WILD/WEED *BETA* POPULATIONS IN THE IMPERIAL VALLEY, CALIFORNIA

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INTRODUCTION

In North America, wild populations of *Beta vulgaris* subsp. *maritima*, *Beta macrocarpa*, and respective hybrids with cultivated beet are found in California. These likely originated from contaminated seed imported from Europe (Biancardi et al., 2012). Section *Beta* includes the wild species *B. macrocarpa*, and *B. v.* ssp. *maritima*, and the cultivated sugar beet, *Beta vulgaris* subsp. *vulgaris* (Frese, 2010). Successful hybridization amongst species of section *Beta* varies. Sugar beet will readily cross-fertilize with *B. v.* ssp. *maritima*, but there is conflicting evidence for successful hybridization between sugar beet and *B. macrocarpa* (de Bock, 1986; Bartsch and Ellstrand, 1999; Jung et al., 1993; Frese, 2010). The relationships among the three species of section *Beta* have been investigated with PCR-based marker and DNA sequencing techniques. Previous research suggests a close relationship between *B. v.* ssp. *vulgaris* and *B. v.* ssp. *maritima* with a more distant position of *B. macrocarpa* in the phylogenetic tree (Letschert, 1993; Shen et al., 1998; Villain, 2007).

When commercial production areas are adjacent to wild beet populations, gene flow from cultivated beets has the potential to alter the genetic composition of the nearby wild populations (Bartsch and Ellstrand, 1999). Carsner reported populations of *B. v.* ssp. *maritima*, *B. macrocarpa*, and respective hybrids with cultivated beet in the Imperial Valley, California in 1938 and they continue to be identified in California. Plant and root characteristics of Imperial Valley wild beets were compared with collections of *B. v.* ssp. *maritima* and *B. macrocarpa* from European coastlines. The wild beets found in the Imperial Valley differ from typical *B. v.* ssp. *maritima* and other wild beets found in California and are most similar to *B. macrocarpa* (McFarlane, 1975). In 2011, plants were collected from wild *Beta* populations adjacent to commercial sugar beet fields and while many samples had clear morphological characteristics of *B. macrocarpa*, several showed *B. v.* ssp. *maritima*-like characteristics. This distinction is critical because *B. v.* ssp. *maritima* will readily cross hybridize with cultivated sugar beet while *B. macrocarpa* hybrids occur less frequently and often result in infertile progeny. Further research is needed to evaluate wild beets in the Imperial Valley to understand the origin of populations, determine the species, and explore whether or not gene flow occurs between these wild beets and cultivated beet. Herbarium samples, leaf tissue and seed of weed beet in and around commercial sugar beet fields were collected with the objectives of assigning taxonomy based on morphology and determining genetic variation by genotyping.

MATERIALS AND METHODS

Locating and sampling wild beet populations

Wild beet populations growing in and adjacent to commercial sugar beet fields in the Imperial Valley, CA were identified and located by the agriculture department of Spreckels Sugar (Brawley, CA) April 4-6, 2011. Collected populations were located in commercial sugar beet fields throughout the agricultural production area in the Imperial Valley and, when possible, were at least 3 km apart. GPS coordinates, elevation, and site description were recorded at each collection location. Irrigation canals and drainage ditches adjacent to sugar beet fields were checked for wild beet populations. The eastern edge of the Sonny Bono Salton Sea National Wild Life refuge and north along the eastern shore of the Salton Sea were checked for wild beet populations. Banks of the Alamo and New rivers from Brawley to the Salton Sea were also checked for wild beet populations.

Thousands of wild beet plants were present at each site and seed stage varied greatly. When ripe seed was present, the most mature seed was collected from 20 to 50 plants per site. An average of 43.2 g of seed was collected per site. Plants were randomly selected from within and along the edges of the sugar beet fields. Images of the wild beet plants and fields and one to three herbarium samples were collected at each site. Whole plants were collected for herbarium samples but because most plants were very large, some secondary stems were trimmed when the samples were pressed. At least 100 mg young leaf tissue was collected from between four to eight plants randomly located throughout each site.

Herbarium sample characterization

Morphology of seed and herbarium samples were compared to several *B. v*. ssp. *maritima* plants of National Plant Germplasm System accessions growing in the greenhouses at the Western Regional Plant Introduction Station (WRPIS), Pullman, WA and identification was made using the Letschert (1993) taxonomic key. Images and notes including general seed ball size, if there were seed balls low on the main stalk, if the upper bracts were large or small were recorded for each herbarium sample. Seven herbarium samples were not kept because of mold: IVCA11-01; -10;-11a,b,c;-13 a,b; and -15. Based on their morphology, the taxonomic identity of some herbarium samples was unclear. If a sample had more characteristics of *B. macrocarpa* but some confounding characteristics it was identified as "*B. macrocarpa*-like" and likewise with the samples with more *B. v*. ssp. *maritima* characteristics. To clarify taxonomic identification, collected seed from each population was planted and one to five seedlings each were grown in the WRPIS greenhouse. The number of plants, average number of nodes (leaf and secondary stems) from the soil line to first seed, average bract length (5 bracts per plant on the upper half of the flower stalk), average glomerule diameter (from the measured bracts), hypocotyl color, and presence or absence of leaf hairs were recorded. In addition, images and herbarium samples were collected for each accession.

Molecular characterization

DNA was extracted from 50-100 mg of frozen leaf tissue harvested from wild beet plants using the Qiagen DNeasy 96 Plant Kit (Qiagen Inc, Valencia, CA). Supplied protocol was modified slightly. A Qiagen/Retsch MM300 mixer Mill was used for tissue disruption and centrifugation steps were run at 3500 x *g*. For DNA elution, incubation time was increased to three minutes prior to centrifugation.

Sixty expressed sequence tag-simple sequence repeat (EST-SSR) markers were used for genotyping, which were developed from the sugar beet GenBank EST database at NCBI (McGrath, 2007 and Imad Eujyl, personal communication). Known *B. v*. ssp. *maritima* and *B. macrocarpa* accessions were included as checks: five individuals from *B. macrocarpa* accessions PI 540559, PI 198405, and PI 546448 (collected in France, Spain, and California (Imperial County), respectively) and two individuals from *B. v*. ssp. *maritima* accessions PI 518354, PI 518345, and W6 44498 (collected in England, England, and Morocco, respectively). PCR was performed with M-13 tailed forward primers labeled with FAM dye (Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture (10 μl) consisted of approximately 20 ng template DNA, 1X PCR buffer (New England BioLabs Inc., Ipswich, MA), 0.2 mM of each dNTP, 0.05 μM of forward labeled primer, 0.2 μM of M-13, 0.25 μM of reverse primer, and 0.05 U of *Taq* DNA polymerase (New England BioLabs Inc., Ipswich, MA). Thermocycler conditions included an initial denaturing period of 5 min at 95^{o}C, followed by 35 cycles of 95^{o}C for 50 s, annealing at 58^{o}C or 60^{o}C for 50 s, extension at 72^{o}C for 90 s, and a final extension period for 10 min at 72^{o}C. PCR products were electrophoresed using ABI 3500 Genetic Analyzer following the manufacturer's protocol (Applied Biosystems, Foster City, CA). Electrophoresed product sizes were analyzed using GeneMapper® v4.1 software (Applied Biosystems, Foster City, CA).

RESULTS

Throughout the Imperial Valley production area, 25 commercial sugar beet fields with wild beet populations were visited and locality data recorded (Figure 1). At the time of our collection, no wild beet populations were identified in irrigation canals or drainage ditches adjacent to sugar beet fields. Nor were wild beet populations identified along the eastern edge of the Sonny Bono Salton Sea National Wild Life refuge or north along the eastern shore of the Salton Sea. Banks of the Alamo and New rivers from Brawley to the Salton Sea were also free of wild beet populations.

None of the wild *Beta* plants taken for herbarium samples had morphological characteristics of *B. v.* ssp. *vulgaris*. While many samples had clear morphological characteristics of *B. macrocarpa* (14 samples), several did not. Many samples were collected from plants growing amongst sugar beet plants, resulting in abnormal stem elongation. If a sample had more characteristics of *B. macrocarpa* but some confounding characteristics it was identified as "*B. macrocarpa*-like" (7 samples) and likewise with the samples with more *B. v.* ssp. *maritima* characteristics (7 samples). Stem elongation due to growing location was factored into the identifications.

The collected seed was incorporated into the National Plant Germplasm System *Beta* collection (IVCA11-01 to IVCA11-27). To clarify taxonomic identification, collected seed from each population was planted and between one and five plants per accession were grown in individual pots in the greenhouse. The average number of nodes from the soil line to first seed ranged from three to nine nodes. Average bract length ranged from 14.9 to 41.3mm. Average glomerule diameter (from the measured bracts) ranged from 5.6 to 9.6mm. All samples had red hypocotyl color and glabrous leaves. All plants grown from the collected seed in the WRPIS greenhouses had clearer *B. macrocarpa* morphology.

Of the 60 EST-SSRs used for genotyping, 20 did not amplify a product and 18 amplified monomorphic products. There was great variability amongst the 22 markers in the number of individuals from which product was amplified. The number of individuals successfully amplifying a product ranged from 12-179 across all markers. The total number of alleles amplified per marker ranged from 2-13. Thirteen markers had alleles that amplified in only one individual. Too few marker data were available for accurate genetic diversity analyses.

DISCUSSION

It is likely that when wild beets were reported in the Imperial Valley in 1928, Carsner incorrectly identified them as *B. v.* ssp. *maritima* descendents. This is plausible as, at the time, wild beets in other California sugar beet areas had been identified as *B. v.* ssp. *maritima* and that original identification was based on morphology of plants at time of collection. In 1975, McFarlane grew Imperial Valley wild beets under greenhouse conditions and, based on morphology, the wild beets were determined to be *B. macrocarpa*. This type of characterization has limitations in species identification as trait expression is strongly influenced by the environment (Laurentin, 2009). Our herbarium and seed sample results agree with this and suggest it is critical to grow out collected seed under non-competitive, greenhouse conditions to clarify taxonomy. In 1999, Bartsch and Ellstrand used allozyme biochemical markers and not morphology to compare genetic diversity of CA wild beets with that of cultivated beet and wild relatives. These markers avoid environmental influence, but are unable to detect low levels of variation. In this study, we used simple sequence repeat (SSR) markers. As DNA-based markers, they are not influenced by the environment, but are also found throughout the genome and able to identify low levels of variation. Our results indicate particularly low levels of polymorphism amongst wild beets in Imperial Valley and that few of the available SSRs developed for sugar beet will successfully transfer to *B. macrocarpa*. McFarlane identified plants with morphology intermediate of sugar beet and *B. macrocarpa* suggesting hybridization. Bartsch and Ellstrand identified otherwise sugar beet-specific allozyme banding patterns in wild populations. To accurately identify the wild beet species in Imperial Valley, determine levels of hybridization, and potentially trace its origin we must develop tools capable of characterizing genetic variation present in populations. New technologies of sequencing and marker genotyping are needed and will enable new approaches towards improvement, such as clarifying relationships within the

species *Beta* and the accurate identification of hybrids between wild and cultivated beet (Morrell et al., 2012).

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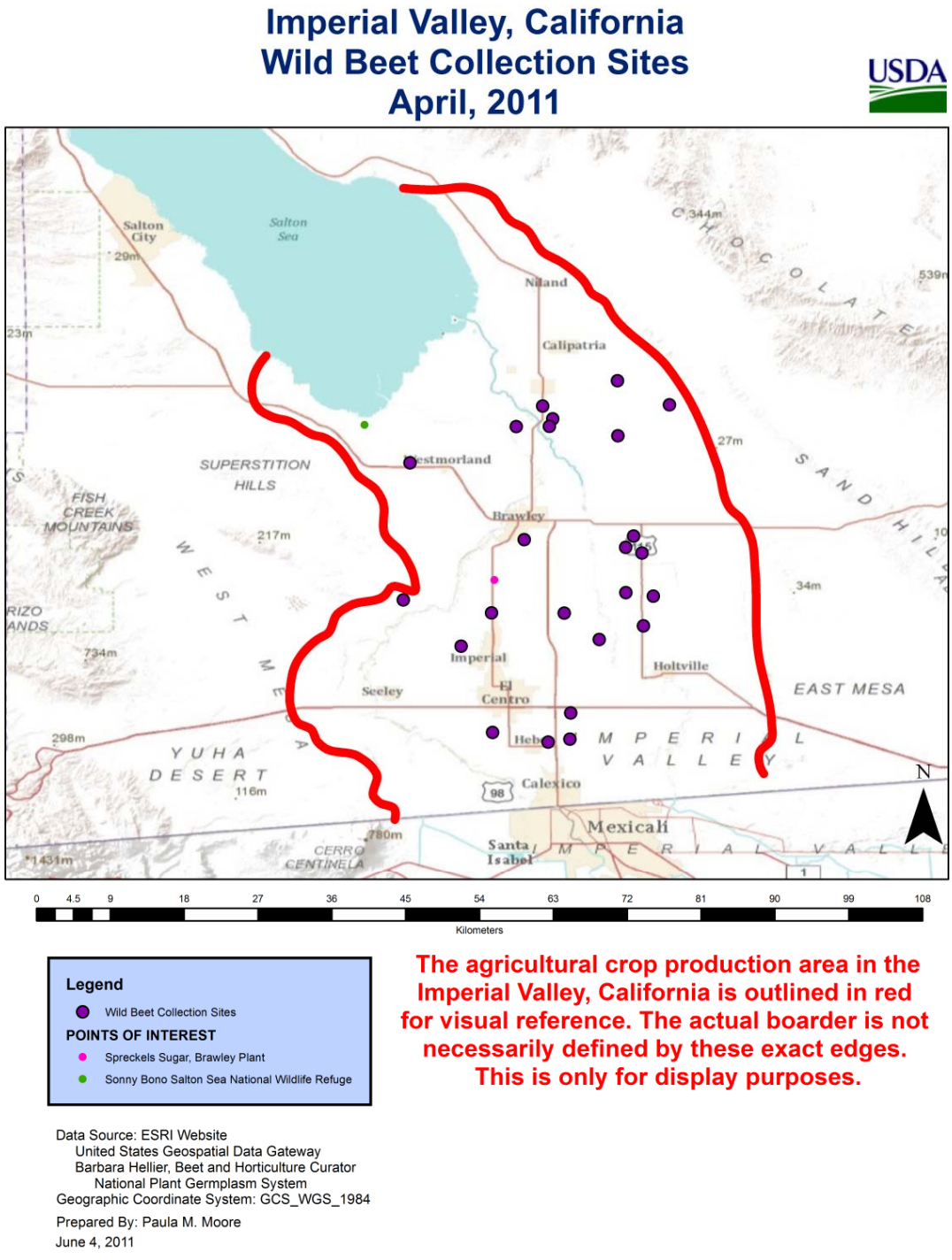

Figure 1. Locations of the 25 commercial sugar beet fields with wild beet populations throughout the Imperial Valley, CA production area visited during the 2011 collecting trip.