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Mark K. Ehlenfeldt
USDA, Agricultural Research Service, mark.ehlenfeldt@ars.usda.gov

James J. Polashock
USDA, Agricultural Research Service, james.polashock@ars.usda.gov

James R. Ballington
North Carolina State University, jrbsrb@ncsu.edu

Vaccinium corymbodendron Dunal as a bridge between taxonomic sections and ploidies in *Vaccinium:* A work in progress

Mark K. Ehlenfeldt [1], James J. Polashock[1], and James R. Ballington [2]

[1] USDA-ARS, Genetic Improvement of Fruits and Vegetables Laboratory, at P.E. Marucci Center for Blueberry and Cranberry Research & Extension, Chatsworth, NJ 08019

[2] Horticultural Science Department, North Carolina State University, Raleigh, North Carolina 27695 (retired)

Abstract: The species *V. corymbodendron* of section *Pyxothamnus* has shown value as a potential bridge between taxonomic sections and ploidies in *Vaccinium* when involved as either a first generation or second generation parent. Tetraploid *V. corymbodendron* has hybridized successfully with 2*x* and 4*x* section *Cyanococcus* species and with 2*x* section *Vitis-idaea*. Hybridizations with other sections are currently being tested. Second generation allotetraploid *V. corymbodendron* - *V. vitis-idaea* hybrids have hybridized successfully with 4*x* section *Oxycoccus* (cranberry), 4*x* section *Cyanococcus* (blueberry), and 2*x* section *Vitis-idaea* (lingonberry). It appears that these allotetraploid hybrids may allow gene movement among these diverse sections at the 4*x* level. Further test-crosses are being made to evaluate the range of crossability of 4*x V. corymbodendron* and the *V. corymbodendron* allotetraploids with other taxonomic sections of *Ericaceae*.

Index words: blueberry, lingonberry, cranberry

Introduction

V. corymbodendron Dunal. (Section *Pyxothamnus*), a species native to high altitude locations in Peru, Colombia, Venezuela, and Guyana, is of interest to conventional blueberry breeding because it possesses several characters of potential value. First, *V. corymbodendron* plants flower at high-altitude locations at times when nighttime temperatures drop below freezing (Luby et al., 1991), suggesting a process for floral cold tolerance. *V. corymbodendron* is also notable for buds with high numbers of flowers per bud, as well as a loose floral structure that may be amenable to machine harvest (Luby et al., 1991). Additionally, plants of *V. corymbodendron* have the potential to develop an upright tree-like bush structure with a monopodial base.

We previously reported on efforts to utilize this germplasm, in which crosses of *V. corymbodendron* with diploid species of section *Cyanococcus* yielded high numbers of triploid offspring. In such crosses, all tested offspring were 3*x*. Many questions followed about the nature of the crossing behavior of *V. corymbodendron.* We also reported that one paradoxical exception to the observed crossing behavior was the production of a highly fertile 4*x* hybrid of 4*x V. corymbodendron* with a species of a different section, 2*x V. vitis-idaea* L. (section *Vitis-idaea*; lingonberry) (Ehlenfeldt and Ballington, 2017a; Ehlenfeldt and Ballington, 2017b). We report here on both crosses with *V. corymbodendron* and on crosses utilizing S_1 *V. corymbodendron – V. vitis-idaea* offspring.

Methods and Materials

Working with both *V. corymbodendron* and lingonberry (*V. vitis-idaea*) in New Jersey is a considerable challenge, since neither is well adapted to hot, humid New Jersey summers. Both flower sparingly and sporadically, nonetheless with persistence we succeeded with numerous crosses.

The original clone of 4*x* (*V. corymbodendron* × *V. vitis-idaea*), US 1184, was a slow growing plant that took several years to come into flower. When it finally flowered, there were neither *V. corymbodendron* nor *V. vitis-idaea* flowers or pollen available to test on this plant. It seemed, therefore, most logical to test its self-fertility, and the few flowers present were self-pollinated. From these self-pollinations, approximately 20 offspring were produced. In the meantime, US 1184's ploidy was determined by flow cytometry to be 4*x* Soon after these initial evaluations, the mother plant, US 1184, perished. US 1184 morphologically appeared intermediate to the two parents, but most notably produced spherical fruit that ripened to a deep red color, rather than the black or blue-black that might be expected.

Considerable variation existed among the US 1184 selfs. Although all resembled US 1184, there were considerable differences in vigor and propensity to flower. Ultimately, three S_1 clones stood out as being the most vigorous, prolific, and fertile - US 1930, US 1933, and US 1993. When these came into flower, we once again had no 4*x* lingonberry nor 4x *V. corymbodendron* to test these against, so they were pollinated with 4*x V. marcocarpon* (courtesy of Dr. N. Vorsa, Rutgers University) and 4*x V. corymbosum*. As various other cross combinations became feasible due to coincidences of flower timing, other evaluations were made of the crossing behavior of both pure *V. corymbodendron*, and the S_1 *V. corymbodendron* – *V. vitis-idaea* derivatives. Because of the small, crowded, and somewhat fragile nature of the flowers on both types of parents, no emasculation was used. Flowers were hand pollinated using a sharpened pencil point to transfer pollen. A summary of crosses follows.

Results

Crosses with 4x *V. corymbodendron*

Morphological appearance of hybrids with 4*x V. corymbodendron* as a parent – *V. corymbodendron* has a relatively small, uniformly flat leaf with fine lobe-like scalloping along the edges with a matte surface, and no waxiness. Hybrids with *V. corymbodendron* are often recognizable by *V. corymbodendron*'s influence. Hybrid leaves are usually reduced in size (similar to that seen in *V. darrowii*-derived hybrids), the shape is typically reminiscent of *V. corymbodendron*, and leaf surfaces have a relatively flat aspect with little surface texture and flat (not undercurved) margins.

2*x V. corymbosum* (forma *V. atrococcum* Gray) × 4*x V. corymbodendron* – This cross was made to determine if *V. corymbodendron* produced triploids when used as a male with diploid section *Cyanococcus* species, as it had when used as a female, i.e. producing triploid offspring (Ehlenfeldt and Ballington, 2017a). Although success of this cross combination was not high, eight progeny were produced. Six appeared to be morphologically intermediate to the parents and were determined by flow cytometry to be triploids. Two seedlings appeared to be essentially like the female parent, and have been determined by flow cytometry to be diploid, and hence almost certainly selfs.

4*x* *V. corymbosum* × 4*x* *V. corymbodendron* – After earlier experiments had determined *V. corymbodendron* to be 4*x*, we felt the need to determine whether 4*x V. corymbodendron* could cross with what might be considered a more logical, natural partner, 4*x V. corymbosum*. This cross was done with 'Duke', 'Bluecrop', and ARS 99-72 as females, and although success varied with the *V. corymbosum* cultivar, overall, this cross was quite easily done and produced many progeny. These plants appeared intermediate to the parents, and limited testing has found all offspring evaluated thus far to be tetraploid.

2*x* *V. macrocarpon* × 4*x* *V. corymbodendron* – These crosses were intended to test the possibility of triploid production much like that seen in the 2*x V. corymbosum* (*V. atrococcum*) crosses, previously mentioned. Although many offspring were generated, all appear to be morphologically identical to 2*x V. macrocarpon.* We continue to evaluate these seedlings, but currently consider this combination to be unsuccessful.

4*x* *V. corymbodendron* × 2*x* *V. vitis-idaea* – These crosses repeat the initial hybridization of lingonberry with *V. corymbodendron*. Fruit maturation is still in progress. Seed quality is uneven. A few seed have been rated as good; many others were rated as poor. Overall, the quality is sufficiently poor so as to question whether any will yield viable hybrids. Nonetheless, this cross has worked before.

Crosses with 4*x* S_1(*V. corymbodendron – V. vitis-idaea*) hybrids – US 1930, US 1933, and US 1993

Morphology of 4*x* S_1(*V. corymbodendron – V. vitis-idaea)* hybrids – The S_1 hybrids of *V. corymbodendron – V. vitis-idaea* are fairly distinct in their morphology, having leaves that are relatively small (3 cm x 1.5 cm), pointed and lance-shaped to obovate (Fig. 1a). Leaves under ideal conditions are dark green, lightly textured in a pattern reminiscent of lingonberry, very slightly serrate, and possessing slightly down-turned edges. Leaves are also very regular in size, and in the greenhouse shoots appear to develop as indeterminate growth, rather than distinct flushes. Flowers are intermediate, but more similar to those of *V. corymbodendron,* than to the open flowers of *V. vitis-idaea* (Fig. 1b). Our hybrids generally have elongate, oval-shaped flowers with a slightly constricted throat. Flowering occurs in branched clusters, and flowering appears to be indeterminate. Ripe fruit of these hybrids is a medium red, similar to lingonberry (Fig. 1c).

Figure 1. A 4x S_1 V. corymbodendron – V. vitis-idaea hybrid - a) leaves, b) flowers, and c) fruit.

4*x* S_1(*V. corymbodendron – V. vitis-idaea)* × 4*x V. macrocarpon* (section *Oxycoccus*) – The three S_1 hybrids were crossed as females with three different 4*x V. macrocarpon* selections. These 4*x V. macrocarpon* plants came from Dr. N. Vorsa (Rutgers University) and were verified as tetraploids by flow cytometry. These hybridizations had a high degree of success, and many offspring were produced. All plants thus far evaluated are tetraploid. These hybrids are morphologically distinctive. Plants possess a vigorous trailing habit, and very uniformly sized and uniformly spaced, lance-shaped leaves.

4*x V. macrocarpon* × 4*x* S_1(*V. corymbodendron – V. vitis-idaea*) - These crosses intended to test the reciprocity of previous crosses have thus far have proven unsuccessful.

4*x* S_1(*V. corymbodendron – V. vitis-idaea)* × 4*x V. corymbosum* (section *Cyanococcus*) - Several S_1 hybrids were crossed as females with several different 4x *V. corymbosum* cultivars. Although fruit set was reasonable, only a small number of hybrids resulted from these crosses. As juveniles, these hybrids have been quite slow growing, but are becoming established and their growth is accelerating. These hybrids have relatively small leaves at this point, but appear distinctly different from the mother plants and have a flatter leaf morphology than might be expected of selfs. 4*x* S_1(*V. corymbodendron – V. vitis-idaea)* hybrids never fail to surprise. Six plants evaluated by flow cytometry thus far have been determined to be 6*x*. It is unknown at this point if the presumed unreduced gametes came from the maternal or paternal side. Knowing this might considerably increase the chances of success in future crossing plans. Molecular studies should ultimately resolve parentage issues. Due to the low success of this cross, most of our pollinations in Spring 2018 onto 4*x* S_1(*V. corymbodendron – V. vitis-idaea*) were aimed at pollinating with 4*x V. corymbosum* cultivars.

4*x V. corymbosum* (section *Cyanococcus*) × 4*x* S_1(*V. corymbodendron – V. vitis-idaea)* - These crosses thus far have proven difficult to unsuccessful. One apparent hybrid has the pedigree ‘Reveille’ × US 1930. This plant has large leaves and appears little different from pure *V. corymbosum.* Without further verification of hybridity, this plant must be considered likely to be a self.

4*x* S_1(*V. corymbodendron – V. vitis-idaea*) × 2*x V. vitis-idaea* (section *Vitis-idaea*) and reciprocal – In this case, we did not have a 4*x* lingonberry available, so success, if any, in these crosses was expected to be dependent upon the functioning of 2n gametes. Since the presence of 2*n* gametes in lingonberry is thus far undetermined, we acquired a wide selection of lingonberry cultivars, and used them as both males and females. As expected, these crosses had a low level of success, but three putative hybrids (based on morphology) were identified. Phenotypes of these hybrids appeared to vary widely. Our three putative hybrids had the pedigrees: ‘Red Sunset’ × US 1930, ‘Red Sunset’ × US 1933, and US 1930 × ‘Sanna’. Regrettably, the first of these hybrids died. Among the other two, the second, with lingonberry as a female, was determined to be 4*x*, and the third, with lingonberry as the male was 3*x*.

4*x* S_1(*V. corymbodendron – V. vitis-idaea*) × 4*x V. corymbodendron* (section *Pyxothamnus*) – This combination represents a backcross to *V. corymbodendron*. These crosses were easily accomplished and the offspring posses a hybrid appearance. The most notable morphological feature of these offspring is a rosy tinge to young growth, which was not seen in the other hybrids generated.

6*x* *V.* × *hybrid* 'Nocturne' x 4*x* S_1(*V. corymbodendron* – *V. vitis-idaea*) – These crosses were initially done on a limited scale. We had previously found 'Nocturne' to be a highly self-fertile 6*x* parent (Ehlenfeldt and Kramer, 2012), so it was hoped 'Nocturne' might be a receptive parent for 4*x* S_1 hybrid pollen. Only a few seedlings were derived from these crosses. These offspring appeared to be somewhat atypical for 'Nocturne'; however, 'Nocturne' has such a diverse species background that it was initially unclear whether these hybrids might simply be unusual 'Nocturne' recombinants. These hybrids grew slowly, but in their second year, several were evaluated by flow cytometry. Ploidy levels were found to be 5*x*, suggesting they were the expected hybrids.

Discussion and Conclusions

Crosses of somewhat similar nature among these three major *Vaccinium* crop species have been done previously (Crist, 1977; Vorsa et al., 2009; Zeldin, 2015; Ehlenfeldt, personal communication, Vorsa, personal communication); however, a critical difference between earlier crosses and these are the resultant ploidy levels. Most previous crosses occurred between diploids, and resulted in sterile or near sterile hybrids; the current crosses have occurred primarily at the tetraploid level, and initial indications are that some measure of fertility will exist in most offspring.

Even at the tetraploid level, important questions remain: Despite initial indications, are any or all of these advanced hybrids fertile? If fertile, are any fertile enough to be considered "cropable"? Can we find rare, vigorous combinations for weaker type combinations, i.e. S_1 × 4*x* blueberry? Will heterologous genome recombination occur in the allotetraploids? Ongoing research will answer many of these questions.

The other important consideration is what are the prospects for short-term commercial value from these crosses. For crosses with *V. macrocarpon*, the highly defined morphology and ecology of 2*x V. macrocarpon* suggest that introgressed allotetraploids might not be suitable as crop plants under current cranberry management systems. Our introgressed allotetraploids are more upright, stockier, and more woody, than diploid cranberry, and the tetraploids may not tolerate flooding as is practiced in cranberry. If this experimental germplasm could be reduced to the diploid level, however, considerable interesting possibilities might exist.

For crosses to *V. corymbosum*, mainstream germplasm is utilized primarily at the 4*x* level, and *V. corymbosum* already has considerable variability in its accessible base; nonetheless, additional variability available from new crosses might be expected to be useful if it brings new traits and is selected carefully. Ease of use, and the utility of the derived allopolyploid hybrids may ultimately depend upon whether the hybrids produced are consistently 4*x* (from 4*x V. corymbosum* × 4*x V. corymbodendron*) or 6*x* (from 4*x* S_1 × 4*x V. corymbosum*). Our current sample size is very small, but future evaluations are expected to clarify the situation.

In our opinion, *V. vitis-idaea* may benefit most in the short-run from introgression of new germplasm. Many temperate growers have experimented with lingonberry, only to find it too susceptible to root disease to be a viable option. An allotetraploid lingonberry with appropriate amounts of germplasm introgressed from other *Vaccinium* species might open new options in lingonberry or lingonberry-like fruit production.

It is premature to say that *V. corymbodendron* has allowed the full bridging of these three commercially-important *Vaccinium* sections; however, we have demonstrated that *V. corymbodendron*, and its derivatives can bridge to each of these respective sections. Thus, it seems clear that with additional effort it should be possible to promote movement of genes between these three sections to the benefit of the commercial crop genomes.

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