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Hybridization of highbush blueberry (*Vaccinium corymbosum*) in section *Cyanococcus* with two *Vaccinium* section *Bracteata* species native to subtropical Asia

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ABSTRACT

The heat- and drought-sensitive highbush blueberry (*Vaccinium corymbosum*, section *Cyanococcus*; hereafter referred to as *cor*), is one of the main cultivated species of blueberry. In contrast, *Vaccinium boninense* (*bon*) and *Vaccinium wrightii* (*wri*), in the *Bracteata* section, are native to subtropical Asia, including Japan. The development of new blueberry cultivars combining the qualities of these species is of considerable interest at present. However, it is difficult to obtain hybrids between species from different sections or with different ploidy levels. Furthermore, our earlier attempt to cross *cor* with *bon* and *wri* did not produce any hybrids (Miyashita, 2017). We hypothesized that the rate of success of interspecific hybridization in *Vaccinium* is reduced by emasculation, which is commonly performed before pollination. Therefore, the present study aimed to evaluate the crossability between *cor* and the diploid wild species (*bon* and *wri*) and to produce new intersectional hybrids. We made 15 combination crosses between six types of *Vaccinium* plants, including *cor*, *bon*, and *wri* (five species and an interspecific hybrid), with two crossing methods (emasculation and non-emasculation) for each combination included prior to pollination. The hybridity of the resulting seedlings was identified by polymorphism analysis using simple sequence repeat markers for blueberry. Hybrids were obtained from 10 of the 15 combinations. All these combinations were of the cross type such that both parental section and ploidy level were same, or that either section or ploidy level were different. In seven of the 10 combinations, hybrids were obtained only with the non-emasculation method. However, four combinations of the cross type where both section and ploidy level differed between parents failed to produce any hybrids. These results suggested that intersectional interploid cross type has lower crossability than that of either intersectional or interploid type, and that the non-emasculation method is more effective than the conventional emasculation method to obtain hybrids. However, the non-emasculation crossing did not prevent the production of nonhybrid seedlings, indicating the need to confirm the hybridity of each seedling. Subsequently, six reciprocal cross combinations between *cor* and the three polyploid or hybrid plants derived from *bon* and *wri* were made using the non-emasculation method. This yielded six hybrids from two combinations. To our knowledge, these are the first hybrids derived from *cor* and two *Bracteata* species native to subtropical Asia. These hybrids might be useful as breeding materials to enhance heat and drought tolerance in highbush blueberry.

1. Introduction

Highbush blueberry (*Vaccinium corymbosum*; hereafter referred to as *cor*) is a major species of cultivated blueberry. It is adapted to cold climates and does not tolerate heat or drought well. Stable, profitable cultivation of *cor* in warm areas, such as the southern part of the Kanto region in Japan, is difficult because the yield and quality are markedly reduced under the prevalent climatic conditions in the region. To obtain a high yield of high-quality fruit from *cor* in warm regions, it is necessary to develop new cultivars that are adapted to the climate of these

Abbreviations: *bon*, *Vaccinium boninense*; *bra*, *Vaccinium bracteatum*; *cor*, *Vaccinium corymbosum*; *dar*, *Vaccinium darrowii*; *dar/ell*, interspecific hybrid 'TO-303' (*Vaccinium darrowii* × *Vaccinium elliottii*); *dar/ell-bon*, TO-303 × *Vaccinium boninense* intersectional hybrids; RFI, relative fluorescence intensity; SSR, simple sequence repeat; *vir*, *Vaccinium virgatum*; *wri*, *Vaccinium wrightii*; *4x bon*, a tetraploid of *Vaccinium boninense*; *4x bon/wri*, tetraploids obtained by chromosome doubling of *Vaccinium boninense* × *Vaccinium wrightii* interspecific hybrids

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Table 1
Vaccinium plants used for crosses in Experiment 1.

Section and Species		Abbreviated name	Ploidy level	Accession	Common name	Flowering period[c]
Cyanococcus	*V. corymbosum*	*cor*	4*x*	Berkeley, Spartan	Highbush blueberry	April–May
	V. virgatum	*vir*	6*x*	Brightwell, Tifblue	Rabbiteye blueberry	April–May
	V. darrowii	*dar*	2*x*	Native Blue	Darrow's evergreen blueberry	April–May
	V. darrowii × *V. elliottii*	*dar/ell*	2*x*	TO-303[a]	–	April–May
Bracteata	*V. boninense*	*bon*	2*x*	M-1, M-8	Muninshashanbo[b]	November–May
	V. wrightii	*wri*	2*x*	G-1	Giima[b]	December–April

[a] Interspecific hybrid produced by crossing between the diploid wild species native to southern USA, by Georgia State University; *V. darrowii* (FL-4B) × *V. elliottii* (Knight).
[b] Japanese name.
[c] In Tachikawa-shi, Tokyo.

regions.

Interspecific hybridization is the most common method used in blueberry breeding (Lyrene and Ballington, 1986). In the United States, interspecific crossing of blueberries and native wild species has successfully produced cultivars that are suited to various environments. In particular, the successful production of interspecific hybrids between *cor* and *Vaccinium darrowii* (hereafter referred to as *dar*), which is native to the southern United States, set the precedent for the development of *cor* cultivars for warm areas of the United States (Ballington, 2009; Lyrene, 1997). The new *cor* cultivars, called the southern highbush blueberry varieties, were developed by crossing blueberry species (mainly *cor*) with *dar*, which has low chilling requirements and exhibits drought tolerance. Their creation has made it economically feasible to cultivate *cor* in the warm, southern areas of the United States such as Florida. In Japan, many southern highbush blueberry cultivars have been introduced in recent years for cultivation in warm areas. However, unlike Florida and other similar regions, which are warm all year round, temperatures in the Kanto area and other regions south of Kanto in Japan are cold during the winter and early spring. Therefore, a part of southern highbush blueberry cultivars are not very well adapted to these regions. In addition, the harvest period of *cor*, including that of southern highbush blueberry, overlaps with the rainy season in the warm regions of Japan, resulting in a decline in fruit quality.

There are 19 wild *Vaccinium* species in Japan (Yamazaki, 1989, 1993) with various distributions and characteristics. Among them, *Vaccinium boninense* (hereafter, *bon*) and *Vaccinium wrightii* (*wri*) are native in subtropical regions (Ito and Sugawara, 2009, 2010); thus, they are expected to have a relatively high tolerance to both heat and drought. These wild species mature late in comparison with *cor* and could, therefore, be useful in breeding new highbush blueberry cultivars that can be harvested after the rainy season.

Generally, the closer the genetic relationship between the species involved in a cross, the higher the interspecific cross-compatibility. Galletta (1975) demonstrated that interspecific hybrids could generally be bred by using *Vaccinium* species with the same ploidy level; conversely, hybridizations are difficult to achieve between species with different ploidy levels. Thus, until now, it was considered unlikely that *cor*, which is a tetraploid species of the *Cyanococcus* section ($2n = 4x = 48$), could be successfully crossed with *bon* or *wri*, two diploid species of the *Bracteata* section. Regarding *Bracteata* species native to Asia including Japan, in addition to *bon* and *wri*, there is *Vaccinium bracteatum* (hereafter, *bra*). Although Tsuda et al. (2013) produced intersectional hybrids by intraploid crossing with *bra* and *cor*, there are no successful cases of interploid crosses between *Bracteata* species and blueberries. Therefore, it is important to clarify the effect of different ploidy levels on these intersectional crossing. Furthermore, *bra* is distributed in the warm-temperate zones (Hayashi et al., 1987), and it matures early in comparison with *bon* and *wri* (Miyashita, 2017). Therefore, it is considered important to individually evaluate characteristics, including crossability of these *Bracteata* species as blueberry breeding material.

Although we have successfully crossed many species of *Vaccinium* and have obtained various hybrids from crosses between species with different ploidy levels or from different sections, the success rate of these crosses has been remarkably low (Miyashita, 2017). Moreover, we have not been able to obtain hybrids between blueberry species with ploidy levels of 4*x* or 6*x* and diploid wild species (such as *bon*) from different sections. In those crossing experiments, anthers and petals were always removed prior to pollination for emasculation. In later experiments, we attempted to perform crosses by non-emasculation, which resulted in both improved fruit yield and increased number of seeds per fruit.

In the present study, first, crossings between six types of *Vaccinium* plants, including *cor*, *bon*, and *wri* were made, and the influence of the difference in cross type (interspecific and intersectional, homoploid and heteroploid, and non-emasculation and emasculation) on the success of the hybridization was evaluated. Then, we crossed *cor* with polyploid and hybrid plants derived from *bon* and *wri*, resulting in new intersectional hybrids.

2. Materials and methods

The crossings were carried out separately for Experiments 1 and 2.

2.1. Plant materials and crossings in Experiment 1

A total of six *Vaccinium* species or hybrids were examined: *Vaccinium corymbosum* (highbush blueberry; *cor*), *Vaccinium virgatum* (rabbiteye blueberry; *vir*), *Vaccinium darrowii* (*dar*), the interspecific hybrid 'TO-303' (*Vaccinium darrowii* × *Vaccinium elliottii*; *dar/ell*), *Vaccinium boninense* (Muninshashanbo; *bon*), and *Vaccinium wrightii* (Giima; *wri*) from the *Bracteata* section (Table 1). *dar/ell* was produced by crossing between the diploid wild species of the *Cyanococcus* section native to southern USA. Plants used in the crosses were cultivated either in the field or under greenhouse conditions in Tachikawa-shi, Tokyo, Japan.

We made 15 combination crosses among the six aforementioned *Vaccinium* plants, with two cross methods, emasculation and non-emasculation, for each combination prior to pollination. In each method, more than 11 flowers were cross-pollinated.

The crossings were performed during the flowering period of each seed parent, from November 2013 to May 2014, as follows. In the emasculation method, buds with anthers of the orange or red color stage just before blooming were selected, and all petals and anthers were removed, followed by pollination. Meanwhile, in the non-emasculation method, flowers just after blooming were selected and pollinated without these structures removed. In both methods, pollination was performed by pollen collected from the pollen parent just after flowering. When flowering of the seed parent and that of the pollen parent were not synchronized (Table 1), pollen was dried and refrigerated at 5 °C for 1–10 months until crossing. After pollination, flowers were covered for 2 weeks with paraffin paper bags to prevent