M6: A Diploid Potato Inbred Line for Use in Breeding and Genetics Research

S. H. Jansky,* Y. S. Chung, and P. Kittipadukal

ABSTRACT

M6 (Reg. No. GP-1, BS 228) is a diploid self-compatible inbred line of the potato wild relative Solanum chacoense. It is a vigorous, homozygous breeding line derived by self-pollinating the diploid wild potato relative S. chacoense for seven generations. While most wild Solanum species are self-incompatible, this clone is homozygous for the dominant self-incompatibility inhibitor gene Sli. In addition, it is homozygous for 90% of single-nucleotide polymorphism markers in the Infinium Array developed by the SolCAP consortium. M6 is vigorous and both male and female fertile, producing seeds in crosses to diploid cultivated and wild potato germplasm. These traits enable us to systematically develop diploid inbred lines, which was not possible in potato breeding until the discovery of Sli. M6 produces tubers under both short and long photoperiods, unlike other wild potato relatives. In addition, M6 has several desirable traits, including high dry matter content, good chip processing quality, and resistance to soft rot and Verticillium wilt. M6 is being used to develop recombinant inbred line populations.

Advances in potato (Solanum tuberosum L.) breeding and genetics have been slow compared with other crops. Over a century of potato breeding in the United States has not led to yield gains (Douches et al., 1996). Breeding gains in potato are limited by low recombination, a long generation cycle, polyploidy, inbreeding depression, and poor adaptation of wild potato germplasm (Visser et al., 2009; Lindhout et al., 2011). Because of these challenges, new breeding methods to improve progress have not been developed or adopted. The creation of diploid inbred lines in potato offers a strategy to address many limitations faced by potato breeders (Birhman and Hosaka, 2000; Phumichai et al., 2005).

Diploid cultivars are self-compatible, but because they are tetraploid, they must be self-pollinated for at least 20 generations to reach 99% homozygosity. The approach to homozygosity in diploids is much more rapid, but most diploid germplasm in potato is self-incompatible (Cipar et al., 1964; Hawkes, 1958). Consequently, whereas inbred lines have been fundamental tools for breeding and genetic analyses in other major crops, notably maize (Zea mays L.), rice (Oryza sativa L.), wheat (Triticum aestivum L.), soybean (Glycine max (L.) Merr.), and tomato (Solanum lycopersicum L.), they have not been readily available for potato. Self-compatible diploid germplasm provides new opportunities for the exploitation of genetic resources previously unavailable to potato researchers, such as recombinant inbred lines and introgression lines. In the future, researchers may be able to use diploid breeding to rapidly respond to specific market needs by releasing inbred lines or F1 hybrids as cultivars (Lindhout et al., 2011).

Wild relatives of potato offer a rich genetic resource that is readily available to potato breeders (Hawkes, 1945; Hanneman, 1989; Bradshaw and Ramsay, 2005; Visser et al., 2009). The major limitation to the use of these wild Solanum relatives for potato improvement is their poor adaptation to primary production areas in North America, Europe, and Asia. Most wild Solanum species are found in equatorial regions of South America, with a few occurring in China, India, and the Philippines. The wild relatives that have been used by potato breeders include Solanum pseudocapsicum, S. rimosum, S. chacoense, and S. macrocnemum.
and Central America (Hijmans et al., 2002) and produce tubers at photoperiods of 12 h or less. When grown under the long photoperiods of temperate regions, wild *Solanum* species typically do not tuberize (Hermundstad and Peloquin 1985). On the other hand, the cultivated potato (*S. tuberosum*) grown in temperate regions is believed to be derived from Chilean germplasm and produces tubers under moderate to long photoperiods (Rios et al., 2007; Spooner et al., 2007). In dihaploid × wild species hybrids, tuber production under a long photoperiod is a dominant trait (Hermundstad and Peloquin, 1985; Yerk and Peloquin, 1989; Jansky et al., 2004; Jansky, 2010; Kittipadukal et al., 2012). A transcription factor that controls the tuberization response to photoperiod has recently been identified (Kloosterman et al., 2013).

Most diploid wild potato relatives are self-sterile due to a gametophytic self-incompatibility system (Pushkarnath, 1942; Pandey, 1962). In some *S. chacoense* germplasm, however, the self-incompatibility system is inactivated by the dominant allele of the S-locus inhibitor gene *Sli* (Hosaka and Hanneman, 1998). When plants carrying the *Sli* gene are first self-pollinated, they exhibit a high degree of inbreeding depression in the form of poor vigor, flower bud abortion, and sterility (Birthman and Hosaka, 2000). However, vigorous, fertile clones are produced after several generations of self-pollination.

Here we introduce the potato clone M6 (Reg. No. GP-1, BS 228) (previously designated chc 523-3) created by seven generations of self-pollination of the wild diploid potato species *Solanum chacoense*. The clone was developed by Robert Hanneman, Jr. (deceased; USDA–ARS VCRU, Madison, WI). Subsequent evaluations were performed by the authors of this paper. M6 is vigorous and fertile. Morphologically, it is indistinguishable from *S. chacoense* plants that have not been inbred. In addition, it exhibits several desirable agronomic traits, including high dry matter content, good chip processing quality, photoperiod adaptation, and resistance to soft rot, caused by *Pectobacterium carotovorum*, and Verticillium wilt, caused by *Verticillium dahliae*. *Solanum chacoense* is a vigorous, widely adapted, highly fertile species that is easily crossed with cultivated potato (Den Nijs and Peloquin, 1977). In addition, because *S. chacoense* is divergent from cultivated potato, it provides a source of new genetic diversity and traits not found in existing cultivars.

**Methods**

To evaluate female fertility, flowers were emasculated before anthers dehisced and bulked pollen from diploid clones was applied to M6 stigmas. Fruit was retained on plants for 3 wk after pollination, then removed and stored at room temperature for 3 wk. Seed was then extracted and plump seeds counted. Male fertility was measured in a similar way, except that pollen was applied to M6 stigmas by a transverse cut, rinsing it in tap water, and frying it in 190°C. Male fertility was measured by cross-pollination with 2n pollen grains from diploid clones.

To characterize the photoperiod required for tuberization, M6 was evaluated along with two diploid cultivated potato clones (US-730 and US-W973) and another diploid wild species (*S. microdontum*) clone in a growth chamber (Kittipadukal, 2010). They were evaluated for tuber production under five photoperiods (18, 16, 14, 12, and 10 h) in a completely randomized design using a stem-cutting technique (Ewing, 1978).

To determine homozygosity, DNA was extracted from young leaf tissue of greenhouse-grown plants using the Qiagen DNeasy Plant Mini Kit (Qiagen), quantified using the Quant-iT dsDNA Assay Kit (Invitrogen), and adjusted to a concentration of 50 ng µL⁻¹. Single-nucleotide polymorphism (SNP) data were generated using an Illumina iScan Reader (Illumina) and the Infinium 8303 Potato Array according to the manufacturer’s suggested protocol. Data were analyzed using Illumina Genome Studio software (Illumina).

Tuber dry matter content was determined using specific gravity of freshly harvested tubers from 2012 and 2013 greenhouse harvests. It was measured using the formula [weight in air/weight in air – weight in water].

Three-hill plots of M6, along with a clone of the wild species *S. microdontum* (PI 500041) and two *S. tuberosum* dihaploids, US-W730 and US-973, were planted in the field at the University of Wisconsin Leilah Starks Potato Research Farm at Rhinelander, WI, on 12 May 2008. The dihaploids are diploid cultivated potato clones derived by parthenogenesis from the tetraploid breeding line WisAg231 (Peloquin et al., 1960). Within-plot spacing was 30 cm; between-plot spacing was 90 cm. Standard cultural practices were used. The trial was planted in a completely randomized design, with two replications. Clones were scored for the initiation of flowering weekly from 30 June to 18 Aug. 2008. A clone was considered to be flowering if at least one open flower was observed in a plot. The trial was repeated at the same location in 2009. Plots were harvested on 24 Sept. 2008. At harvest, tuber size and stolon length data were collected. Tuber size ratings focused on the largest tuber in a plot and were recorded as follows: 0 = no tuberization, 1 = swelling at the end of the stolon, 2 = 1- to 5-cm tubers, 3 = 5- to 8-cm tubers, 4 = >8-cm tubers. Stolon length ratings were 1 = stolons <60 cm, 2 = stolons 60 to 90 cm long, and 3 = stolons >90 cm long.

The trial was repeated at the same location in 2009. Plots were planted on 6 May and harvested on 9 September. Plant spacing was 30 cm between plants (within a plot) and 120 cm between plots. Plots were scored weekly for the initiation of flowering from 24 June until 9 September.

Chip color was determined from tubers from greenhouse-grown plants stored at 4°C and 6°C for 2 to 5 mo. Each tuber was evaluated by taking a 2-mm-thick slice from the center of a transverse cut, rinsing it in tap water, and drying it in 190°C vegetable oil until bubbling ceased. Chip color was then evaluated visually using a scale of 1 (light) to 9 (dark).

Tubers generated from two greenhouses on the University of Wisconsin–Madison campus (Walnut Street and Dairy Forage) were planted back onto M6 stigmas to test for self-compatibility. To determine whether M6 is homozygous or heterozygous for *Sli*, seed from self-pollination of M6 was sown and self-pollinations were attempted on 126 plants.
and from the field at the Hancock, WI, Agricultural Experiment Station were evaluated for soft rot resistance 1 wk after they were harvested. Before inoculation, tubers were washed with distilled water and dried overnight. Pectobacterium carotovorum isolate WPP14 was cultured, and 10 μL of prepared bacterial suspension (1.0 × 10^6 colony forming units [cfu] mL^-1) was inoculated into each tuber in a hole with a depth of 5 mm created by a pipette tip (Yap et al., 2004). This method measures resistance after wound damage under storage conditions (Koppel, 1993). Then, after a 72-h incubation in the dark at 100% relative humidity at room temperature, tubers were sliced at the wounded site and the diameter of decay was measured. Increased susceptibility is indicated by larger lesion diameter.

Two replications of five-hill plots of M6 were planted in a Verticillum dahliae nursery at the Hancock, WI, Agricultural Research Station in 2012. At the end of the growing season, basal stem segments were collected, dried, ground, and plated on selective medium. After a 14-d incubation period, the number of colonies per plate was counted as a measure of stem colonization by the pathogen. M6 was also tested using polymerase chain reaction–based markers for the Ve gene ortholog that confers resistance to Verticillum wilt, described in Uribe et al. (2014).

Because high tuber glycoalkaloid content is an undesirable trait in wild potato species, levels of the two most common types of glycoalkaloids were measured. Two tuber samples from the 2009 Rhinelander field plot were peeled and lyophilized after harvest. Alpha-solanine and β-solanine were quantified by reverse-phase liquid chromatography. Approximately 2 g of lyophilized, dried sample was added to an acidified ion pairing solution (0.02 M 1-heptanesulfonic acid sodium salt, monohydrate in 2% [v/v] acetic acid) and extracted for 3 min with a Polytron tissue homogenizer. The resulting extract was centrifuged to pellet tissue debris, and a 10-mL aliquot of the supernatant was passed through a methanol-activated SPE cartridge (Waters Corp, catalog no. WAT036810). The cartridge was eluted with an acetonitrile-water wash (20:80, v/v) as a mobile phase. After vacuum drying, the sample was eluted from the cartridge with a tetrahydrofuran-water-acetonitrile (50:30:20, v/v) solution, and 20 μL was injected into the liquid chromatography system, equipped with a diode array detector for analysis. Separation was accomplished on a C-6 analytical column, with a buffered (pH 3.5) mobile phase.

### Characteristics

M6 has been used as a male and female parent in crosses to diploid clones. It flowers profusely and sheds abundant pollen. When used as a male parent in crosses to 22 diploid clones, 213 pollinations produced 7981 seeds (37 seeds per pollination). When used as a female parent in crosses to three diploid clones, 24 pollinations produced 480 seeds (20 seeds per pollination). The production of 2n pollen grains is very rare in M6. However, it produces approximately one seed per pollination when crossed as a female to tetraploid clones. The production of hybrid seeds from crosses to tetraploids indicates that M6 produces some 2n eggs (Erazzú & Camadro, 2006).

M6 is highly self-compatible. Large numbers of spontaneous fruits from self-pollination are typically observed on greenhouse-grown plants. M6 was self-pollinated to produce 126 offspring. When those 126 plants were then self-pollinated, one was male sterile and the remaining 125 produced seeds. In addition, M6 has been crossed to many diploid clones in our program, and all attempts to self-pollinate fertile offspring from those crosses have been successful. Consequently, it appears that M6 is homozygous for Sli. This is a significant finding because previously it was believed that Sli could be present only in heterozygous form due to linkage to an unknown deleterious gene (Hosaka and Hanneman, 1998).

An Illumina SNP array has been created by the SolCAP consortium (Felcher et al., 2012). The array detected 7845 high quality SNPs, of which 7060 (90.0%) were homozygous in M6. If all SNPs were heterozygous in the clone that was originally self-pollinated, then only 0.8% of SNPs would be expected to be homozygous after self-pollination. Consequently, there is a high level of residual heterozygosity in M6. Single-nucleotide polymorphisms in the Infinium array were generated based on transcriptome sequencing of six potato cultivars (Felcher et al., 2012). Markers were selected for maximum genome coverage and include SNPs in 3018 candidate genes. It is possible that since wild species were not included during SNP discovery, some ascertainment bias may exist. However, introgressions of wild species germplasm into cultivated potato is common, and among-species ascertainment bias is likely to be minimal (Hirsch et al., 2013).

In the field, M6 was later to initiate flowering than a clone of another potato wild relative, *S. microdontum* (clone 57-10, PI 500041) and two diploid *S. tuberosum* clones (US-W730 and US-W973) (Table 1). It continued flowering throughout the summer and senesced later than the *S. tuberosum* clones and at the same time as the *S. microdontum* clone. It produces long stolons, approximately 1 m in length. In comparison, stolons on the *S. tuberosum* clones are less than 1 m, whereas those of *S. microdontum* are nearly 1 m.

Table 2 shows percentage tuberization of M6, *S. microdontum* 57-10, US-W730, and US-W973. As was expected for wild *Solanum* species, *S. microdontum* did not tuberize until the photoperiod was shortened to 12 h. M6, however, produced tubers at a 14-h photoperiod, as did the cultivated potato clones. The 14-h photoperiod is sufficient for tuber production in the field, and we have produced tubers of M6 in the field in central Wisconsin. In contrast, in field trials, we routinely observe that other wild *Solanum* species do not produce tubers.

### Table 1. Field performance of M6, a clone of the wild species Solanum microdontum, and two diploid cultivated potato clones (US-W730 and US-W973) combined across 2 yr (2008 and 2009).

<table>
<thead>
<tr>
<th>Clone</th>
<th>Flowert</th>
<th>Maturity‡</th>
<th>Stolon length§</th>
<th>Tuber size¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>wk</td>
<td>wk</td>
<td>1–3</td>
<td>0–4</td>
<td></td>
</tr>
<tr>
<td>M6 (S. chacoense)</td>
<td>12.7 a</td>
<td>18.6 a</td>
<td>2.6 a</td>
<td>1.7 b</td>
</tr>
<tr>
<td>57-10 (S. microdontum)</td>
<td>11.3 b</td>
<td>18.5 a</td>
<td>2.5 b</td>
<td>0.0 c</td>
</tr>
<tr>
<td>US-W730 (S. tuberosum)</td>
<td>10.5 b</td>
<td>14.8 b</td>
<td>1.0 c</td>
<td>2.0 b</td>
</tr>
<tr>
<td>US-W973 (S. tuberosum)</td>
<td>9.1 c</td>
<td>13.2 c</td>
<td>1.0 c</td>
<td>3.1 a</td>
</tr>
</tbody>
</table>

† Weeks to first flower.

‡ Weeks to senescence.

§ 1 = short, 3 = long.

¶ 0 = no tubers, 4 = large tubers.

Within a column, numbers followed by different letters are significant at the 0.05 probability level.
Average tuber size of M6 is small, which is typical for wild potato species. When eight greenhouse-grown plants were harvested in November 2011, average tuber number and weight were 11 and 18.5 g, respectively. In the 2009 field trial, each plant produced an average of four tubers weighing 1.8 g each.

Two important processing traits are specific gravity and fry color after cold storage (Dale and Bradshaw, 2003). Specific gravity of M6 is very high; the specific gravity of greenhouse-grown tubers was 1.098 to 1.100. The processing industry expects tubers to be in the 1.080 to 1.090 range. Consequently, M6 will be an especially valuable parent for increasing the specific gravity of breeding lines. When M6 tubers were stored at cold temperatures (4–6°C) for 2 to 6 mo, chip color scores were 6 to 7, on a scale of 1 (very light) to 9 (very dark). Scores of 5 or below are considered acceptable for chip processors. While not tested, they would likely be acceptable when stored at the more moderate temperatures currently used by the chip processing industry (9–11°C).

M6 tubers contained 28.3 mg glycoalkaloids 100 g⁻¹ fresh wt.. This is above the acceptable level of 20 mg 100 g⁻¹ fresh wt. (Sinden and Webb, 1974). However, progeny from crosses to low glycoalkaloid breeding lines are likely to have acceptable levels for human consumption (Sanford et al., 1996). It will be important to monitor glycoalkaloid levels in breeding lines derived from M6.

**Disease Response**

Lesion diameter following inoculation with *Pectobacterium carotovorum* revealed less soft rot disease development in M6 than in the susceptible cultivar Atlantic. Mean lesion diameter for the soft rot resistance experiments at the Walnut Street Greenhouse, Dairy Forage greenhouse, and Hancock Agricultural Research Station was 4.3, 4.7, and 4.0 mm, respectively, for M6, and 11.0, 9.5, and 9.8 mm, respectively, for Atlantic.

### Table 2. Effect of photoperiod on percentage tuberization of M6, a clone of *Solanum microdontum*, and two diploid *S. tuberosum* clones.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Photoperiod</th>
<th>Tuberization</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. chacoense</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>10</td>
<td>100.0 a†</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>94.4 a</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>100.0 a</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>11.1 b</td>
</tr>
<tr>
<td><em>S. microdontum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57-10</td>
<td>10</td>
<td>100.0 a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>61.1 bc</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.0 d</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.0 d</td>
</tr>
<tr>
<td><em>S. tuberosum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US-W730</td>
<td>10</td>
<td>100.0 a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>88.9 ab</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>100.0 a</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>33.3 bc</td>
</tr>
<tr>
<td>US-W973</td>
<td>10</td>
<td>100.0 a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100.0 a</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>29.4 b</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>14.3 b</td>
</tr>
</tbody>
</table>

† Numbers followed by different letters are significant at the 0.05 probability level.

M6 expresses resistance to Verticillium wilt. In 2012, only 1 cfu was cultured from a 100-mg stem sample in one replication, and none were found in the second replication. For comparison, an average of 81 cfu 100 mg⁻¹ stem tissue was found in the stems of the resistant cultivar Ranger Russet. Resistance is likely due to an ortholog of the tomato Ve gene, as M6 is homozygous for a pair of cleaved amplified polymorphic sequence markers based on that gene (Uribe et al., 2014).

**Breeding and Genetics Applications**

M6 has been crossed as a female to several diploid potato clones, including interspecific hybrids with resistance to early blight (caused by *Alternaria solani*), common scab (caused by *Streptomyces scabies*), late blight (caused by *Phytophthora infestans*), and cold-induced sweetening. It has also been crossed with a *S. bukasovii* clone that produces high tuber starch amylose, with a diploid *S. tuberosum* clone (US-W4) that produces hybrid offspring with high tuber yield and large tuber size, and with DM1-3, the doubled monoploid clone that provided the basis for the first published potato genome sequence (Xu et al., 2011). Hybrids have been self-pollinated to generate F₂ populations. The DM1-3 by M6 F₂ is likely one of the first true F₂ populations developed in potato. It exhibits tremendous segregation for plant morphology and tuber traits. All F₂ populations are being self-pollinated to homozygosity to create recombinant inbred line populations, which will form the basis of a nested association mapping population (NAM). After its development, the NAM will be released as a breeding and genetics resource for the potato community.

M6 and its derivatives are also likely to be useful for the generation of introgression lines. When wild relatives are used as sources of novel traits in breeding programs, genes for poor adaptation may preclude the identification of yield-enhancing genes. For example, a weedy relative of cultivated rice, *Oryza rufipogon*, is inferior to modern rice cultivars because of poor adaptation. However, an accession of this species was found to carry chromosomal segments that enhanced yield (Xiao et al., 1996). More recent research in rice used inbred lines developed from *O. sativa* ssp. *indica* and *japonica* to identify yield-enhancing genes (Marathi et al., 2012). Similarly, in tomato inbred lines carrying chromosomal regions from the wild relative *Solanum pennellii*, a quantitative trait locus responsible for high sugar yield was identified within an invertase gene (Fridman et al., 2004). Potato offers a parallel situation, with a small fraction of its accessible genetic diversity currently found in cultivars. The dissection of genetic variation in diploid hybrids between wild and cultivated potato will also likely reveal alleles that contribute to enhanced yield.

Inbred lines will also provide valuable tools for functional genomics studies in potato. The *Tnt1* retrotransposon has been used for insertional mutagenesis in M6 (Duangpan et al., 2013). Self-pollination of plants carrying insertions revealed distinct morphological variants. This system may ultimately be used to tags all genes in the potato genome.

**Availability**

M6 is available from the NRSP-6 Potato Genebank. Recognition of this clone is requested when it is used in
published breeding and genetics studies or in the development of new germplasm.

References


Chapter 4: University of Wisconsin–Madison.