Seed Number per Berry, Endogenous Gibberellins and Abscisic Acid Content in Relation to Berry Ripening in Highbush Blueberry©

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To study the maturing mechanisms of blueberry, the effects of self- and cross-pollination on berry ripening, seed number per berry, and changes in gibberellin-like substances and abscisic acid (ABA) contents were determined. Days to ripening from flowering became greater in the order, 'Collins', 'Berkeley', 'Darrow' in open and cross pollination. Though the brownish seed number per berry tended to be higher in the berry after cross-pollination than in that after self-pollination, the variations are dependent on the cultivar. Endogenous gibberellins (GAs) in open-pollination fruit was higher in Stage I compared to those of Stage II or III in all cultivars. The level of GAs in Stage I may be higher in early maturing cultivars such as 'Collins' than in late maturing cultivars. On the other hand, the ABA content was the lowest in Stage I and the highest in Stage II or III, and was the reverse of the change in GAs. These results suggested that the endogenous GAs and ABA may affect the ripening of blueberry. However, it was not clear about the effect of seed number per berry on endogenous GAs and ABA contents in this experiment.

INTRODUCTION

Individual berries within a cluster usually ripen at different time in blueberry (*Vaccinium corymbosum* L.), so that growers must harvest each ripe berry by hand. Tamada (2008) estimated that an adult can harvest only 30–40 kg per day, so two or three individuals per 10 acres are needed during harvest season. In general, the maturity of fruit depends on the date of flowering. Several studies reported that the date of ripening tended to be earlier in fruit from the early blooming flowers than those from later blooming flowers within the same flower cluster in blueberry (Suzuki et al., 1998; Suzuki and Kawata, 2001). However, there are several reports that ripening is affected by seed number per berry, so that the days to ripening from flowering shorten due to increased seed number per berry (Darrow, 1958; Lang and Danka, 1991). Furthermore, it is known in highbush blueberry that the percentage of fruit set is usually higher in cross pollination than in self pollination, and seed number per berry varied by pollen parent cultivar (Iwasaki et al., 2006).

Although the effects of seeds on fruit maturing were not clear, it was reported that plant hormones such as indole-3-acetic acid (IAA) and gibberellins (GAs) produced by seeds were involved in ripening of sweet cherry (Kondo et al., 2000). Moreover, exogenously applied abscisic acid (ABA) promoted coloring of grape berry (Hiratsuka et al., 2001), and that indicate ABA is also involved in coloring of berry skin.

The objectives of this study were to determine maturing mechanisms, and we investigated the effects of self and cross pollination on berry ripening, seed number per berry, and changes in gibberellin (GA)-like substances and ABA content in blueberry.

MATERIALS AND METHODS

The experiments were carried out with 7-year-old highbush blueberry plants (cultivars 'Collins' 'Berkeley', and 'Darrow', with 3 to 5 plants of each) growing in an experimental field at Meiji University. The plants were potted using mainly peat moss.

Relationship Between Berry Ripening and Seed Number. In the study on the relationship between berry ripening and seed number per berry, one plant of 'Bluetta', 'Collins', 'Darrow', 'Berkeley', and 'Dixie' were transferred to a greenhouse in late February 2006. Anthers were collected from flower buds at just before flowering and the anthers were opened in an incubator maintained at 25 ºC to collect pollen. Ten flower clusters in each cultivar were selected for pollination. Flowering date of each floret was recorded for pollination. There were two other cultivars present except for experiments in this field, and many insects visiting flowers during the flowering period, so that it was easy to accomplish cross pollination.

In early April, cross pollination with two cultivars of pollen parent and self pollination were carried out in all cultivars. Twenty to thirty flower clusters were selected in each cultivar and two to eight florets just before flowering were hand-pollinated with cross or self pollination in each cluster. A berry was regarded as ripened when completely purple, and days to ripening from flowering were determined by days after flowering.

After harvest, berry weight, skin color, degrees Brix, and brownish seeds number per berry were determined. Brownish seeds were dried, sterilized, and treated with 100 ppm GA_3 for 6 days. Then treated seeds were incubated in a growth chamber (25±1 ºC and 16-h light period). After 2 months of incubation, germinated seeds were counted. Based on these data, correlation coefficients were calculated, and then a multiple regression model was adopted in order to identify the factors that caused variation in berry ripening of blueberry. Flowering date, brownish seed number per berry, and number of germinated seeds as independent variables, while days to ripening from flowering as dependent variable were used for a multiple regression analysis.

Determination of Endogenous GA-like Substances and ABA Content. On three cultivars described above, berries were sampled at each time from Stage I, II, and III in open-pollinated flowers, and then the samples were stored at -80 ºC until analysis. About 5 g of fresh berries were homogenized in 100 ml of 80% methanol and left to stand in a freezer for 24 h. One ml of the extracts was used for ABA analysis. The ABA content was determined by immunoassay with commercially available kit. For the rest of the extract, methanol was removed by evaporator at 35 ºC, and GA-like substances were extracted by ethyl acetate and phosphate buffer, then the extracts were fractionated by thin layer chromatography. Gibberellin-like substances were determined as GA_3 equivalent by bioassay using the dwarf rice cultivar 'Tangin-bozu'.

RESULTS AND DISCUSSION

Days to ripening from flowering became greater in the order, 'Collins', 'Berkeley', 'Darrow' in open- and cross-pollinated flowers. Days to ripening from flowering in 'Collins' was lowest in the berry from cross pollination compared to that from self pollination, while it was lowest in fruit from self pollination in 'Berkeley'. Days to ripening from flowering in 'Darrow' were the highest in the berry after cross pollination by 'Bluetta'. The standard deviation of days to ripening from flowering varied among the cultivars, and there were no tendency in variation of the days to ripening from flowering among the pollen parents (Table 1). Though the number of brownish seeds per berry tended to be higher in the berries after cross pollination than in that after self pollination, the variations were dependent on the cultivars. Also, the brownish seed number per berry in 'Berkeley' was higher in the berry after open pollination compared to that after self or cross pollination. There were no differences in the germination rate of brownish seeds among the cultivars or by the pollination methods (Table 1). Based on these results, a multiple regression model was adopted using number of days to ripening from flowering as dependent variable, while flowering date, brownish seed number per berry, and germinated seed number per berry as independent variables showed significant multiple regression coefficients in all cultivars. However, the standardized partial regression coefficient of seed number per berry was higher than that of germinated seed number, and was suggested that seed number per berry was more effective on berry ripening than germinated seed number (Table 2).

In the previous investigations using 'Berkeley' fruits from cross pollination by 'Collins' ripened earlier than those from self or open pollinations. The seeds number per berry and percentage of germinated seeds were also higher in the fruits from cross pollination than those from self- or open pollination, so that maturing of blueberry seemed to be affected by seeds number per berry (Iwasaki et al., 2006). However, the results in this experiments were different from those of previous research and suggested the other factors (e.g., air temperature during flowering period) may have affects on berry ripening.

Content of endogenous GA-like substances in open pollination was higher in Stage I compared to those of Stage II or III in all cultivars. In Stage I, endogenous GA-like substance content in 'Darrow' was higher than that of 'Collins' (Fig. 1). In sweet cherry, it was reported that the GAs in seed increased gradually before fruit maturation when the fruit was actively enlarging, and then decreased dramatically towards harvest (Kondo et al., 2000), so that GAs seemed to be high in premature fruits such as Stage I in blueberry. Farther it was suggested that the level of GAs in Stage I may be higher in early-maturing cultivars than in late-maturing cultivars.

On the other hand, the ABA content, which was lower in Stage I and higher in Stage II or III, showed the tendency that was reverse to the change of GA-like substances. Further, ABA contents of the berry in Stage I and II were the highest in 'Collins' and the lowest in 'Darrow' (Fig. 2). Abscisic acid is considered to be involved in the coloration of grape berry (Hiratsuka et al., 2001). Since the main pigments of grape skin are known to be anthocyanins (Shiraishi and Watanabe, 1988), ABA also may be involved in blueberry coloration. Matsushima et al. (1989) suggested that ABA promoted coloration via accumulation of sugars in grape skin. Therefore, accumulation of sugars in Stage III also seemed to be important factor in relation to coloration in blueberry (Hiratsuka et al., 2001).

Table 1. Differences in days to ripening from flowering, berry weight and Brix among the treatments.

♀	♂	Days to ripening from flowering	Berry weight (g)	Brix (%)	Brownish seeds (no.)	Germinated seeds (no.)	Rate of germinated seeds
'Collins'	(open-pollination)	62.1±7.01	1.2	11.4	1.2	0.8	0.76
'Collins'	'Collins'	60.6±3.21	1.5	11.5	2.2	1.4	0.67
'Collins'	'Darrow'	58.9±3.43	1.5	11.4	3.7	2.7	0.79
'Collins'	'Bluetta'	60.7±4.85	1.5	11.5	3.4	2.8	0.86
'Berkeley'	(open-pollination)	67.6±5.60	1.5	9.8	17.1	8.2	0.51
'Berkeley'	'Berkeley'	74.8±3.62	1.4	9.6	4.3	2.6	0.61
'Berkeley'	'Dixi'	72.1±4.32	1.6	10.2	10.8	6.8	0.65
'Berkeley'	'Bluetta'	73.8±5.92	1.5	10.1	10.4	6.8	0.75
'Darrow'	(open-pollination)	74.2±9.95	1.4	10.6	6.7	4.4	0.73
'Darrow'	'Darrow'	75.9±6.57	2.0	10.4	7.5	5.8	0.81
'Darrow'	'Berkeley'	74.3±5.64	1.9	10.3	11.7	8.9	0.77
'Darrow'	'Bluetta'	77.8±7.80	1.8	9.5	8.1	5.9	0.78

These results suggested that the endogenous GAs and ABA may affect the ripening of blueberry. However, it was not clear about the effect of seeds number per berry on endogenous GAs and ABA contents in this experiment.

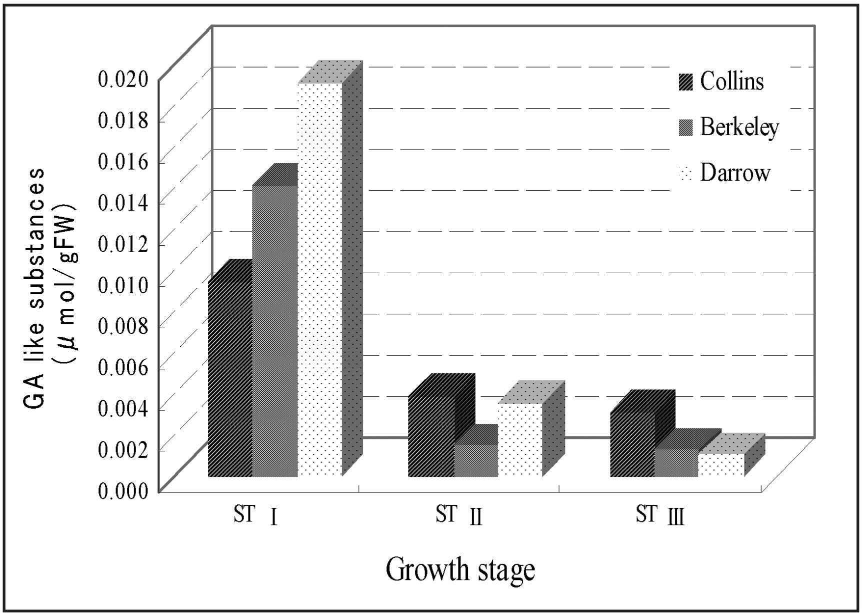

Figure 1. Changes in gibberelin-like substances during berry growth of three Blueberry cultivars.

Table 2. Results of multiple regression analysis using days to ripening from flowering as the dependent variable, while flowering date, brownish seed number per berry, and number of germinated seed as independent variables.

	Multiple regression coefficient		Standardized partial regression coefficients					
			Flowering date		Brownish seeds (number)		Germinated seeds (number)	
'Collins'	0.649	(p = 0.0008)	-0.209	(p = 0.1721)	-0.51	(p = 0.0667)	-0.045	(p = 0.8643)
'Berkeley'	0.760	(p = 0.0008)	-0.210	(p = 0.3332)	-0.61	(p = 0.0681)	0.010	(p = 0.9686)
'Darrow'	0.867	(p = 0.0300)	-0.468	(p = 0.1646)	-0.87	(p = 0.1274)	0.423	(p = 0.4530)

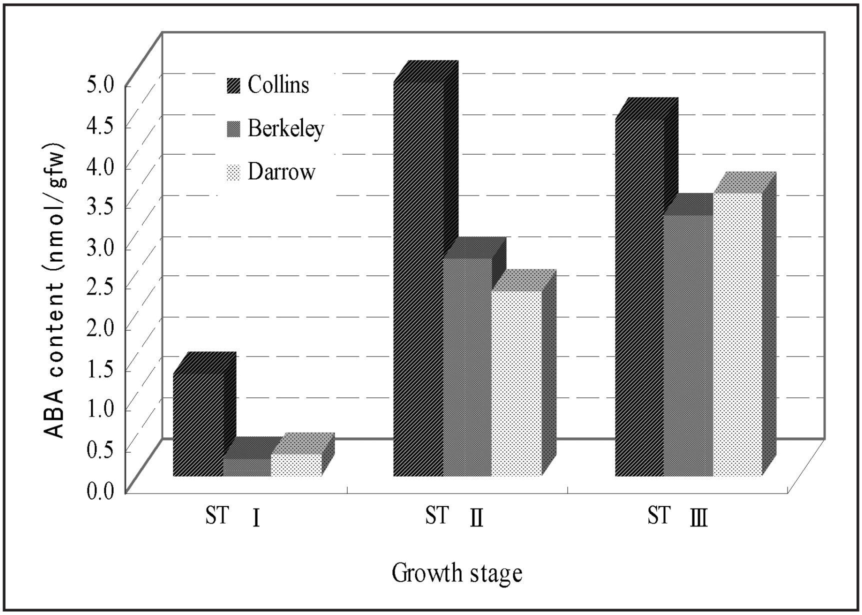

Figure 2. Changes in abscisic acid contents during berry growth of three Blueberry cultivars.

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