



The Barren Berry phenotype: untangling cranberry (*Vaccinium macrocarpon* Ait.) genetic contamination in central Wisconsin and beyond

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Abstract

Wisconsin is the world's leading producer of cranberries (*Vaccinium macrocarpon* Ait.; $2n=2x=24$). The state produces over twenty-thousand acres that contribute to more than 50% of the global total production, with more than one billion dollars in value. Cranberry growers in the “central sands” of Wisconsin have been experiencing yield decline due to vegetative unproductive genotypes, popularly known as “Barren Berry”, which consistently remain vegetative and produce no fruit. The purpose of this study was to compare visual inspection in the field to DNA fingerprinting for the early detection of unproductive/barren genotypes. Additionally, the study served as a survey of unproductive genotypes in central Wisconsin, the largest growing region in the world. A total of 839 cranberry leaf samples, from 14 growers representing plantings to four cultivated varieties, were submitted for DNA testing of two visually determined phenotypes: unproductive or barren ($n=646$; those that produced little to no fruit), versus productive or fruiting ($n=193$). We conducted genetic fingerprinting on the leaf tissue using nine microsatellite markers previously shown to differentiate cranberry genotypes. This study identified a barren berry genotype unique to central Wisconsin, which we denoted “Barren Berry 1”. This genotype accounted for 43% of samples submitted as the barren berry phenotype. Genetic fingerprinting revealed sixty-five different genotypes in beds which were thought to be monocultures of just four cultivated genotypes. Early detection of genetic contamination through visual inspection, genetic testing, and responsible propagation can drastically aid in the management and longevity of cranberry beds, and in turn save time and money to the growers.

Keywords SSRs · Microsatellites · DNA fingerprinting · Genetic uniformity · Clonal purity · Barren berry · Cranberry bed management

Introduction

The American cranberry (*Vaccinium macrocarpon* Ait.; $2n=2x=24$) is a fruit-bearing woody perennial plant, native to North America (Durand 1942). Cranberries were first cultivated in 1810 on the peatlands on Cape Cod, Massachusetts, which were later surrounded by ditches and dikes (raised earthen dams) to enhance production and ease management (Eck 1990). The state of Wisconsin began cultivating cranberries in 1853 (Matusinec et al. 2022; Schlautman et al. 2018), and rapidly became a global producer by

the 20th century, producing 5.01 million barrels or 227,249 tons of fruit in 2023 and contributing ~50% of the global cranberry commerce (Vorsa and Zalapa 2019; USDA NASS 2023). Currently, the cranberry yield per acre in Wisconsin is the best in the world at 250 barrels per acre or 11.34 tons per hectare (USDA NASS 2023).

In the wild cranberries are adapted to bog environments, which are poor nutrient and stressful environments, and thus their most important mode reproduction is asexual (Vander Kloet 1988; Eck 1990; Polashock et al. 2014; Vorsa and Zalapa 2019). However, under favorable conditions in the

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wild (i.e., nutrient availability), cranberries reproduce sexually, and due to clonality, and tolerance to inbreeding, selfing is a predominant mode of reproduction (Polashock et al. 2014; DeVetter et al. 2015; Bolivar-Medina et al. 2019; Rodriguez-Bonilla et al. 2020). Under commercial production and due to their clonal and selfing ability, cranberries are grown as a monoculture, and any deviation of the desired cultivar is undesirable since cranberry genotypes exhibit marked phenotypic differences including dormancy, plant architecture, flowering, and fruiting (Eck 1990; Diaz-Garcia et al. 2019; Vorsa and Zalapa 2019; Lopez-Moreno et al. 2023). Thus, maintaining the genetic purity of beds is crucial to optimize agronomic management, harvest, and post-harvest storage. Several studies have found reported yield potential decreases when the genetic contamination is with aggressive vegetative genotypes (Roper et al. 1995; Matusinec et al. 2022). Most new plantings or renovations of cranberry beds are carried out using cuttings often mowed from existing beds to allow for vegetative or clonal reproduction of the desired cultivar (Schlautman et al. 2018). Genotype purity of the mowed beds is often a key factor contributing to variation in crop yields (Matusinec et al. 2022), and plantings of new uncontaminated hybrid cultivars produce ~2–4 times more fruit than mixed genotype native varieties (Hoekstra et al. 2020; Vorsa and Zalapa 2019).

Cultivar-specific phenotypic variation makes it feasible for growers to identify genetic contamination in cranberry beds by visually inspecting plant characteristics in the field (e.g., leaf color, flowering, canopy thickness, yield, etc.) (Matusinec et al. 2022; Roper and Vorsa 2010). Visual observation has shown promise as an accurate and cost-effective tool for identifying areas of genetic contamination compared with more rigorous detection methods such as analysis of molecular markers, as demonstrated by Matusinec et al. (2022). For instance, results from the pilot study of Matusinec et al. (2022) were a key factor contributing to the decision to renovate most of the beds of the Wisconsin Cranberry Research Station (WCRS) and demonstrated the usefulness of molecular fingerprinting for genetic contamination determination to inform decisions on cranberry farm renovations (Matusinec et al. 2022).

In this study, we conducted a large-scale assessment of genetic contamination in commercial cranberry beds in central Wisconsin, popularly known among growers as the “Wisconsin Cranberry Country.” We combined visual inspection of productivity and molecular fingerprinting for identification of pure cultivar. Our goal was to determine if visual inspection of productivity can be used to identify unproductive genotypes. Unproductive genotypes are thought to be a problem in central Wisconsin (Roper et al. 1995; Matusinec et al. 2022), but they may also be affecting

cranberry production in northern Wisconsin, southeastern Massachusetts, New Jersey, states in the Pacific Northwest, and internationally. If present, unproductive genotypes may be a cause of decreased cranberry yields with an unknown impact, yet a rigorous analysis of visual inspection as a tool for identifying barren genotypes is lacking. To fill this gap, we compared visual inspection of productivity and molecular testing for identifying genetic contamination across 14 cranberry growers in central Wisconsin, which represent one of the most productive cranberry production areas worldwide. We report a breakdown of predominant genotypes associated with the unproductive/barren phenotype of Central Wisconsin and beyond, including the discovery of a new genotype, denominated Barren Berry 1 (BB1), associated with the barren phenotype.

Materials and methods

Tissue collection and phenotype identification

To assess genetic contamination in central Wisconsin and its association with the unproductive/barren phenotype, 839 plant tissue samples were collected from 14 growers. The samples were collected based on visual evaluation of cranberry bed productivity in the field during the month of September 2021 shortly before the cranberry harvest so that a clear fruit presence or absence could be identified. (Table 1; Fig. 1A). Based on the two phenotypes collected (productive vs. unproductive), we expected the productive samples to be predominantly pure known cultivars, while the unproductive samples to be unknown genotypes. To test for genetic contamination, the cranberry samples were collected from beds originally planted to one of four cultivars: ‘Stevens’ ($n=552$), ‘Ben Lear’ ($n=146$), ‘Searles’ ($n=113$), or ‘HyRed’ ($n=28$). ‘Stevens’ is the most popular cultivar in Wisconsin and around the world with ~40% of all acreage, ‘Ben Lear’ and ‘Searles’ are native cultivars (i.e., selected from the wild) still widely grown in Wisconsin, and ‘HyRed’ is a new cultivar gaining popularity in Wisconsin (Vorsa and Zalapa 2019). The 14 growers were divided into three general geographic regions or sections based on the assumption that growers in proximity to one another are more likely to share vegetative propagules to plant newly constructed beds or to renovate existing ones (Fig. 1A). To this end, we grouped the 14 growers into three sections based on a radial distance of 15 km to three towns, Tomah, City Point, and Wisconsin Rapids (i.e., growers within 15 km to each town were grouped together; the exact location of each grower was hidden due to privacy concerns; Fig. 1A). Regardless of the cultivar, each grower visually assessed their plantings based on qualitative differences

Table 1 Genetic contamination identified using nine microsatellite markers among 839 cranberry samples collected at 14 grower sites in central Wisconsin. Three grower sections or regions were designated based on geographical proximity of growers sampled (Fig. 1A). N=number of samples submitted in two categories productive and unproductive (i.e., barren). Samples were genetically analyzed and divided into cultivars, cultivars, self/crosses, Barren Berry 1 (BB1), Perry Red (PR), and unknown (UNK) genotypes

Grower#	Section	Total (N)	Productive (N)	Unproductive (N)	Cultivar	Self/Cross	BB1	PR	UNK
1	1	119	35	84	31%	4%	45%	9%	11%
2	1	33	8	25	9%	12%	76%	0%	3%
3	1	16	4	12	25%	19%	0%	50%	6%
4	1	82	20	62	26%	48%	0%	2%	24%
5	2	96	24	72	36%	0%	58%	0%	5%
6	2	32	8	24	63%	0%	0%	34%	3%
7	2	64	16	48	9%	36%	19%	16%	20%
8	2	152	20	132	18%	4%	68%	9%	1%
9	2	32	8	24	3%	9%	75%	0%	13%
10	3	37	6	31	24%	0%	0%	57%	19%
11	3	16	4	12	19%	13%	0%	0%	69%
12	3	16	4	12	94%	0%	0%	0%	6%
13	3	80	20	60	58%	0%	0%	36%	6%
14	3	64	16	48	36%	2%	16%	22%	25%
Totals		839	193	646	30%	10%	34%	14%	12%

of fruiting (productive) versus non-fruiting (unproductive) phenotypes. (Fig. 1B). From the 14 growers, a total of 193 samples were collected as productive or fruiting (Table 1; Fig. 1B, right), while 646 samples were collected as unproductive (i.e., barren phenotype which lacked fruit; Table 1; Fig. 1B, left). The unproductive phenotype usually also had a hairy or bushy (vegetative) growth habit, which could be easily distinguished from the productive areas of the cranberry bed. Lady Bug IPM, LLC, a cranberry crop consultant located in Pittsville, WI, was responsible for collecting samples along with each participating grower, and problematic areas were determined in conjunction with the grower. Samples with or without fruit (i.e. productive and unproductive, respectively) were collected to include both uprights (i.e., reproductive vertical stems) or runners (i.e., stem that grows parallel to the soil surface) of 4–5 cm long. The plant material collected corresponded to the current year's growth and contained at least 10 leaves. Samples were labeled with site name, date, bed identification, cultivar, and phenotype (unproductive or barren and productive or fruiting), kept cool on ice in the field, and then frozen in laboratory until further processing for DNA extraction.

DNA extraction and polymerase chain reactions (PCRs)

Genomic DNA extractions for microsatellite analysis of the 839 samples, collected from the 14 growers (Fig. 1A), were performed using leaf tissue per a CTAB method (Doyle 1991) modified to isolate high quality, clean DNA (Matusinec et al. 2022). PCRs were performed according to Schlautman et al. (2018) using carboxyfluorescein (FAM)

and hexachlorofluorescein (HEX) M13 tags (5'-CACGT TGTAACGAC-3'). Briefly, reactions were performed in 8 µL total volume using 3.5 µL 1× JumpStart REDTaq ReadyMix (Sigma, St. Louis, MO, USA), 1.0 µL of 15 ng/µL DNA, 2.0 µL of ddH₂O, 0.5 µL of 5 µM forward primer, 0.5 µL of 50 µM reverse primer, and 0.5 µL of 0.5 µM M13-FAM, M13-HEX, or M13-NED primer. Thermocycling conditions included a 3 min melting step of 94 °C, followed by 33 cycles of 94 °C for 15 s, 55 °C for 90 s, and 72 °C for 2 min, and a final extension step of 72 °C for 30 min. One microliter each of FAM and HEX labeled PCR product was mixed with 10 µL formamide and a carboxy-X-rhodamin (ROX; GeneFlo-325 ROX; CHIMERx, Milwaukee, WI, USA) ladder, and the pool-plexed mix was sent to the University of Wisconsin Biotechnology Center DNA Sequencing Facility for fragment analysis using an ABI 3730 fluorescent sequencer (Pop-6 and a 50 cm capillary array; Applied Biosystems, Foster City, CA, USA). Allele genotyping was performed using the GeneMarker software v 1.91 (SoftGenetics LLC, State College, PA, USA). To avoid technical and human error during genotyping, PCR were repeated as needed (up three times) and multiple scorers were used to ensure the repeatability of the results.

Data analysis

Nine simple sequence repeat (SSR) or microsatellite loci were used to genotype the samples collected from the 14 participating growers. The primers used were vm04084, vm26877, vm28527, vm31701, vm38401, vm39030, vm51985, vm52682, and vm78806 (Supplementary file 1). The nine microsatellite markers were initially found to

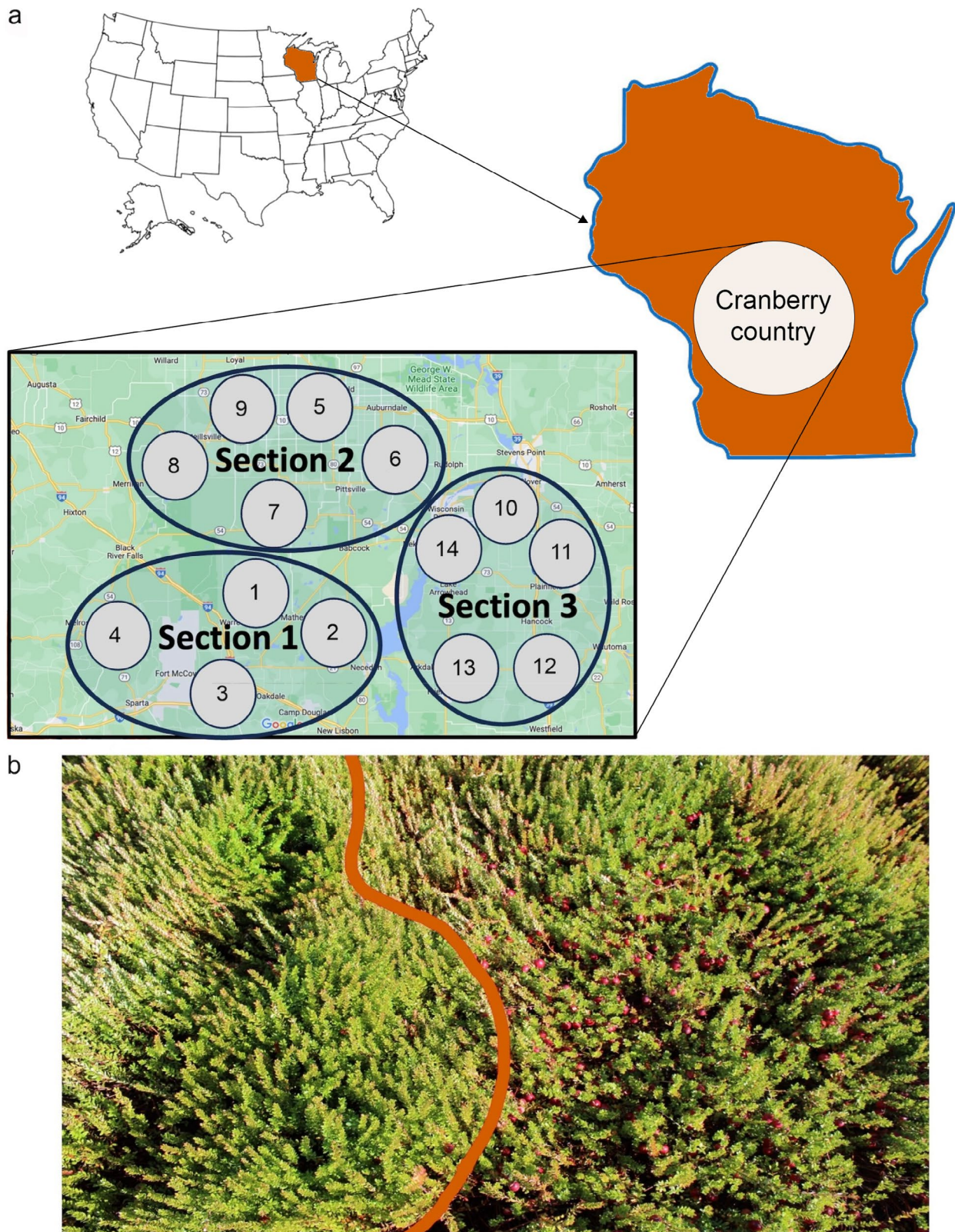


Fig. 1 (A) Central Wisconsin cranberry growing area denoted in the gray circle. The Wisconsin cranberry country is the world's most prominent producer of cranberries. Three grower sections or regions were designated based on geographical proximity of growers sampled.

(B) The phenotype of 'Barren Berry 1' (left, unproductive) versus that of the industry standard 'Stevens' (right, productive; Note the abundant cranberries on the right side of the picture). The exact location of the growers is not shown due to privacy concerns

differentiate cranberry cultivars by Fajardo et al. (2013) and were previously identified by Zhu et al. (2012). These SSRs have been used extensively in cranberry to differentiate and assess the cultivated genetic diversity including the USDA National Clonal Repository (Schlautman et al. 2018) and to assess genetic contamination in commercial plantings in Wisconsin (Matusinec et al. 2022). They have also been used to assess the genetic diversity of natural wild cranberries and their relatives and to detect clonal versus reproductive populations in Wisconsin and the US National Forests (DeVetter et al. 2015; Rodriguez-Bonilla et al. 2019, 2020). Genotype diversity was examined using GenAEx 6.5 (Peakall and Smouse 2006). Unique clones were initially detected by calculating a simple genetic distance matrix between pairs of individuals based on the nine microsatellite loci as estimated by GenAEx 6.5. Then, the alleles of each genotype were individually examined to determine clones. When two genotypes had identical allelic constitutions at all nine microsatellite markers used, they were considered clones. When necessary, missing data points were imputed based on the clonal information using the data matrix consisting of the 839 samples. The final pairwise genetic distance between plants was estimated and used to perform principal coordinate analysis (PCoA) using a standardized covariance matrix of all 839 genotypes identified at the Wisconsin Cranberry Research Station (WCRS) (Matusinec et al. 2022). For cultivar identification, genotypes at the WCRS were compared to a database of cranberry cultivar variants present at the National Clonal Germplasm Repository (NCGR; Schlautman et al. 2018). Additional PCoAs were performed based on the 65 unique genotypes identified in the study (Supplementary file 1) plus other diverse cranberry cultivars from NCGR and other studies using the same SSRs (Fajardo et al. 2013; Schlautman et al. 2018; Matusinec et al. 2022). Selfed genotypes of known cultivars were identified manually possessing homozygous allelic constitutions based on the nine microsatellite markers used. Similarly, progeny of known genotypes were identified manually possessing one allele in common per loci at all nine microsatellite markers used. Based on this database and the genetic test results of this study, samples were divided into two categories (1) productive or likely productive and (2) unproductive or likely unproductive. We grouped known cultivar genotypes and their derivative self/cross genotypes as productive and likely productive. The unproductive or likely unproductive genotypes included all the unknown genotypes identified herein (i.e., not matching any cultivated genotype in NCGR) plus a new genotype associated with the barren phenotype, denominated Barren Berry 1 (BB1), identified in the current study, and Perry Red, which was previously associated with the unproductive phenotype (Matusinec et al. 2022).

Binomial exact test

To assess the growers' ability to visually differentiate pure cultivars from contaminated unproductive genotypes in the field, we performed a Binomial exact test, which is a statistical method used to determine whether the observed success rate in a binomial experiment (trials with two possible outcomes, e.g. productive vs. unproductive) significantly deviates from a specified theoretical expectation derived from the null hypothesis (i.e., the growers can differentiate between productive and unproductive genotypes in the field based on phenotypic evaluation). In an exact binomial test, a test statistic (TS) is calculated:

$$\begin{aligned}
 TS &= \\
 &\text{Probability Mass Function (PMF) for } x \\
 &= 1 - PMF(PMF(X = x)) \\
 &= C(n, x) * p^x * (1 - p)^{(n-x)}, \text{ where } C(n, x) \\
 &= n! / (x!(n - x)!)
 \end{aligned}$$

n represents the total number of trials, x represents successful outcomes, and p represents the probability of success on any single trial. This test statistic is then compared to the chosen critical value of significance ($p < 0.05$) obtained from a two-sided test. If the computed test statistic is less than the critical value, then the null hypothesis is accepted. Two tests were conducted, (1) to assess if growers can visually differentiate pure cultivars from unproductive genotypes in the field, and (2) to assess if growers can visually differentiate unproductive genotypes from pure cultivars in the field.

Results

Overall genetic contamination analysis

Our genetic analysis results, representing 839 samples from 14 growers, yielded a total of 65 unique genotypes. Cultivar genotypes constituted 30% ($n=250$) of all samples while self/crosses of cultivar represented 10% ($n=86$) (Table 1; Fig. 2A). The unproductive or likely unproductive genotypes represented 60% ($n=503$) of the samples (i.e., Barren Berry 1, Perry Red, and unknown genotypes) (Table 1; Fig. 2A). Of these unproductive samples, 34% were Barren Berry 1 ($n=283$, first time identified in this study), followed by 14% Perry Red ($n=120$), which has been identified as an unproductive cultivar in central Wisconsin (Matusinec et al. 2022). Unknown genotypes, which were not genotypically matched to any named cultivar genotype in our database, represented 12% ($n=100$) of the total samples.

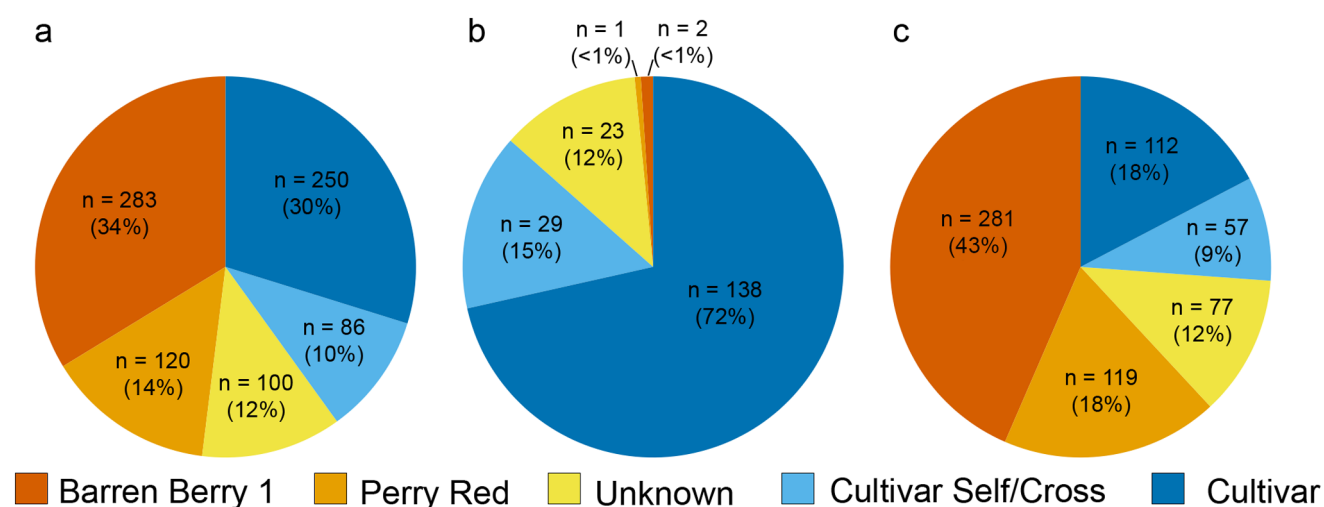


Fig. 2 Genetic contamination identified using nine microsatellite markers among 839 cranberry samples collected at 14 grower sites in central Wisconsin (Tables 1 and 2). (A) Barren Berry 1 ($n=283$; 34%), Perry Red ($n=120$; 14%), unknown genotypes ($n=100$; 12%), and pure cultivars ($n=250$; 30%), cultivar self and crosses ($n=86$; 10%). (B) Partitioning of samples collected as perceived pure cultivar ($n=193$), actual pure cultivars ($n=138$; 72%), cultivar self and

crosses ($n=29$; 15%), unknown genotypes ($n=23$; 12%), Perry Red ($n=1$; <1%), and Barren Berry 1 ($n=2$; <1%). (C) Partitioning of samples collected as perceived genetically contaminated ($n=646$), Barren Berry 1 ($n=281$; 43%), Perry Red ($n=119$; 18%), unknown genotypes ($n=77$; 12%), cultivar crosses or self ($n=57$; 9%), pure cultivars ($n=112$; 18%)

Table 2 Cultivar identity and genetic contamination identified using nine microsatellite markers among 839 cranberry samples collected at 14 grower sites in central Wisconsin. Submitted cultivar bed, N=number of samples submitted in two categories productive and unproductive (i.e., barren). Samples were genetically analyzed and divided into cultivars (match or other), cultivars self/crosses, Barren Berry 1 (BB1), Perry Red (PR), and unknown (UNK) genotypes

Cultivar* (Bed)	Total (N)	Productive (N)	Unproductive (N)	Genotype Match	Other Cultivar	Cultivar Self/Cross	BB1	Perry Red	Unknown
'Stevens'	552	123	429	24%	12%	1%	40%	14%	9%
'Ben Lear'	146	36	110	27%	2%	34%	0%	14%	23%
'Searles'	113	28	85	0%	4%	27%	54%	0%	15%
'HyRed'	28	6	22	25%	0%	0%	0%	75%	0%
Totals	839	193	646	21%	9%	10%	34%	14%	12%
Productive	193	193	0	53%	19%	15%	1%	1%	12%
Unproductive	646	0	646	12%	6%	9%	43%	18%	12%

To further explore these results, we partitioned the samples into two categories: (1) those samples perceived by growers as (productive) pure cultivars (Tables 1 and 2; Fig. 2B), and (2) those samples perceived by growers as (unproductive) genetically contaminated (Tables 1 and 2; Fig. 2C). Of the 839 samples, 193 samples were submitted as perceived pure cultivar by phenotypic inspection in the field (Table 1; Fig. 1B). In comparison, DNA fingerprinting revealed that 138 (72%) of those samples were pure cultivars (Table 2; Fig. 2B). To assess if growers can visually differentiate pure cultivars from unproductive genotypes in the field, we performed an exact binomial test of the 138 confirmed cultivar samples versus the 193 samples submitted as pure. The result was significant at $p < 0.001$, indicating that growers can visually identify pure cultivars from contaminated genotypes through visual inspection (Fig. 1B).

Overall, 646 of the 839 samples were submitted as unproductive (Tables 1 and 2). Our DNA fingerprinting results identified that 281 samples (43%) belonged to Barren Berry 1 (Fig. 2C), Perry Red accounted for 119 unproductive samples (18%), while unknown genotypes accounted for 77 samples (12%) (Fig. 2C). Thus, confirmed unproductive genotypes accounted for 73% of samples submitted as unproductive (Table 2). To assess if growers can visually differentiate unproductive genotypes from pure cultivars in the field, we performed a second exact binomial test of the 477 confirmed unproductive samples versus the 646 samples submitted as unproductive. The result was significant at $p < 0.001$, confirming that growers can visually distinguish unproductive genotypes from cultivars through visual inspection in the field (Fig. 1B).

Geographical genetic contamination analysis

To analyze the spatial distribution of contaminating genotypes in central Wisconsin, we divided the 14 growers into three regions or groupings based on distance proximity (Table 1; Figs. 1A and 3). Every grower in all three sections showed some level contamination by unproductive genotypes (i.e., Barren Berry 1, Perry Red, and unknowns) (Fig. 3A). Seven of the growers in all three sections showed contamination by Barren Berry 1 with most of the contamination concentrating in Sect. 2 (Fig. 3B). While nine of the growers showed genetic contamination by Perry Red (three growers per section, Fig. 3C), contamination by unknown genotypes appeared in all growers and all sections (Fig. 3D).

We further explored a historic national and international data using the same SSR primer set available through a free fingerprinting service to growers by the USDA-ARS Cranberry Genetics and Genomics Lab (Supplementary Table 1). The data set was composed of 2,912 samples submitted for DNA fingerprinting by 58 different grower contacts between the years 2011–2022. The grower contacts represent mostly

Wisconsin and its growing areas, but samples were also submitted by growers in Minnesota, New Jersey, Oregon, Washington, and Europe. Barren Berry 1 and Perry Red represented 130 and 467 of genetically contaminated samples while unknowns accounted for 517, altogether representing ~38% percent of the total submitted samples. Based on this data set, Barren Berry 1 was first identified in 2016 in Wood County (Sect. 2 in Table 1; Fig. 2) Wisconsin, and no cases were detected in other growing regions outside Wisconsin. Thus, the Barren Berry 1 genotype was detected in eight growers mostly in central Wisconsin (Jackson, Monroe, Juneau and Wood counties), but also in the northern county of Vilas and in the western county of Crawford. Based on our historic data set, the Perry Red genotype was identified for the first time in Wisconsin in 2011 in Monroe County. The Perry Red genotype was widely spread-out among 27 growers with geographical representation in Central Wisconsin (all three sections in Table 1; Fig. 3), in La Crosse County, and in the state of Washington, USA. Finally, unknown genotypes were a problem for 44 grower

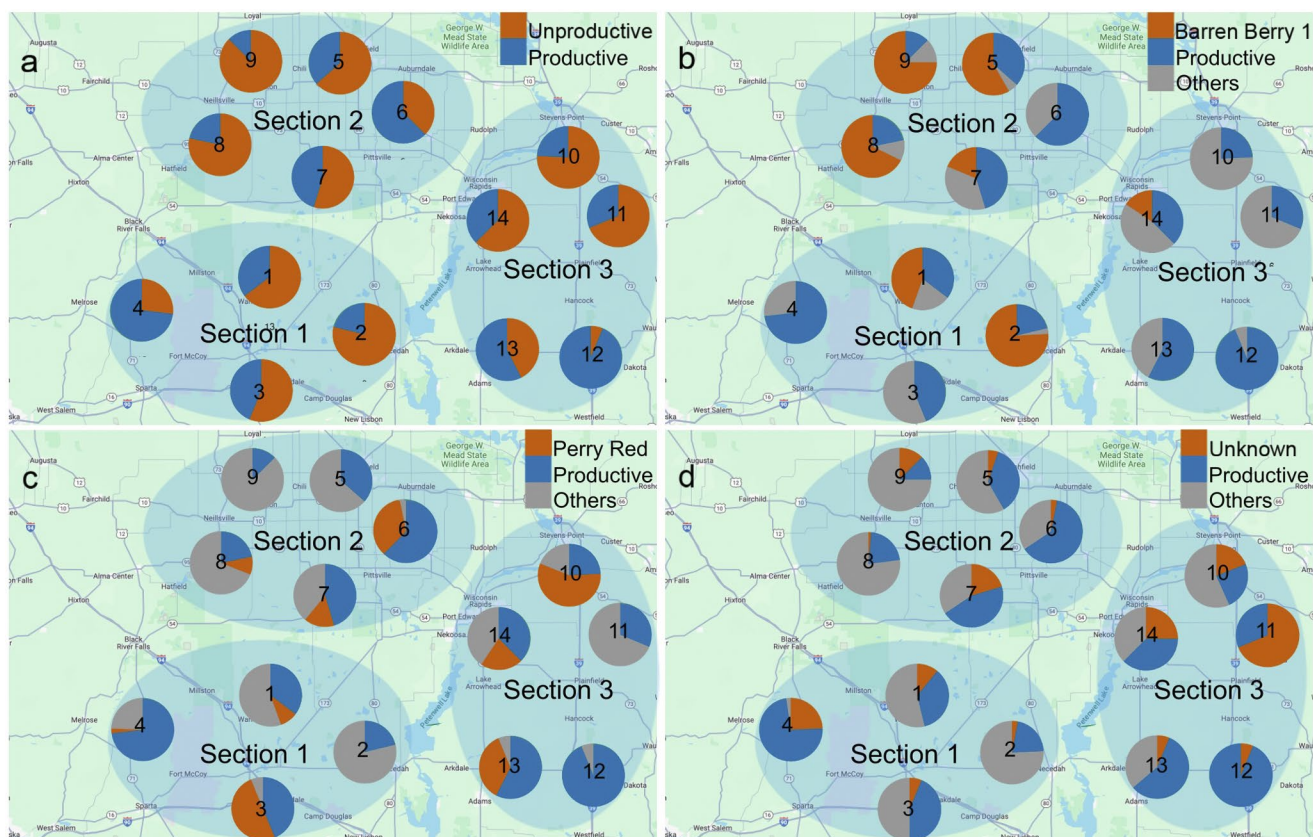


Fig. 3 Geographical distribution of participating cranberry growers and genetic analysis results based on nine microsatellite markers (Table 1). 3 A. Number of samples per grower representing unproductive genotypes (Barren Berry 1, Perry Red, and unknown) and productive genotypes (cultivar, crosses, and self). 3B. Number of samples per grower representing Barren Berry 1 detected per grower (shown in

red) and productive genotypes (cultivar, crosses, and self). 3 C. Number of samples per grower representing Perry Red detected per grower and productive genotypes (cultivar, crosses, and self). 3D. Number of samples per grower representing unknown genotypes detected per grower and productive genotypes (cultivar, crosses, and self). Exact location of each grower has been hidden due to privacy concerns

contacts in all growing regions represented, except for New Jersey (Supplementary Table 1).

Cultivar contamination analysis

Additionally, we compared genetically contaminated samples to the expected cultivars in those beds. Table 2 shows the expected cultivar and the breakdown of productive and unproductive submitted samples. The fingerprinting analysis revealed a correspondence between the number of samples submitted as a productive phenotype and the number of samples that genetically matched the expected cultivar name, except for ‘Searles’ (Table 2). We further broke down the contamination by cultivar, showing that most of the samples in this study were submitted from ‘Stevens’ beds ($n=552$) in two categories: productive ($n=123$) and unproductive ($n=429$). ‘Stevens’ beds were mostly contaminated with Barren Berry 1 (40%), followed by Perry Red (14%), and unknowns (9%). A considerable number of ‘Stevens’ bed samples did not match the correct genotype, but 12% of were actually other commercial cultivars. ‘Ben Lear’ bed samples ($n=146$; productive $n=36$; unproductive $n=110$) were contaminated with the unproductive Perry Red (14%) and unknowns (23%), but a considerable number of samples were determined to be self/cross (34%). For samples submitted from Sealers beds, there were none matching the correct ‘Searles’ genotype in our database; however, a considerable number of samples appeared to be self/crosses of the cultivar ‘Searles’ (Table 2). While most of the unproductive

genetic contamination in ‘Searles’ beds was due to Barren Berry 1, the unproductive genetic contamination in ‘HyRed’ beds was mostly Perry Red.

Principal coordinate analysis

To understand the genetic relationships among Barren Berry 1, Perry Red, and the 839 samples in this study, we conducted a PCoA analysis. Figure 4 displays the relationships of all the samples to Barren Berry 1 and Perry Red and the correct genotypes of the ‘Stevens’, ‘Ben Lear’, ‘Searles’, and ‘HyRed’ (cultivars represented in this study). The PCoA of the 839 samples resulted in 47.73% of variance ($\text{PCoA } 1=26.88\% + \text{PCoA } 2=20.85\%$) explained by the first two axes (Fig. 4). The Barren Berry 1 position represents 283 samples, the Perry Red position represents 120 samples, the ‘Stevens’ position represents 131 samples, the ‘Ben Lear’ position represents 39 samples, ‘HyRed’ represents 7 samples, and the ‘Searles’ genotype was not found among the samples of this study, but it is shown for comparison (Table 2). The Barren Berry 1 genotype was part of a differentiated cluster of samples, and the closest cultivar to that cluster was ‘Ben Lear’. Looking at the genotypes of Barren Berry 1 and ‘Ben Lear’ (Supplementary Table 2), they share six alleles at six markers. The Perry Red genotype was slightly differentiated from the main genetic cluster but remained connected and far away from the positions of the ‘Stevens’, ‘Ben Lear’, ‘Searles’, and ‘HyRed’ genotypes. Finally, to better assess the genetic relationships,

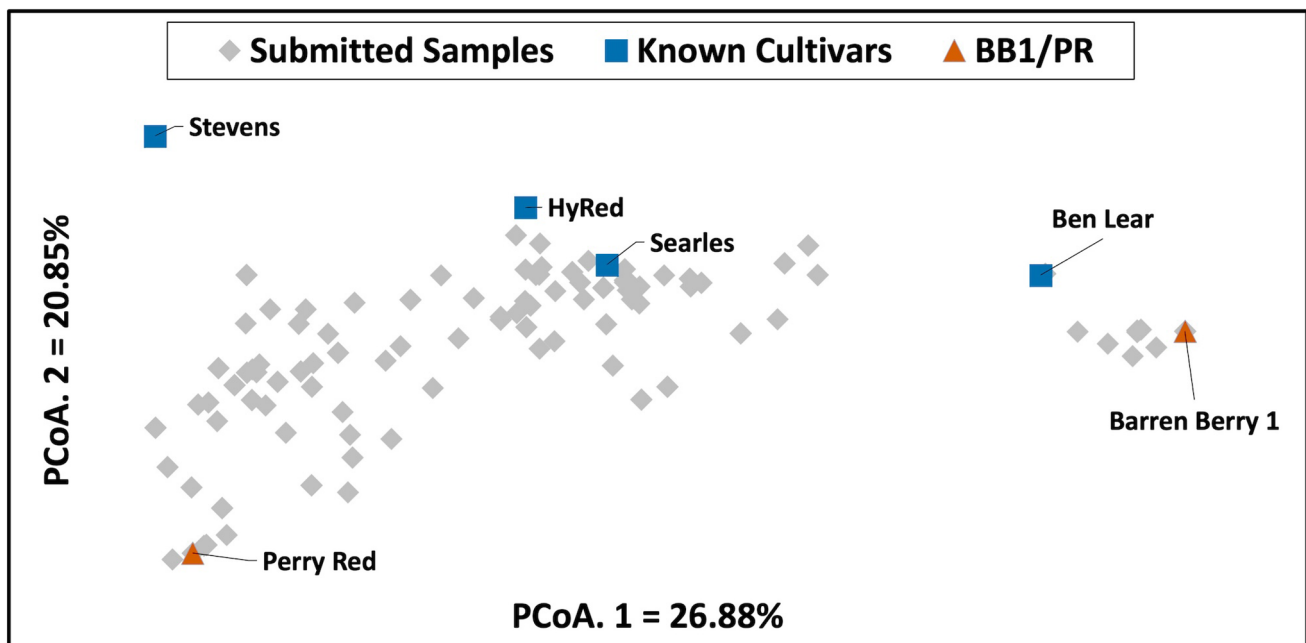


Fig. 4 Principal coordinate analysis (PCoA) of 839 cranberry samples submitted as productive or unproductive/barren (Table 1; Fig. 2) based on nine microsatellite markers. Cultivar genotypes of the submitted

beds are shown in blue. The genotypes of Barren Berry 1 and Perry Red are shown in Red

we conducted two additional PCoA with all the redundant samples removed from the data set (Supplementary Fig. 1) and another one with additional genotypes of representative and diverse commercially grown cultivars (Supplementary Fig. 2). The resulting PCoAs included the 65 unique genotypes discovered in the 839 samples through the fingerprinting analysis, and an added ‘Searles’ genotype for reference. Besides ‘Stevens’, ‘Ben Lear’, ‘Searles’, and ‘HyRed’, other cultivars detected in the samples submitted by growers, included 73 samples (Table 2), ‘Howes’ = 61, ‘Potter’s Favorite’ = 7, ‘Stankavich’ = 3, and ‘Harju’ = 2. The total variation explained for the two PCoAs were 26.07% and 24.24%, respectively. In these two PCoAs, there were no differentiated clusters, and the genotypes of Barren Berry 1 or Perry Red appeared as part of the main cluster (Supplementary Figs. 1 and 2). However, as expected, the Barren Berry 1 genotype grouped closer to ‘Ben Lear’ and its derivatives varieties while Perry Red clustered remained close to eastern native varieties.

Discussion

The American cranberry is one of Wisconsin’s most important crops, contributing over one billion dollars in revenue annually (USDA NASS 2023; Vorsa and Zalapa 2019). Genetic purity is very important to the overall production and health of a cranberry bed as it is managed as a monoculture. Maintaining genetic purity is crucial for ensuring ongoing cranberry bed productivity as well as proper horticultural management and post-harvest storage protocols (Eck 1990; Diaz-Garcia et al. 2019; Lopez-Moreno et al. 2023). Unproductive yield patterns have been reported by growers in cranberry beds in all growing regions in the U.S. and have also been observed in Southern Chile and Canada (Fajardo et al. 2013; Schlautman et al. 2018; Matusinec et al. 2022; Millar et al. 2024; Maureira personal communication). In this study, we analyzed 839 samples using nine microsatellite markers submitted by growers as productive and unproductive/barren. We compared visual inspection and molecular testing for identifying genetic contamination across 14 cranberry growers in central Wisconsin. We report the genotypes of the main unproductive contaminants of cranberry beds in Central Wisconsin and beyond.

Visual inspection versus genetic testing

Visual inspection has been useful in identifying genetic contamination at the Wisconsin Cranberry Research Station (Matusinec et al. 2022). Based on visual inspection in the current study, Wisconsin growers were successful in identifying pure cultivars (Table 2; Fig. 2B). The unproductive

or likely unproductive genotypes detected in the samples submitted as pure cultivars accounted for less than 13% (i.e., unknown genotypes, BB1, and PR). Similarly, growers were successful in identifying unproductive or barren genotypes in the field; pure cultivars accounted for only 18% in the samples submitted as unproductive (Table 2; Fig. 2C).

Barren Berry 1

We discovered a new genotype associated with a barren phenotype of central Wisconsin, which we denominated Barren Berry 1 (Supplementary Table 2). Of the 646 samples submitted as unproductive or barren samples, 43% were Barren Berry 1 (Table 1; Fig. 2). This newly identified genotype creates a new concern for growers in terms of productivity. To date, Barren Berry 1 is the first cranberry genotype known to be almost completely vegetative with no fruit production. We did not find any self or first-generation crosses related to Barren Berry 1, which supports the idea of that this genotype is primarily vegetative. To our knowledge, this is the only invasive barren genotype affecting fruit crops recorded in the literature. According to grower descriptions, the barren phenotype is “extremely aggressive and forms large clumps of hairy or bushy vine” (vegetative growth habit), which could be easily distinguished from the productive part of the cranberry bed (Fig. 1B).

Barren Berry 1 appears to produce an abundance of vegetative growth and prioritizes its clonal reproduction over fruit production. Cranberries originate in natural bogs, which are typically very low nutrient environments (Eck 1990; Vander Kloet 1988); thus, in the wild cranberry vines seldom produce fruit or are at most ‘biennial-bearers’ (Vorsa 1994; Vorsa and Zalapa 2019). Cranberry vegetative reproduction and biennial-bearing are thought to be evolutionary adaptations allowing cranberries to survive in poor nutrient environments (DeVetter et al. 2015; Bolivar-Medina et al. 2019; Vorsa and Zalapa 2019). Moreover, cranberries are often found in the wild in large clonal pockets throughout Wisconsin and across their natural range (Rodriguez-Bonilla et al. 2020). However, some wild populations have been found to harbor clonal diversity (Rodriguez-Bonilla et al. 2020). Through human selection, cranberries have been domesticated and bred to produce abundant fruit and “rebud” each year (i.e., avoid biennial-bearing) (Vorsa and Johnson-Cicalese 2012; Vorsa and Zalapa 2019). Focusing on vegetative growth allows Barren Berry 1 to be extremely vigorous and aggressive compared to cultivars in fruit production in terms of perennial development (such as bud development, fruit setting, ripening, etc.) and resource use efficiencies (such as carbohydrates) due to seasonal constraints (DeVetter et al. 2015; Obeso 2002).

The first barren berry report in the cranberry literature is Roper et al. (1995), who found a genetic dissimilarity based on dominant markers among barren and productive ‘Searles’ vines. Based on codominant marker fingerprints, we now speculate that the Barren Berry 1 genotype originated from the wild, likely in Wisconsin. This genotype appears more closely related to Western genotypes such as ‘Ben Lear’ and its derivative cultivars in the PCoAs (Fig. 4; Supplementary Figs. 1 and 2). ‘Ben Lear’ is one of Wisconsin’s first wild selection cultivars first discovered in 1901 (Eck 1990). In the geographical analysis, Barren Berry 1 appears to be more prevalent in Sect. 2, and it seems to be expanding into Sects. 1 and 3 (Fig. 3B). Additionally, in a national and international historical fingerprinting database, Barren Berry 1 was detected for the first time in 2016 also in Sect. 2, with additional presence in the northern and western cranberry growing regions of Wisconsin, but it was not detected in any other grower regions outside of Wisconsin (Supplementary Table 1). Thus, the Barren Berry 1 genotype likely entered grower’s marshes in Wisconsin during management practices, such as through flooding from its natural source (Eck 1990; Matusinec et al. 2022). Alternatively, Barren Berry 1 could have originated through a process of de-domestication where a wild genotype of cranberry crossed with that of a cultivated variety (e.g., ‘Ben Lear’), and the resulting genotype expressed a more wild-like phenotype (Dongya et al. 2021). The expansion of Barren Berry 1 is likely happening due to the practice of mowing and sharing vine among growers for renovation of new cranberry beds (Matusinec et al. 2022). Barren Berry 1 appears to be completely barren or non-reproductive under cultivated conditions, but that may not be the case in the wild. This genotype is working its way into commonly cultivated cranberry plantings, and its response to high nitrogen input appears to turn it to a vegetative state rather than reproductive (DeVetter et al. 2015). In weed cropping systems it is well known that weeds take input and compete with agronomic crops for nutrients and environment (Korres 2018). Cranberries must be fertilized at the correct levels and timing for optimal production, as naturally, cranberries seldom produce fruit, or are known to be biennial bearers (Strik et al. 1991; Eck 1976; Davenport and Vorsa 1999). ‘Mullica Queen’ for example, a new variety that is being readily accepted for its superior fruit qualities and yields can accept heavy fertilizer inputs and turn that into production for growers (Jeranyama et al. 2023). ‘Stevens’, on the other hand, receiving a similar regime of fertilizers would turn vegetative and avoid flowering altogether. Barren Berry 1’s reproductive abilities will need to be further tested under controlled conditions in a greenhouse or field experiment. ‘Stevens’ was the first hybrid cultivar released in the 1950s and has become the most popular cultivar in Wisconsin and around the world with ~40% of

all acreage. ‘Ben Lear’ and ‘Searles’ are 120-year-old native cultivars still widely grown in Wisconsin, and ‘HyRed’ is a new (2023) cultivar gaining popularity in Wisconsin (Vorsa and Zalapa 2019). Based on our data, the expansion of Barren Berry 1 in Wisconsin is affecting all important cultivars in the state, even recently released cultivars such as ‘HyRed’ (Table 2).

Other genetic contaminants

Perry Red

In this study, we identified several additional non-cultivated genotype contaminants in grower’s beds in Central Wisconsin. One such genetic contaminant was an unproductive genotype, denominated Perry Red, which accounted for 18% of the contamination among unproductive grower-submitted samples. Perry Red was previously identified and associated with low productivity in Wisconsin during the renovation of the Wisconsin Cranberry Research Station (Matusinec et al. 2022). Based on the PCoA Perry Red is more closely related to eastern wild selection cultivars such as ‘Howes’, ‘Early Black’, and ‘Stankavich’ (Fig. 4; Supplementary Figs. 1 and 2). In the geographical analysis (Fig. 3C) and national and international fingerprinting data set (Supplementary Table 1), Perry Red appears more widespread in all growing regions and was observed in Washington state. The origin of Perry Red is unknown, but the name was adopted from a genotype match with an accession at the USDA-ARS national cranberry collection in Corvallis, OR (Schlautman et al. 2018). From historical information, Perry Red was first selected in 1888 from wild stands in Massachusetts (Dana 1983; Eck 1990). All historical accounts suggest that the cultivar was once productive and commercially grown, but it is likely that the correct genotype was lost mis-propagation with the unproductive genotype in the east coast of the US. Previous work suggests that Perry Red was introduced in ‘Stevens’ beds through contaminated bales of vegetative propagule and planted in an alternating fashion (Matusinec et al. 2022). More recently, Perry Red was identified in Massachusetts (Millar et al. 2024), which supports the idea that the genotype originated in the east and was transferred to Wisconsin through the practice of sharing of vegetative propagules among growers.

Unknown genotypes

Contamination by unknown genotypes unrelated to cultivars appeared in all growers and all growing regions (Fig. 3D) and in other states and internationally (Supplementary Table 1). In this study, we detected a total of 65 unique genotypes (Supplementary Figs. 1 and 2), and of

those 32 were unknown genotypes (i.e., genotypes that do not match any named cultivar in a genotype database utilizing the same nine microsatellite markers). Although not all unknown genotypes may be unproductive or barren, unknown genotypes are likely less productive than known cultivars based on their overrepresentation in the samples submitted as unproductive (Table 1). Unknown genotypes likely originate from a wild gene bank, typically located nearby, and are naturally flowing through the waterways as seed or vegetative propagule. Unselected wild genotypes tend to be more vegetative and biennial-bearing, traits that promote survival in the wild and would confer advantage in a cultivated system (Matusinec et al. 2022). One example of volunteerism reducing yields in a cultivated system is “weedy” rice, which invades the standard rice cropping systems from nearby wild stands (Kanapeckas et al. 2016; Qiu et al. 2017).

Contamination with other cultivars and self/crosses

Other unexpected cultivars detected in the samples submitted by growers, included 73 total samples and corresponded to the following genotypes: ‘Howes’=61, ‘Potter’s Favorite’=7, ‘Stankavich’=3, and ‘Harju’=2 (Table 2). All of these cultivars are wild selections known to have at least 2–3 times lower yield performance than ‘Stevens’ (Eck 1990; Vorsa and Zalapa 2019). Thus, contamination with these cultivars could also result in lower yield and the perception as unproductivity by growers. Previous studies have found that some genotypes can derive from cultivar selfing and/or crossing with cultivars (Sarracino and Vorsa 1991; Huevel and DeMoranville 2009; Matusinec et al. 2022). In the current study, self/crosses were designated as likely productive based on their close relationship with commercial cultivars. However, it is possible that some of these self/crosses maybe be unproductive when the number of submitted unproductive samples can only be explained by adding up some the self/crosses to the list of unproductive genotypes (Table 1). Finally, the Searles genotype was not identified in the current study. This is likely because of the low number of samples submitted as productive Searles ($n=28$); most of the samples productive Searles samples were other cultivar genotypes or self/crosses (Table 2).

Other causes of low productivity

When analyzing samples submitted as unproductive based on their phenotype in the field, it was noted that a 18% of those samples were confirmed to be known cultivars (Table 2). This is likely due to inappropriate management practices affecting productivity such as nutrient or carbohydrate availability at critical times (Jeranyama et al. 2023).

Biennial-bearing allows cranberries to survive in poor nutrient environments such as bogs (Bolivar-Medina et al. 2019). Under commercial conditions, however, cranberries are managed to avoid biennial-bearing by feeding them nitrogen in small doses during flowering and fruit production (DeVetter et al. 2015). Also, cranberry cultivars are selected and bred to avoid biennial-bearing, particularly cultivars released after 1960 such as ‘Stevens’ and ‘HyRed’ (Vorsa and Zalapa 2019). In the future, it will be important to assess growers’ management practices to determine if biennial-bearing may be causing low yield when genetic contamination is not an issue.

Future work

The competitive advantage of the BB1 and PR genotypes lies or their vegetative nature, which allows them to out-compete fruiting genotypes. We have isolated and propagated the BB1 and PR genotypes in our greenhouse. We will conduct experiments to confirm their vegetative nature. We will explore nitrogen fertilizer and hormonal treatments to see if normal flower production is possible based on different treatments and what is the reproductive ability of male and female flowers and what is the faith pollinations if they are possible. We will attempt selfing of both genotypes as this is the primary sexual reproduction in our clonal system. We will also attempt crossing and will try to produce fruit and seed in a control setting via selfing and crossing. If we succeed in making them flower, we are going to try crossing the two genotypes and retest the vegetative nature and see if the offspring segregate for reproductive ability. Other, genetic mapping, sequencing, and RNA-seq experiments are also planned based on the genotypes of BB1 and PR and possible segregating populations.

Conclusions and grower recommendations

As previously reported, our study of submitted unproductive/barren samples from growers showed that visual inspection in the field is a useful tool for growers to assess genetic contamination of their plantings. Together, visual inspection and genetic fingerprinting of grower fields can explain discrepancies in the performances of cranberry beds and enable growers to make informed decisions for renovations and new plantings. Generally, the further the bed deviates from monocultures of cranberry cultivars with known performances, the less predictable and lower the yield will be. Cranberry genetic contamination can arise from vegetative propagation, selfing, and outcrossing, and based on our data, genetic contamination is occurring by all three means in growers’ fields in Wisconsin. Genetic contamination is likely also originating from the wild gene bank nearby as

evidenced by the predominance of unrelated unknown genotypes in our cultivated sample. Even the genotypes closely related to cultivated germplasm can affect productivity negatively. Only cranberry true-to-type cultivars specifically chosen by the grower for their known productivity and phenotypic attributes are likely to deliver the desired results, while management strategies are unlikely to make an unproductive genotype productive. Barren Berry 1, Perry Red, unknowns, and other off types are all likely a detriment to cranberry production. If left unaddressed, genetic contamination will spread and could become very costly to growers due to its negative effect on production and renovation costs. The genetic fingerprinting of unproductive genotypes (especially Barren Berry 1) presented in this study allows growers to more easily identify unproductive genotypes during bed renovation. One of the main reasons that cranberry contaminants are so common is that there are currently no regulations on the propagation of unpatented cranberry germplasm such as ‘Stevens’. We recommend that growers responsibly buy, share, or sell only tested propagule. Furthermore, if a bed contains small areas of contamination, it can be addressed by the grower through vine destruction subsequent replanting or overseeding with vegetative propagule of the affected surrounding area. Visual inspection, genetic testing, and responsible propagation can substantially aid in the management and longevity of cranberry beds, and in turn save time and money to growers.

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Data availability The SSR primers and the 65 unique genotypes based on the SSRs are included in Supplementary File 1.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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