization. *Brassica campestris* and *R. sativus* represent the former and *B. oleracea* the latter (24); our results agree with this concept. Both intergeneric hybrid 'K-11' and interspecific hybrid 'Shören' were shown to be seed vernalization type plants. Kagawa (14) reported that interspecific hybrids between the seed and the green plant vernalization types of *Brassica* crops are to be the

seed vernalization type, i.e., the mode of inheritance of seed vernalization type is dominant over green plant vernalization type. The phenomenon of the flowering response not only in 'Shören' but also in 'K-11' may be explained by Kagawa's hypothesis. The low percentage of flowering in seed-vernalized plants of 'Shören' and 'K-11' in spite of the 100% appearance of flower buds and the high percentage of flowering in their parental species, suggest that the sensitivity of these hybrids to seed vernalization is weaker and less stable than that of their seed vernalization type parents.

In Cruciferae crops, interspecific hybrids have been used to introduce genes for disease resistance (3, 4, 6, 9) and to overcome cross incompatibility (16). It is considered that genes for flowering response could be also transferred. In the subtropics such as Bangladesh, India or Pakistan, rapeseed plants (*Brassica napus*) fail to

complete their reproductive phase (2) or flower too late to produce an economically competitive seed yield (22). Production of cabbage seeds is difficult in tropical and subtropical countries because cabbage is a cold-temperature crop, even though it is an important and popular vegetable there (8). This points out the necessity to breed seed vernalization type interspecific hybrids between cabbage and Chinese cabbage. This is made possible by transmitting the seed vernalization character of Chinese cabbage into cabbage (12). Therefore, it is supposed that *Brassicoraphanus* and Hakuran could be used as bridge plants to transfer the genes controlling vernalization from *B. campestris* or *R. sativus* into *B. oleracea* to breed early flowering cultivars. However, it has been shown difficult to obtain hybrids by crossing *Brassicoraphanus* and Hakuran with cabbage because of seed abortion and cross incompatibility in our experiment (Long *et al.*, unpublished). Alternative breeding methods to overcome these difficulties is necessary.

Increase in endogenous GA after vernalization is reported in some plants (7, 25, 26, 27). Amagasa *et al.* (1) reported that endogenous GA content in the amphidiploid obtained from *B. oleracea* × *B. campestris* always showed an intermediate amount between those in its parents. They concluded that there is a difference between the two parents in the potential of GA production, the inheritance of which is incomplete dominance. The results in our experiment indicates that the increase in the endogenous GA by seed vernalization is independent of the flowering response to the treatment. The potential of GA production in these species might be controlled by more complicated genetic mechanisms.

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Brassicoraphanus およびハクランにおける 花成反応と内生ジベレリン

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摘 要

Brassicoraphanus (*Brassica oleracea* × *Raphanus sativus*), ハクラン (*B. × napus*) およびそれらの両親種の春化处理に対する花成反応を内生ジベレリンとの関連で調査した。種子春化处理に対しては両雑種とも鈍感ではあるが種子春化型となった。緑植物春化処

理に対しては種子反応型親に似た花成反応を示して早生化することが認められた。内生ジベレリン量は種子春化处理により両雑種およびそれらの両親種において増加し、花成反応とは独立しているようであった。