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Dissertação

***Solanum malmeanum*, a promising wild relative for potato breeding**

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Pelotas, 2021

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Solanum malmeanum, a promising wild relative for potato breeding

Dissertação apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Ciências (área do conhecimento: Fitomelhoramento).

Orientador: Dr. Gustavo Heiden

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Solanum malmeanum Bitter

Igami 2020

Resumo

NICOLAO, Rodrigo. ***Solanum malmeanum*****, um parente silvestre promissor para o melhoramento da batata**. 2021. 123f. Dissertação (Mestrado em Agronomia/Fitomelhoramento) – Programa de Pós-Graduação em Agronomia, Universidade Federal de Pelotas, Pelotas, 2021.

Solanum malmeanum (*Solanum* sect. *Petota*, Solanaceae) é um parente silvestre da batata (*S. tuberosum*), nativo do Cone Sul da América do Sul, com ocorrência na Argentina, Brasil, Paraguai e Uruguai. *S. malmeanum* foi historicamente confundida ou tradicionalmente considerada coespecífica com *S. commersonii*, tornando-a negligenciada quanto a estudos aplicados de uso no melhoramento. Com o objetivo de elucidar o potencial da espécie e fornecer bases para promover o use deste recurso genético no melhoramento, a dissertação está organizada em dois capítulos. O Capítulo 1 apresenta uma revisão de literatura, de espécimes de herbários e de bancos de germoplasma. As informações disponíveis foram atualizadas, de modo a facilitar o reconhecimento e dar apoio para a utilização desse parente silvestre nos programas de melhoramento. São apresentados dados sobre taxonomia, distribuição geográfica, ecologia, biologia reprodutiva, e informações de resistência aos estresses bióticos e abióticos. Além disso, são discutimos métodos para superar as barreiras reprodutivas e de hibridação, visando o uso aplicado nos programas de melhoramento de batata. O Capítulo 2 apresenta estudos de biologia reprodutiva e hibridações intra e interespecíficas controladas com base em acessos do Banco Ativo de Germoplasma de Batata da Embrapa Clima Temperado. O estudo demonstrou que cinco acessos diploides de *S. malmeanum* (BGB015, BGB017, BGB446, BGB447, BGB471) e três acessos triploides (BGB021, BGB080, BGB448) produzem pólen fértil, enquanto dois acessos triploides (BGB081, BGB084) não produzem pólen. Dois acessos triploides (BGB021, BGB448) produzem frutos partenocárpicos induzidos ou de forma espontânea. Autopolinização e apomixia estão ausentes em todos os acessos avaliados. Cinco acessos diploides (BGB015, BGB017, B446, BGB447 e BGB471) e dois acessos triploides (BGB080 e BGB448) são alógamos e férteis quando genitores femininos em cruzamentos com *bulk* de pólen, enquanto três acessos triploides (BGB021, BGB081, BGB084) não produzem sementes nessas condições e reproduzem-se apenas vegetativamente. Destaca-se o reconhecimento de um acesso auto-compatível (BGB447) com potencial para o desenvolvimento de linhagens endogâmicas visando o melhoramento diploide. Os acessos diploides de *S. malmeanum* hibridizaram com o acesso BGB001 2x (1EBN) *S. commersonii*, produzindo sementes normais e viáveis. Os acessos BGB017, BGB021, BGB080, BGB446, BGB447 e BGB448 também hibridizaram com o acesso BGB444 2x (2EBN) *S. chacoense*, porém a produção de sementes e viabilidade foram limitados. O cruzamento direto entre *S. malmeanum* (1EBN) e 4x (4EBN) *S. tuberosum* (cultivar BRSANA e linhagem dp progrma de melhoramento C1750-15-95) é inviável e confirmou a incompatibilidade reprodutiva esperada. Contudo, o sucesso parcial de *S. malmeanum* quando cruzado com *S. chacoense* sugere que existem mecanismos que podem superar a barreira do EBN e que possibilitam a introgressão de genes de interesse via cruzamentos indiretos mediados por espécies-ponte.

Palavras-chave: batata-silvestre · melhoramento de batata · melhoramento vegetal · parente silvestres · recursos genéticos

Abstract

NICOLAO, Rodrigo. ***Solanum malmeanum*, a promising wild relative for potato breeding**. 2021. 123f. Dissertação (Mestrado em Agronomia/Fitomelhoramento) – Programa de Pós-Graduação em Agronomia, Universidade Federal de Pelotas, Pelotas, 2021.

Solanum malmeanum (*Solanum* sect. *Petota*, Solanaceae) is a wild relative of the cultivated potato (*S. tuberosum*), native from the Southern Cone of South America and distributed in Argentina, Brazil, Paraguay and Uruguay. *S. malmeanum* was historically mistakenly identified or traditionally considered as conspecific with S. *commersonii*, becoming a neglected genetic resource regarding its applied uses in breeding. To elucidade the potential of this species towards the use in potato breeding, this dissertation is organized into two chapters. Chapter 1 presents a review of literature, specimens from herbaria and gene banks. The available information was updated to allow the recognition and aid the applied use of this wild potato in breeding. Data on taxonomy, geographic distribution, ecology, reproductive biology, and information about resistance to address abiotic and biotic stresses are provided. Moreover, methods to overcome barriers of reproduction and hybridization are discussed, for the efficient application in potato breeding program. Chapter 2 present studies on reproductive biology and cross-ability relationship based on intra- and interspecific crosses under controlled conditions using plant accessions of Embrapa Clima Temperado Potato Gene Bank. The essays demonstrated that five diploid accessions of *S. malmeanum* (BGB015, BGB017, BGB446, BGB447, BGB471) and three triploids (BGB021, BGB080, BGB448) produce fertile pollen, while two triploid accessions (BGB084, BGB448) do not produce pollen. Two triploid accessions (BGB021, BGB448) produce spontaneous and induced parthenocarpic fruits. Self-pollination and apomixis are absent in all evaluated accessions. Five diploid accessions (BGB015, BGB017, B446, BGB447, BGB471) and two triploid ones (BGB080 and BGB448) are allogamous and fertile as female genitor when bulk pollinated, while three triploid accessions (BGB021, BGB081, BGB084) do not produce seeds from same conditions and reproduce only vegetatively. The reconize of a self-compatible accession (BGB447) with potential for the development of diploid inbred lines is highlighted. The diploid accessions of *S. malmeanum* easily hibridize with BGB1 2x (1EBN) *S. commersonii*, producing normal and viable seeds. The accessions BGB017, BGB021, BGB080, BGB446, BGB447 and BGB448 also hybridize with BGB444 2x (2EBN) *S. chacoense*, but the number of seeds and germination were limited. Direct crosses between *S. malmeanum* (1EBN) and 4 (4EBN) *S. tuberosum* (cultivar BRSANA and breeding line C1750-15-95) fails and confirms the expected reproductive incompatibility. However, the partial success of *S. malmeanum* when crossed with *S. chacoense* suggests the presence of mechanisms that could help to overcomes barriers of hybridization residing on EBN and facilitates the introgression of genes via bridge-crossings.

Keywords ·crop wild relatives· genetic resources · pre-breeding wild potatoes

List of Figures

General Introduction

Chapter I – *Solanum malmeanum*, a neglected wild relative for potato breeding

Chapter II How to unlock the reproductive isolation of *Solanum malmeanum*, a promising wild relative to broaden the potato crop genetic basis?

List of tables

Chapter I – *Solanum malmeanum*, a neglected wild relative for potato breeding

Chapter II How to unlock the reproductive isolation of *Solanum malmeanum*, a promising wild relative to broaden the potato crop genetic basis?

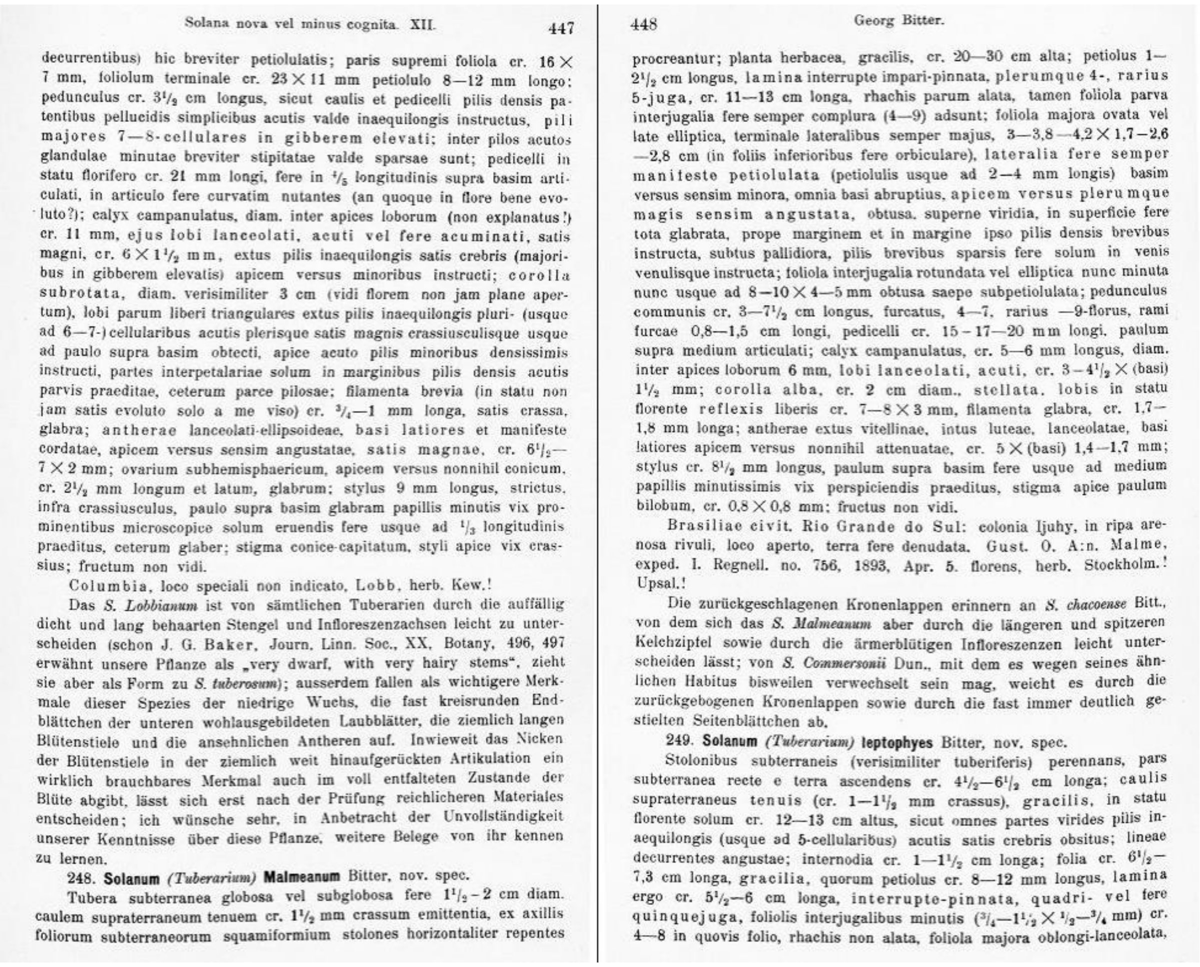
Summary

General introduction

Solanum malmeanum Bitter (1913) is a wild relative of the potato crop *S. tuberosum* Linnaeus (1753), belonging to the Solanaceae family. The recorded history of this species starts in 1893, when the Swedish botanist Oskar Andersson Gustaf Malme (1864-1937) was at Ijuí, Rio Grande do Sul state, Brazil. Malme was one of the two first grantees of the Regnellian Fund for plant collection expeditions in Brazil and neighboring countries (SANTOS, 2016). Meanwhile exploring the vegetation of Southern Brazil, he found and collected some wild potatoes growing on a sandy bank of a stream on open grassland (BRÜCHER 1999). These specimens were herborized and sent to the Herbarium S of the Natural History Museum of Stockholm, Sweden (Figure 1). Only twenty years later, Bitter (1913) had the opportunity to study these samples and based on them published the discovery of a new wild potato species named in honor of Malme, who collected the studied samples on which the original description (Figure 2) of *S. malmeanum* was based.

After the discovery of *S. malmeanum* and the description as a specific taxon by Bitter (1913), different taxonomical approaches have been applied to it. Correll (1962) considered *S. malmeanum* as an infraspecific form of *S. commersonii*, because he believed that it could be originated from a hybridization between *S. chacoense* Bitter (1912) and *S. commersonii* Dunal in Poiret (1814), treating it as *S. commersonii* form *malmeanum* (Bitter 1913) Correll (1962). Later, Hawkes and Hjerting (1969) considered *S. malmeanum* differently and classified it as *S*. *commersonii* subspecies *malmeanum* (Bitter 1913) Hawkes & Hjerting (1969), attesting that it does not hybridize with *S. chacoense* and arguing that it presents a wide morphological variability along its geographical distribution. Mentz and Oliveira (2004) studied some plants of *S. malmeanum* from Brazil, however, they did not provide a conclusive opinion and considered *S. malmeanum* as a form of *S. commersonii* the same way as Correll (1962). Recently, Spooner et al. (2016) provided the most comprehensive taxonomic treatment of potatoes (*Solanum* sect. *Petota* Dumortier 1828) and considered *S. malmeanum* as a specific taxon as the original approach by Bitter (1913). They differentiate *S. malmeanum* from *S. commersonii* based on morphological traits, mainly by the rosette to semi-rosette habit and white corolla color found in the former, when compared to the habit commonly prostate and corolla color purple, lilac or bluish-white typical of the later.

Figure 1 *Solanum malmeanum* (Solanaceae): specimens collected in 1893 (*Malme 756*), based on which Bitter described this new potato wild relative species for science. Image courtesy of the Herbarium S Catalogue of the Swedish Museum of Natural History, Stockholm, Sweden.



decurrentibus) hic breviter petiolulatis; paris supremi foliola cr. 16 × 7 mm, foliolum terminale cr. 23 × 11 mm petiolulo 8—12 mm longo; pedunculus cr. $3^1/_2$ cm longus, sicut caulis et pedicelli pilis densis patentibus pellucidis simplicibus acutis valde inaequilongis instructus, pili majores 7—8-cellulares in gibberem elevati; inter pilos acutos glandulae minutae breviter stipitatae valde sparsae sunt; pedicelli in statu florifero cr. 21 mm longi, fere in $^4/_5$ longitudinis supra basim articulati, in articulo fere curvatim nutantes (an quoque in flore bene evoluto?); calyx campanulatus, diam. inter apices loborum (non explanatus!) cr. 11 mm, ejus lobi lanceolati, acuti vel fere acuminati, satis magni, cr. 6 × $1^1/_2$ mm, extus pilis inaequilongis satis crebris (majoribus in gibberem elevatis) apicem versus minoribus instructi; corolla subrotata, diam. verisimiliter 3 cm (vidi florem non jam plane apertum), lobi parum liberi triangulares extus pilis inaequilongis pluri- (usque ad 6—7-) cellularibus acutis plerisque satis magnis crassiusculisque usque ad paulo supra basim obtecti, apice acuto pilis minoribus densissimis instructi, partes interpetalariae solum in marginibus pilis densis acutis parvis praeditae, ceterum parce pilosae; filamenta brevia (in statu non jam satis evoluto solo a me viso) cr. $^3/_4$—1 mm longa, satis crassa, glabra; antherae lanceolati-ellipsoideae, basi latiores et manifeste cordatae, apicem versus sensim angustatae, satis magnae, cr. $6^1/_2$—7 × 2 mm; ovarium subhemisphaericum, apicem versus nonnihil conicum, cr. $2^1/_2$ mm longum et latum, glabrum; stylus 9 mm longus, strictus, infra crassiusculus, paulo supra basim glabram papillis minutis vix prominentibus microscopice solum eruendis fere usque ad $^1/_3$ longitudinis praeditus, ceterum glaber; stigma conice-capitatum, styli apice vix crassius; fructum non vidi.

Columbia, loco speciali non indicato, Lobb, herb. Kew.!

Das *S. Lobbianum* ist von sämtlichen Tuberarien durch die auffällig dicht und lang behaarten Stengel und Infloreszenzachsen leicht zu unterscheiden (schon J. G. Baker, Journ. Linn. Soc., XX, Botany, 496, 497 erwähnt unsere Pflanze als „very dwarf, with very hairy stems", zieht sie aber als Form zu *S. tuberosum*); ausserdem fallen als wichtigere Merkmale dieser Spezies der niedrige Wuchs, die fast kreisrunden Endblättchen der unteren wohlausgebildeten Laubblätter, die ziemlich langen Blütenstiele und die ansehnlichen Antheren auf. Inwieweit das Nicken der Blütenstiele in der ziemlich weit hinaufgerückten Artikulation ein wirklich brauchbares Merkmal auch im voll entfalteten Zustande der Blüte abgibt, lässt sich erst nach der Prüfung reichlicheren Materiales entscheiden; ich wünsche sehr, in Anbetracht der Unvollständigkeit unserer Kenntnisse über diese Pflanze, weitere Belege von ihr kennen zu lernen.

248. **Solanum** *(Tuberarium)* **Malmeanum** Bitter, nov. spec.

Tubera subterranea globosa vel subglobosa fere $1^1/_2$ – 2 cm diam. caulem supraterraneum tenuem cr. $1^1/_2$ mm crassum emittentia, ex axillis foliorum subterraneorum squamiformium stolones horizontaliter repentes

procreantur; planta herbacea, gracilis, cr. 20—30 cm alta; petiolus 1—$2^1/_2$ cm longus, lamina interrupte impari-pinnata, plerumque 4-, rarius 5-juga, cr. 11—13 cm longa, rhachis parum alata, tamen foliola parva interjugalia fere semper complura (4—9) adsunt; foliola majora ovata vel late elliptica, terminale lateralibus semper majus, 3—3,8—4,2 × 1,7—2,6—2,8 cm (in foliis inferioribus fere orbiculare), lateralia fere semper manifeste petiolulata (petiolulis usque ad 2—4 mm longis) basim versus sensim minora, omnia basi abruptius, apicem versus plerumque magis sensim angustata, obtusa, superne viridia, in superficie fere tota glabrata, prope marginem et in margine ipso pilis densis brevibus instructa, subtus pallidiora, pilis brevibus sparsis fere solum in venis venulisque instructa; foliola interjugalia rotundata vel elliptica nunc minuta nunc usque ad 8—10 × 4—5 mm obtusa saepe subpetiolulata; pedunculus communis cr. 3—$7^1/_2$ cm longus, furcatus, 4—7, rarius —9-florus, rami furcae 0,8—1,5 cm longi, pedicelli cr. 15 – 17—20 mm longi, paulum supra medium articulati; calyx campanulatus, cr. 5—6 mm longus, diam. inter apices loborum 6 mm, lobi lanceolati, acuti, cr. 3 – $4^1/_2$ × (basi) $1^1/_2$ mm; corolla alba, cr. 2 cm diam., stellata, lobis in statu florente reflexis liberis cr. 7—8 × 3 mm, filamenta glabra, cr. 1,7—1,8 mm longa; antherae extus vitellinae, intus luteae, lanceolatae, basi latiores apicem versus nonnihil attenuatae, cr. 5 × (basi) 1,4—1,7 mm; stylus cr. $8^1/_2$ mm longus, paulum supra basim fere usque ad medium papillis minutissimis vix perspiciendis praeditus, stigma apice paulum bilobum, cr. 0,8 × 0,8 mm; fructus non vidi.

Brasiliae civit. Rio Grande do Sul: colonia Ijuhy, in ripa arenosa rivuli, loco aperto, terra fere denudata. Gust. O. A:n. Malme, exped. I. Regnell. no. 756, 1893, Apr. 5. florens, herb. Stockholm.! Upsal.!

Die zurückgeschlagenen Kronenlappen erinnern an *S. chacoense* Bitt., von dem sich das *S. Malmeanum* aber durch die längeren und spitzeren Kelchzipfel sowie durch die ärmerblütigen Infloreszenzen leicht unterscheiden lässt; von *S. Commersonii* Dun., mit dem es wegen seines ähnlichen Habitus bisweilen verwechselt sein mag, weicht es durch die zurückgebogenen Kronenlappen sowie durch die fast immer deutlich gestielten Seitenblättchen ab.

249. **Solanum** *(Tuberarium)* **leptophyes** Bitter, nov. spec.

Stolonibus subterraneis (verisimiliter tuberiferis) perennans, pars subterranea recte e terra ascendens cr. $4^1/_2$—$6^1/_2$ cm longa; caulis supraterraneus tenuis (cr. 1—$1^1/_2$ mm crassus), gracilis, in statu florente solum cr. 12—13 cm altus, sicut omnes partes virides pilis inaequilongis (usque ad 5-cellularibus) acutis satis crebris obsitus; lineae decurrentes angustae; internodia cr. 1—$1^1/_2$ cm longa; folia cr. $6^1/_2$—7,3 cm longa, gracilia, quorum petiolus cr. 8—12 mm longus, lamina ergo cr. $5^1/_2$—6 cm longa, interrupte-pinnata, quadri- vel fere quinquejuga, foliolis interjugalibus minutis ($^3/_4$—$1^1/_2$ × $^1/_2$—$^3/_4$ mm) cr. 4—8 in quovis folio, rhachis non alata, foliola majora oblongi-lanceolata,

Figure 2 Original description of *Solanum malmeanum* Bitter (Solanaceae) as a new species in 1913: the birth certificate of this Southern South American wild potato to science.

As currently recognized, *S. malmeanum* (Figure 3) is a mostly diploid or sometimes triploid wild potato species. It is characterized as an herbaceous stoloniferous and tuberous, rosette to semi-rosette habit plant. Its underground stems are modified as stolons and bear white to cream and rounded to ovoid tubers. The leaves are green and odd-pinnate, with lateral segment pairs often subequal or decreasing in size gradually toward the base except for the most proximal one or two pairs, which are greatly reduced in size. The flowers are typically stellate and always white. The berries are ovoid or globose, green, or yellowish-green when ripe, aromatic, and glabrous. The seeds are smallish, whereas more than 100 seeds per berry can be produced.

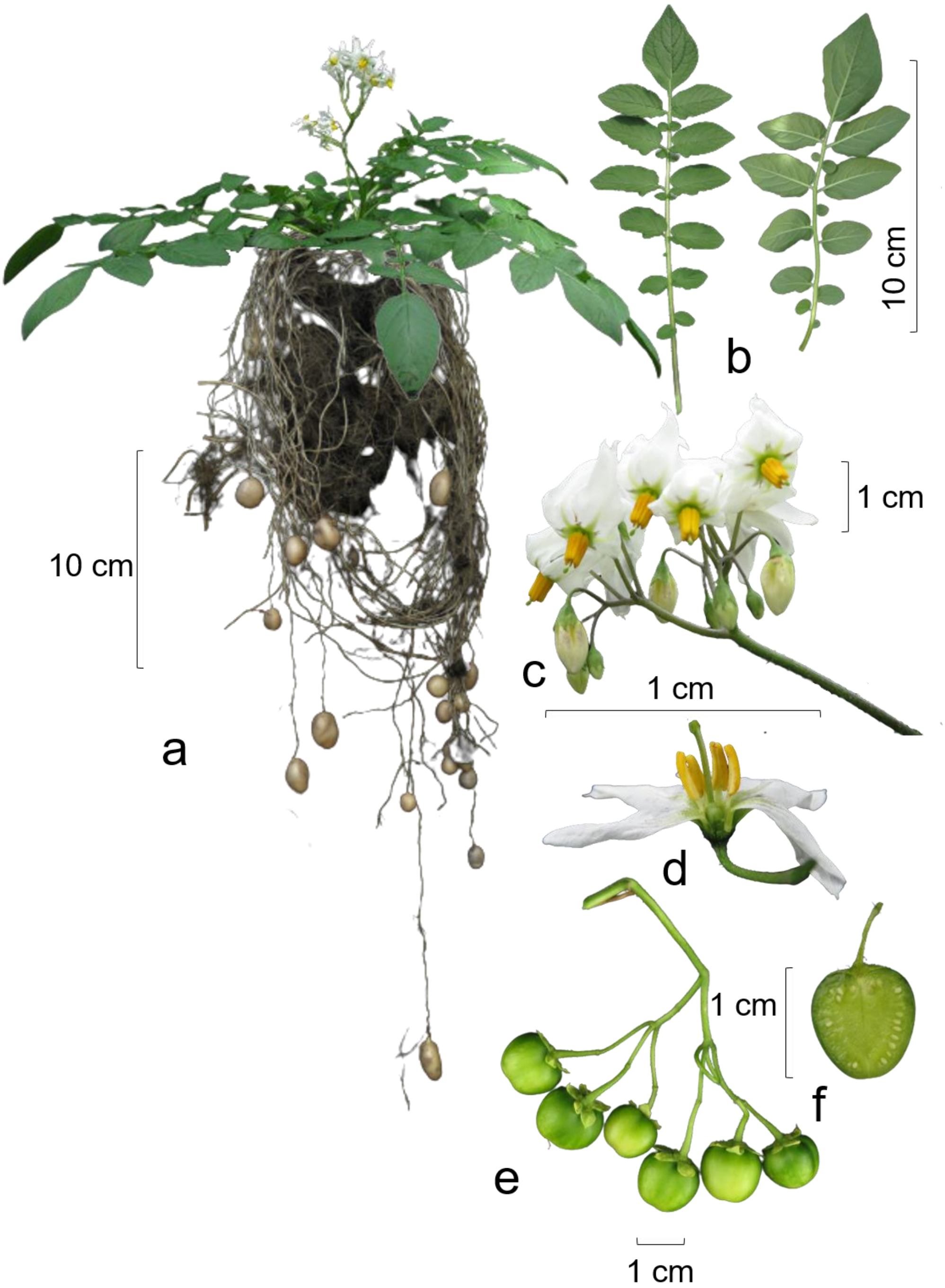


Figure 3 *Solanum malmeanum* (Solanaceae): a) Plant habit depicting overground branches and inflorescence and underground stolons and tubers (BGB471, *Heiden 2384*), b) Leaves abaxial and adaxial surfaces (BGB447, *Costa 60*), c) Inflorescence, flowers and buds (BGB448, *Costa 63*), d) Flower in cross section evidencing the stamens and pistil (BGB443, *Olegario 2*), e) Berries (BGB446, *Costa 63*), f) Open berry showing the fleshy pulp and seeds (BGB017, *Costa et al. 57*). *Photographs by Rodrigo Nicolao.*

The Russian agronomist, botanist, and geneticist Nikolai Vavilov (1887 - 1943), while developing his theory on the centers of origin of cultivated plants, organized a series of expeditions, and collected seeds worldwide creating the world's largest collection of plant genetic resources at that time (KURLOVICH et al. 2000). Vavilov (1928) presented the centers of origin theory on the V International Congress of Genetics in Berlin. He noticed that the erosion of the genetic base of the cultivated crops could lead to an instability of production, and then identified the 'centers of diversity' of species for the main crops (VAVILOV, 1957). Since his seminal works, several expeditions were carried out and efforts are still underway in a global and multinational effort to collect, conserve, characterize and pre-breed plant genetic resources for food and agriculture (FAO, 2009). Crop Wild Relatives (CWR) are a component of the plant genetic resources considered as an invaluable source of genetic variability for the improvement of crops (DEMPEWOLF et al. 2017). Potato wild relatives are potential sources of genes that may contribute to adapt the crop to climate change scenarios including abiotic stresses (e.g., heat, frost, drought, flood, sandy soils) and to cope with biotic stresses (pest and diseases); as well as to contribute with novel quality attributes for industry and nutrition (HAWKES; HJERTINGS 1969; JANSKY et al. 2013).

The first steps to use wild relatives in modern potato breeding started almost two centuries ago. Wild potatoes from South America had been introduced in Europe in 1824. Before 1920 many efforts were made to cross *S. demissum* Lindley (1848) with *S. tuberosum* and by 1932 introgression of resistance genes succeed first for late blight, later for virus (1941) and potato cyst nematode (ROSS 1966). Since then, many resistance genes have been introduced into modern potato cultivars. Nowadays, with the developing of several new biotechnological techniques, the potential of using wild potatoes for breeding the cultivated potato is more promising than ever.

Solanum malmeanum encloses several interesting traits to contribute for the development of new potato cultivars. Some accessions are resistant against bacterial (*Ralstonia solanacearum)* and verticillium wilt (*Verticillium dahlia)*, ring rot (*Corynebacterium michiganensis* subsp. *sepedonicus*), late (*Phytophthora infestans*) and early blight (*Alternaria solanii*), fusarium dry rot (*Fusarium sambucinum*), hapla (*Meloidogyne hapla*) and cyst nematode (*Globodera rostochiensis*), colorado potato beetle (*Leptinotarsa decemlineata*), potato leaf hopper (*Empoasca fabae*), green peach aphid (*Myzus persicae*), potato aphid (*Macrosiphum euphorbiae*), and potato leafroll virus (PLRV) (*Polerovirus* sp.) (RADCLIFFE; LAUFR 1971; FLANDERS; RADCLIFFE 1992; LAFERRIERE et al. 1999; MICHELETTO et al. 1999; MICHELETTO et al. 2000; SIRI et al. 2009; BASHIR et al. 2021; USDA 2021). In addition, it is a genetic reservoir for quality traits for industrial purposes such as high dry matter and protein contents and low reducing sugar content in tubers (ROCHA et al. 2000; CHALÁ et al. 2001; JANSEN et al. 2001). Some authors emphasized its good performance under low temperatures and frost (ROSS; ROWE 1965; HAWKES; HJERTING 1969). Even so, tolerance to heat and cold stress can be positively correlated (Hetherington et al. 1983). Tu et al. (2021), for example, confirmed the potential of this species as a source of genes to resist under low temperature stress by the mean of somatic hybridization between frost resistant *S. malmeanum* and susceptible haploid *S. tuberosum* yielding a frost-tolerant offspring, evidencing the promising role of this wild genetic resource for potato breeding focusing on abiotic stresses.

Solanum malmeanum occurs in Argentina, Brazil, Paraguay, and Uruguay, and is partially sympatric with two other wild potatoes: *S. chacoense* and *S. commersonii* (HAWKES; HJERTING 1969; SPOONER et al. 2016). The barriers of hybridization between these populations are not complete neither fully understood (TARN; HAWKES 1986; EHLENFELDT; HANNEMAN 1988), and thus, genetic flow within and between populations of the same and distinct species could lead to the hypothesized formation of natural hybrids with a wide morphological variability and ploidy levels (CAMADRO et al. 2012). Since the first collection of *S. malmeanum* in southern Brazil and its recognition as a specific taxon, this species remained neglected, and the genetic resources studies and breeding efforts were almost totally directed to *S. chacoense* and *S. commersonii*. Since *S. malmeanum* does not belong to the primary and secondary potato genepool, it does not cross directly with the cultivated potato crop (*S. tuberosum*) due to the post-zygotic barriers of hybridizations lead by the Endosperm Balance Number (EBN) (HANNEMAN 1994). However, introgression of wild potatoes tertiary genepool desirable traits into the potato primary genepool is well documented and several techniques are available and under continuous development for breeders to overcome some of these barriers and introgress the desirable genes via 2n gametes, ploidy manipulation, bridge species, protoplast fusion and other techniques (CARPUTO et al. 2003; JANSKY 2006; JANSKY; HAMERNIK 2009).

Thus, it is fundamental to gather, update and synthesize the information on *S. malmeanum* desirable traits, characterize and evaluate the germplasm available and find pathways to introgress target genes into *S. tuberosum*, broadening the genetic basis of the world third most important food crop. To rescue this wild potato species from the role of a neglected wild relative and shed light on its promising traits to elucidate and unlock the potential of *S. malmeanum* use towards potato breeding, this Master Thesis has two chapters formatted as manuscripts for submission to scientific journals.

Chapter 1 presents a literature and genebanks review to promote the recognition of *S. malmeanum* in collections worldwide and evidence its historical controversial classification; presents data on morphological characterization, phenology, geographic distribution, habitats and ecological preferences, genebanks representativeness, and genetic variability characterization. Besides, it compiles and synthetize the available records of evaluated accessions for agronomical traits (response to abiotic and biotic stresses, quality traits for production and industry), and discusses the available information on cytogenetics, reproductive biology, breeding system and EBN to understand the barriers and strategies to apply its potential uses in breeding programs. Finally, breeding with the use of di-haploid, 2n gametes and ploidy manipulation, as well as the emerging potato diploid hybrid breeding and tools to aid the improvement of new cultivars are discussed with examples of successful trials for introgression.

Chapter 2 presents a first-step breeding behavior characterization by controlled crosses of the ten available accessions of *S. malmeanum* conserved at Embrapa Clima Temperado Potato Gene Bank. The accessions were assessed to evaluate the occurrence of parthenocarpy, apomixis, autogamy, allogamy, and the cross-ability with two wild species 2x 1EBN *S. commersonii* and 2x 2EBN *S. chacoense*, and the potato crop itself 4x 4EBN *S. tuberosum*. The success of crosses yielding fruit and seed production, seed development, and seed germination from each treatment and the reciprocal crosses are presented. Inter and intraspecific crosses with positive and negative results are discussed under the EBN hypothesis and 2n gametes production, pointing the importance of 2n gametes to overcome barriers of hybridization between different EBN groups allowing future essays for the introgression of desirable traits. The detection of some *S. malmeanum* genotypes able to self-fertilize (self-compatibility) is recorded for the first time and the importance of this self-compatible plants for developing endogamic lineages for diploid breeding is highlighted.

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Chapter 1. *Solanum malmeanum*, a neglected wild relative for potato breeding*

Rodrigo Nicolao[1] Caroline M. Castro[2] Gustavo Heiden[2]

Abstract

Crop wild relatives are gaining increasing attention. Their use in plant breeding to broaden the genetic basis of crops are essential to meet industrial demands, global food security, and sustainable production. *Solanum malmeanum* (*Solanum* sect. *Petota*, Solanaceae) is a wild relative of potatoes (*S. tuberosum*) from Southern South America occurring in Argentina, Brazil, Paraguay, and Uruguay. This wild potato has been largely confounded or historically considered as conspecific with *S. commersonii*. Nevertheless, it was just recently reinstated at the species level. Due to the instability of the correct application of this species name and discordant species circumscriptions and morphological borders applied to recognize it, retrieving information on its traits and applied uses is tricky. To overcome these difficulties, we performed a thorough literature reference survey, herbaria specimen's identification revision and genebank database consults to review and update the information available on this neglect potato wild relative towards increasing research on it to fully understand and explore its potential for potato breeding. Limited studies were carried out concerning its reproductive biology, resistance against pests and diseases as well as tolerance for abiotic stresses and evaluation of quality traits. Furthermore, many discrepancies are found at previous reports that treat *S. malmeanum* under different scientific names. The scattered and scarce information available make it less represented in gene banks and genetic studies are missing. We compile, update and present available information for *S. malmeanum* on taxonomy, geographical distribution, ecology, reproductive biology, relationship with its closer relatives, biotic and abiotic stresses resistance traits, and discuss ways to overcome sexual barriers of hybridization and future perspective for its use in potato breeding. As a final remark, we highlight that this species potential uses for potato breeding have been neglected and must be unlocked. Thus, further studies on morphological and genetic variability with molecular tools are fundamental for an efficient conservation and applied use of this promising genetic resource.

Keywords · Breeding ·Crop wild relatives· Genetic resources ·Potato breeding ·Wild potatoes

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Ethics declarations

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Authors' contributions

Nicolao R. did the literature, herbaria and genebank data revision. Nicolao R. and Heiden G. wrote the text. Castro C.M. gave additional input on text writing.

Introduction

Global human population is predicted to reach 10 billion people and the demand for food may double by 2050, or more, when compared to 2000 (Ray et al. 2013; Hunter et al. 2017). In addition, future climate change scenarios, including rising temperatures, irregular rainfall, soil degradation, and water and land scarcity (Rosegrant et al. 2002), favors the negative effects on agricultural fields (George et al. 2017; Raymundo et al. 2018; Pastor et al. 2019) and human health (Patz et al. 2005; Nelson et al. 2018). Potato crop (*Solanum tuberosum* L.) is the third most important food crop after rice and wheat, and prime important non-grain crop. Furthermore, the Food and Agriculture Organization (FAO) of the United Nations proclaimed 2008 as "The Year of the Potato" to highlight its prominence in ensuring global food security for the humankind more efficiently, principally due to its source of calories, fibers, proteins, mineral micronutrients, vitamins B6 and C, potassium, and antioxidant compounds, on less land than any other crop (Birch et al. 2012; Devaux et al. 2014). All modern potato cultivars are tetraploid, vegetatively propagated, and have narrower genetic variation than what is available on its gene pool (Fu et al. 2009; Vincent et al. 2013; Hardigan et al. 2017). The lack of resistance to the main diseases of the cultivated potato makes it one of the most dependent crops on agrochemical applications to combat diseases and pests (Vleeshouwers et al. 2011; Palo et al. 2012). In this scenario, plant breeders require genetic variability for the continuous process of crop improvement to face climate change, increase productivity, and ensure food security.

The domestication process of agricultural crops, including potato (*Solanum tuberosum* L.), which aimed to increase the crop uniformity and productivity, by successive rounds of crosses and selection, caused a significant narrowing of genetic variability, leaving cultivars with less allelic diversity than their wild progenitors (Kolech et al. 2016; Duan et al. 2019; Maccaferri et al. 2019). This phenomenon is also known as 'domestication bottleneck' (Tanksley and McCouch 1997). Along this process, several valuable genetic variants and associated phenotypes have been filtered out of the crop gene pool, for example, insect pests and disease resistance, and abiotic tolerance, as well nutritional quality variability (Hardigan et al. 2017; Drapal et al. 2020). Also, many treats are increasing due to emerging pest-insects and diseases in potato crop, as exemplified either by the outbreak of new isolates of causal late blight oomycete *Phytophthora infestans* (Mont.) de Bary in European and American producing regions (Santana et al. 2013; Göre et al. 2019; Maurice et al. 2019), and the new sequevars of bacterial wilt *Ralstonia solanacearum* in Brazil, Iran, and Peru (Gutarra et al. 2017; Santiago et al. 2017).

These emerging global challenges increase the urgency for expanding the genetic base of crops. Crop Wild Relatives (CWR) encompasses the wild plant taxa or weedy plant species genetically close related to the cultivated crop itself. They are considered unique reservoirs of valuable genetic diversity and adaptative traits for crop improvement (i.e., food, forage, ornamental, medicinal, etc.), such as pests and diseases resistance, abiotic tolerance, nutritional compounds, as well as pharmaceutical and industrial properties (Maxted et al. 2006; Jansky et al. 2013; Zhang et al. 2017; Pradheep et al. 2019; Perrino and Perrino 2020; van Zonneveld et al. 2020). The genetic variability carried by wild plants, especially CWR, is much higher than that of their domesticated counterparts (Warschefsky et al. 2014). The Food and Agriculture Organization (FAO 2010) considers CWR as a subset of Plant Genetic Resources for Food and Agriculture (PGRFA). Furthermore, their important role to guarantee the food security and economic stability in the 21st century is well documented (Maxted et al. 2006; Hajjar and Hodgkin 2007; Jansky et al. 2013; Dempewolf et al. 2014; Dempewolf et al. 2017). So far, around 50,000 species of CWR are known, of which about 10,000 are estimated to be as a high potential resource to guarantee food security and economic stability (Maxted and Kell 2009). Thus, CWR are widely considered an extraordinary source of genetic variability to face both the increase of global population growth and climate change scenarios in parallel to an increment of yield from a sustainable agroecosystem way (Jansky et al. 2013; Dempewolf et al. 2014; Christensen et al. 2017). Even considering the genetic erosion of crops, the crop wild relatives are also vulnerable to habitat loss or fragmentation either due to climate change or anthropogenic disturbances (Foley 2010; Jarvis et al. 2008; Vincent et al. 2019; González et al. 2020). It is estimated that over 70% of the total CWR are in urgent need of collection and conservation in gene banks, and over 95% are insufficiently represented with respect to the full range of geographic and ecological variation in their distributions (Castañeda-Álvarez et al. 2016).

There is probably no other agricultural crop that contains such richness of wild relatives' diversity as the potato gene pool (*Solanum* sect. *Petota*, Solanaceae) (Douches et al. 1989; Hawkes 1990; Pavek and Corsini 2001; Spooner et al. 2014). Over than 100 wild tuber-bearing species are widely distributed on geographical and ecological areas of the Americas, ranging from Southwestern United States (38°N), passing through dry areas of Mexico, until the adjacent mainland areas of Chile (41°S) in the Southern Cone of South America, at altitudes ranging from sea level up to 4.500 m (Hijmans and Spooner 2001; Spooner et al. 2004; Spooner et al. 2014; Spooner et al. 2016; Spooner et al. 2019). About 70% of these species are diploids while autopolyploids and allopolyploids range from the basic 2x up to 6x chromosome numbers (x=12). Regarding the cytogenetic behaviour of diploids and polyploids, Matusbayashi (1991) concluded that, independently of their ploidies, potato species are phylogenetically related because they possess a common (basic) A genome, which, modified to different degrees, originated four additional genomes: B, C, D and E. Furthermore, the basic genome A are assumed to play a minor role as an isolation mechanism (Camadro et al. 2004).

Wild potatoes co-evolved with several adversities of environmental conditions, including humid tropical, and subtropical areas, semidesert conditions (e.g., dry, cold, frost, heat, salinity, etc.), pests and diseases (Hawkes 1958; Hawkes and Hjerting 1969). Therefore, they are widely considered to be an important source of allelic diversity and valuable agronomic traits such as pests and disease resistance, abiotic stress tolerance, and industrial traits (Douches et al. 1989; Hanneman 1989; Flanders and Radcliffe 1992; Spooner and Bamberg 1994; Jansky 2000; Siri et al. 2009; Jansky et al. 2013; Machida-Hirano 2015; Bachmann-Pfabe and Dehmer 2020) and a valuable source of genes to improve field resistance for a broad-spectrum of pest-insects or diseases such as potato late blight conferred by *R-genes* (Smilde et al. 2005; Hein et al. 2009; Sokolova et al. 2011; Chen et al. 2019; Sanetomo et al. 2019; Meade et al. 2020; Rakosy-Tican et al. 2020), extreme resistance reaction to potato virus Y (PVY) which are conferred by the *Ry* genes (Flis et al. 2005; Rogozina et al. 2019), and resistance to potato cyst nematodes (*Globodera rostochiensis*) (Limantseva et al. 2014).

The intentional use of CWR in plant breeding started more than a century ago (Hawkes 1958; Ross 1966). The Russian agronomist, botanist, and geneticist Nikolai Vavilov (1887-1943) began collecting many crop wild species worldwide during the early 20st century, in areas he called ‘centers of diversity’. The use of CWR in breeding programs started in 1900, although it achieved real gains just after 1970 (Prescott-Allen and Prescott-Allen 1986; Ross 1986; Hoyt and Brown 1988; Hajjar and Hodgkin 2007).

Wild potato species can provide a rich diversity of agronomic traits (Hanneman 1999; Bradshaw et al. 2006; Jansky et al. 2013; Villano et al. 2020). More than 100 potato species are recognized (Spooner et al. 2014). In addition, around 70% are diploid, self-incompatible, and cross-incompatible with the cultivated potato (Hawkes 1990; Spooner et al. 2014). Interspecific crosses not always occur because a normal embryo development depends on the EBN that requires a 2: 1 maternal to paternal rate (Johnston et al. 1980; Johnston and Hanneman 1982).

Spooner and collaborators (2014) proposed five crossability groups to guide potato breeders in the efficient use of wild germplasm, based on the EBN along the ploidy, and self-compatible /self-incompatible systems. The first group comprises the cultivated potatoes, landraces, and its wild relatives 4EBN (4x, 6x), the second group is the most representative in species and includes 2EBN (2x, 4x) species, and the third group includes 1EBN (2x) species. Within the 1EBN and 2EBN groups, the self-compatible (SC) species are separated from the self-incompatible (SI) ones in two subgroups. In addition, into the 1EBN and 2EBN groups, the crosses between SC species (as female) with the self-incompatible (as male) are frequently successful, however, the reciprocal crosses usually fail, a phenomenon known as unilateral incompatibility. All 4EBN potato species are self-compatible (Spooner et al. 2014).

Due to the EBN differences, 2x 1EBN species are sexually isolated from 2x (2EBN) and both 4x (4EBN) and haploid (2x=24) (Hanneman 1994; Carputo et al. 1997). The lower EBN species may be intercrossed with the higher EBN ones, if 2n gametes (2n pollen or/and 2n eggs) occur (den Nijs and Peloquin 1977a; den Nijs and Peloquin 1977b; Ehlenfeldt and Hanneman 1984). When 2n gametes are present, it is possible to produce successful reciprocal crosses between 1EBN and 2EBN or between 2EBN and 4EBN, but not between 1EBN and 4EBN.

Tetrasomic inheritance (2n = 4x = 48) of the cultivated potato is infinitely more complicated than disomic inheritance of diploid (2n = 2x = 24) level, mainly due to inbreeding depression and low sexual fertility of tetraploid (Jansky et al. 2014; Hardigan et al. 2017). Most of the genetic resources of potato wild species are diploid (Jansky et al. 2014). Breeding potato at diploid level can promote faster genetic gains than breeding at tetraploid level. Especially for polygenic traits, pre-breeding at the diploid population before to transmit the desirable target trait into the tetraploid population can be very efficacious.

Solanum malmeanum Bitter is a diploid wild potato species, belonging to *Solanum* sect. *Petota* Dumort. ser. *Commersoniana* Buk., currently classified into the tertiary gene pool of potato (*S. tuberosum* L.) and assigned as 1EBN (Hanneman 1994; Spooner et al. 2014). It is native to the Southern Cone of South America occurring in Argentina, Brazil, Paraguay, and Uruguay. This species was collected for the first time in 1893 by the Swedish botanist Gustaf Oskar Andersson Malme along the Ijuí river, at Rio Grande do Sul state in Southern Brazil (53º 54' 53'' W, 28º 23' 16'' S) and named after him when formally described later by the German botanist Friedrich August Georg Bitter in 1913 (Brücher 1999). Taxonomic treatments of wild potatoes by Bitter (1913), Correll (1962), Hawkes and Hjerting (1969), Mentz and Oliveira (2004), and Spooner et al. (2016) based on morphological traits, considered *S. malmeanum* as

S. malmeanum, *S. commersonii* f. *malmeanum*, *S. commersonii* subsp. *malmeanum*, *S. commersonii* f. *malmeanum*, and *S. malmeanum*, respectively. Spooner et al. (2014, 2016) have recently reinstated the taxon at species level. This species is long day adapted, highly flowering and a good pollen producer having a geographical distribution that partially overlaps with *S. chacoense* Bitter and *S. commersonii* Dunal (Correll 1962; Hawkes and Hjerting 1969; Spooner et al. 2016). Phylogenetic analysis using AFLP markers suggested that *S. malmeanum* can be genetically separated from *S. chacoense* and *S. commersonii*, with exception of two of 12 accession of *S. commersonii* that were allocated with the same group of seven *S. malmeanum* accessions (Jacobs et al. 2008).

Solanum malmeanum 2x 1EBN is cross incompatible either with 2x 2 EBN, and *S. tuberosum* haploid 2x 2 EBN, but 2n gametes can overcome this barrier. Introducing new traits from distant wild species (such as those in the 1EBN group, including *S. malmeanum*) to upper groups (as 2EBN or 4EBN species) may require the use of bridge species to allow traits introgression via unreduced gametes (2n pollen or 2n eggs). Several techniques have been developed to overcome sexual hybridization barriers between 2x 1EBN and 2x 2EBN wild potatoes with 4x (4EBN) cultivated potato such as ploidy manipulations, somatic embryo rescue, protoplast fusion, and cell, anther, and pollen culture (Peloquin 1989; Hermundstad and Peloquin 1985; Bradshaw et al. 2006; Jansky 2006).

Due to the changing taxonomic history, several morphological shared traits and geographical distribution patterns that greatly overlaps, *S. malmeanum* has been largely confounded or historically considered as conspecific with *S. commersonii*. Nevertheless, it was recently reinstated at species level. Due to the instability of the correct application of this species name and discordant species circumscriptions and morphological borders applied to recognize it along the history, retrieving information on its traits and applied uses is tricky. To overcome these difficulties, we performed a throughout literature review, genebank database consults and herbaria voucher specimens cited in published studies revision to update the information available on this neglected wild relative towards increasing research on the species to fully understand and explore its potential for potato breeding. Thus, our study aims to compile, synthetize, and update all the disperse information about reproductive biology and cytogenetics, taxonomic treatments and geographical data, biotic and abiotic resistance of *S. malmeanum* and to provide it in an elucidative and comprehensive way.

Material and methods

Species circumscription

Our circumscription of *S. malmeanum* is based on the species original publication by Bitter (1913), the revised taxonomic treatment of Spooner et al. (2016) and the addition of complementary observations from characterized genebank accessions available online (Alelo 2020). When the original data discussed in this review was originally published as *S. commersonii*, but the cited accession or specimen corresponds to *S. malmeanum*, we added the remark “as *S. commersonii*” just after.

Data review

To perform this review, we searched for "*Solanum malmeanum*", "*Solanum commersonii* subsp. *malmeanum*", "*Solanum commersonii* f. *malmeanum*" and their genus abbreviatures in literature references, herbaria and genebank databases as follows.

Literature references. We reviewed the monographs for taxonomic treatments of tuber-bearing *Solanum* species from the American continent conducted by Bitter (1913), Correll (1962), Hawkes and Hjerting (1969), and Spooner et al. (2016), as well as regional taxonomic studies for Argentina by Matesevach and Barbosa (2005), South of Brazil by Mentz and Oliveira (2004), and Paraguay by Elsam et al. (2018). The scientific reports published until March 31st 2021 were searched in the databases Base de Dados da Pesquisa Agropecuária (BDPA 2021), Biblioteca Digital Brasileira de Teses e Dissertações (BDBTD 2021), CAPES Portal de Periódicos da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (2021), Google Scholar (2021), JSTOR (2021), Scientific Eletronic Library Online (SciELO 2021), ScienceDirect system (2021), and printed publications available at Embrapa Clima Temperado library.

Taxonomic and geographical distribution data. To update the taxonomic and geographic data for *S. malmeanum*, we reviewed personally or online the label data of *S. malmeanum* specimens from the herbaria BHCB, CEN, ECT, F, ICN, K, MO, NY, P, R, RB, S, SPF, U, and US (acronyms according to Thiers 2020). Georeferenced records were extracted from the online platform databases GBIF (2021), speciesLink (CRIA 2021), and REFLORA (2021), with removal of duplicate records and manual checking and correction of wrong or doubtful coordinates. The occurrence data were plotted on a map rendered in QGIS (2021).

Genebank databases search. To assess the representativeness of *S. malmeanum* at the germplasm banks we explored the online databases Alelo (2021) by Embrapa and GENESYS © Global Portal on Plant Genetic Resources (2021). To compile the agronomic traits (i.e., biotic resistance and abiotic tolerance levels, and quality traits), we explored the online databases of USDA Agricultura Research Service (2017) U.S. National Plant Germplasm System (NPGS) (2017-2020) and literature cited accordingly.

Criteria for updating taxonomic information. We updated the synonyms of subspecies or form names and checked the retrieved records of *S. commersonii* and *S. malmeanum* for taxonomic accuracy through the revision of voucher specimens deposited in herbaria or other verifiable sources as genebank pictures of the plants under cultivation. The confirmation or corrections of identifications were possible when the studied specimens match the current circumscription of *S. malmeanum* through the observation of diagnostic morphological features present in the physical specimens or in the high-quality digital images retrieved.

Review

Classification history

Since its discovery, *S. malmeanum* has been extensively subjected to diverse taxonomic approaches on previous monographs, treated as specific taxa or as a subspecies or a form of *S. commersonii* (Correll 1962; Hawkes and Hjerting 1969; Mentz and Oliveira 2004; Matesevach and Barbosa, 2005; Spooner et al. 2016; Elsam et al. 2018).

Bitter (1913) named *S. malmeanum* in honor of Malme, and characterized the species as an herbaceous thin plant, measuring 20–30 cm in height, with uneven pinnatisect leaf blade 11–13 cm long, petioles 1–2.5 cm, 4–9 lateral segments (usually referred in literature as '*leaflets*'), the basal ones usually circular, with 4-5 pairs of interstitial segments and terminal odd segment ovate to elliptic measuring 3–3.8–4.2 × 1.7–2.6–2.8 cm, and flowers bearing a white corolla. According to Bitter (1913), *S. malmeanum* presents the corolla lobes folded back, which resemble the corolla lobes of *S. chacoense*, but *S. malmeanum* could be differentiated from *S. chacoense* due the longer and apiculate calyx lobes. Moreover, Bitter (1913) emphasized that *S. malmeanum* can be confused with *S. commersonii* due the similar growth habit. However, it can be distinguished by the corolla lobes curved back and the lateral interstitial leaf segments well marked.

Correll (1962) characterizes *S. malmeanum* (under the name *S. commersonii* f. *malmeanum*) as a small and spreading plant, less than 20 cm tall and rarely 50 cm tall, slightly to densely pilose throughout, and varying in habit from rosette to much elongated, bearing leaves up to 25 cm long, with two or more interstitial segments between each main segment pairs. Additional characters given by him for the differentiation of *S. malmeanum* from *S. commersonii* were the 4–5 lateral leaf segment pairs that are distinctly stalked (referred to as '*petiolulate*'), with frequently two or more interstitial segments, while *S. commersonii* has 2–5 lateral sessile leaf segment pairs without interstitial segments amid leaf segment pairs. Additional characters cited by him for differentiation, were the more conspicuous pubescence and more compact inflorescences of *S. commersonii* when compared to *S. malmeanum*. He justified the downgrading of *S. malmeanum* recognizing it as a "form" of the typical *S. commersonii* because he supposed that it might represent a hybrid of *S. commersonii* and *S. chacoense*. Although if so, the correct name to be applied for this hypothesis would be "*S.* × *malmeanum*" according to the current rules of the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018).

Hawkes and Hjerting (1969) characterized *S. malmeanum* (under the name *S. commersonii* subsp. *malmeanum*) as a small herb, with leaves in a rosette or less commonly widely spaced according to the stem development. The leaves measuring 8–17(–24) cm long, with (0–)2–4(–6) pairs of lateral segments and 2–5 pairs of lateral interstitial segments, and the odd terminal segment broader but generally barely longer than the lateral segments, which decrease in size slowly towards the base of the leaf. According to him, the lateral segments may appear shortly stalked and are very narrowly decurrent on the rachis. The terminal inflorescence is a rather compact cymose umbel, which contains (2–)4–10(–16) flowers and the peduncle is (1.5–)5–13 cm long, not or only once-forked. Flower pedicels are 15–25(30) mm long and articulated about two-third or more above of the base. The corolla is always white, and the lobes only slightly longer than broad. Hawkes and Hjerting (1969) differentiated the two "subspecies" of *Solanum commersonii* based on morphological characteristics whereas they argued that *S. commersonii* subsp. *malmeanum* differs from *S. commersonii* subsp. *commersonii* also in many leaf characters and in some inflorescence and corolla features. They stated that the lateral segments of *S. malmeanum* generally decrease in size gradually towards the base of the leaf and are narrowly decurrent, the petiole is normally short-stalked, the peduncle is unbranched or the branches are not markedly contracted, the pedicel articulation is about median to higher than in *S. commersonii*, and the corolla is always white, with lobes about as long as broad, in opposition to *S. commersonii* that shows the lateral segments decreasing rapidly in size towards the base of the leaf and are often markedly decurrent, being the leaves normally sessile; the peduncle branches are somewhat contracted; the pedicel articulation is low to about median, the corolla is usually purple pigmented, with lobes about 1 and ½ times as long as broad. Like Correll (1962), Hawkes and Hjerting (1969) noted that the sub-sessile or shortly stalked lateral segments of *S. malmeanum* were present in herbarium specimens and in living material, furthermore the compact inflorescences of *S. commersonii* can be very noticeable. Hawkes & Hjerting (1969) considered that Correll (1962) was mistaken in classifying *S. malmeanum* as a form of *S. commersonii* because it consists of an assemblage of biotypes with a distinct geographical distribution which are adapted to a habitat range quite different from that of the typical subspecies, and because *S. malmeanum* and *S. chacoense* were assumed to not hybridize. Despite Hawkes and Hjerting (1969) suggested that the two taxa could almost be considered to differ from each other at the species level, however, they treated them as subspecies belonging to the same species anyway.

Mentz and Oliveira (2004) performed the taxonomic treatment for the *Solanum* species from Southern Brazil and treated *S. malmeanum* in the same way as done by Correll (1962). Also, they attested that *S. malmeanum* (as *S. commersonii* f. *malmeanum*) is distributed in more restricted areas, where it is sympatric with *S. commersonii* (under the name *S. commersonii* f. *commersonii*), which has a wider distribution, stating that only the white color of the corolla is not decisive for the maintenance of the infra-specific form category (Mentz and Oliveira 2004). Matesevach and Barbosa (2005) on the taxonomic treatment of Flora Fanerogámica of Argentina treated *S. malmeanum* as *S. commersonii* subsp. *malmeanum*, the same way as Hawkes and Hjertings (1969).

Spooner and collaborators attributed the specific species concept, while performed and published in parts a thorough taxonomic revision for all the cultivated potatoes (Spooner et al. 2014) and their wild relatives for North and Central America (Spooner et al. 2004), Southern South America (Spooner et al. 2016), and Northern South America (Spooner et al. 2019). In the taxonomic treatment for Southern South America (Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay), Spooner et al. (2016) reinstated the taxonomic concept of *S. malmeanum* at the species level and morphologically circumscribed it based mainly on morphological leaf features. They characterize the species as an herbaceous rosette-forming habit plant with less than 30 cm in height, with leaves 7.5–15.5 cm long and 4–8.5 cm wide, with petioles 1.3–4.5 cm long, bearing 3–5 pairs of lateral segments that gradually decrease in size to the base, except the most proximal 1 or 2 pairs that can be remarkably much smaller; the forked inflorescence with 2–8 flowers, the stellate and always white corollas, and the pedicel of the flower articulated about the midpoint. Spooner et al. (2016) differentiated *S. malmeanum* from *S. commersonii* by its frequently uppermost pair of lateral interstitial segments that do not reduce in size so abruptly toward the leaf base, the more frequently stalked (as "*petiolulate"*) and larger lateral interstitial segments, and by its white corollas, while in *S. commersonii* the corolla is usually brush pigmented. Subsequently, Elsam et al. (2018) on the inventory of genetic resources from Paraguay accepted *S. malmeanum* as a specific taxon, following Spooner et al. (2016).

Taxonomy provides hypothesis of species as the basic units for biological studies and can predict genetic barriers or estimates the genetic variability having a crucial role as a starting point to carry out several further studies on biodiversity and its efficient use as plant genetic resources (Gaston 1992). *Solanum* sect. *Petota* (Solanaceae) has been the subject of extensive taxonomic treatment since the first description of the cultivated potato *S. tuberosum* (Linnaeus 1753). The previously referred taxonomic studies on potatoes and its wild relatives have been carried out using the taxonomic species concept which relies mostly on morphological phenotypes, usually relying on herbaria dried specimens or population samples (accessions) conserved at genebanks (Correll 1962; Hawkes 1990; Hawkes and Hjerting 1969; Spooner and van den Berg 1992; Spooner et al. 2016). However, all these previous taxonomic morphological concepts of *S. malmeanum* can become hampered and difficult to apply mainly due to the wide variability and plasticity of leaf characters, as well as the presence of diploids and triploid cytotypes with distinct morphologies (Tarn and Hawkes 1986).

Morphology: stable × variable characters

The following synthetic morphological description of *S. malmeanum* is based on the compilation of Bitter (1913), Correll (1962), Hawkes and Hjerting (1969), Spooner et al. (2016) and Embrapa Potato Genebank accessions observations (Alelo 2021).

Description. Herbaceous stoloniferous and tuberous semi- to rosette-forming plants, 0.15 to 0.3(–1) m tall (Fig 1a). Underground stems modified as stolons and tubers, predominantly white to cream, rounded to ovoid, (0.5–)1–4.5 cm long, (5–)1–30 cm diam., borne singly at the end of each stolon (Fig 1b). Aerial stems not winged, glabrous to pubescent. Leaves green, 7.5–15.5 cm long, 4–8.5 cm wide, odd-pinnate, glabrous to pubescent adaxially and abaxially; petioles 1.3–4.5 cm long, pubescent; lateral segment pairs 3–5, often subequal or decreasing in size gradually toward the base, except for the most proximal 1 or 2 pairs, which are greatly reduced in size; distalmost lateral segments 3.3–6.5 cm long, 2–4 cm wide, narrowly to broadly ovate, apex acute to rounded, base typically oblique, or cordate, to rounded, subsessile and slightly decurrent or with stalks up to 2 mm long; terminal odd segment 3.2–5.8 cm long, 1.5–3.9 cm wide, broadly ovate to broadly elliptic, apex obtuse to more rarely acute, base cuneate; interstitial segments 0–4, ovate to orbicular, sessile to short-stalked (Fig 1c). Pseudostipules absent or up to 1 mm long, pubescent with trichomes like those of the stem. Inflorescences 5–10 cm long, usually forked, with (2–)4–8(–16) flowers, the axes pubescent; peduncles 1.5–6(–13) cm long; pedicels 8–30 mm long in flower and fruit, articulated at about midpoint. Flowers (Fig. 1d) with calyx 3–7 mm long, pubescent; lobes 1–6 mm long, long-acute to attenuate, acumens 1–2 mm long; corolla 1.8–2.9 cm diameter, white, typically stellate, acumens ca. 0.2 mm long; anthers 4–5.5 mm long; style 9–12 mm long, exceeding stamens by 2–5 mm, stigma clavate to capitate. Berries 2.5–3 cm long, 2–2.5 cm wide, ovoid or globose, green or yellowish-green when ripe, aromatic, glabrous (Fig 1e). Seeds smallish, 1×1 mm**,** more than 100 seeds per berry (Fig 1f).

A wide morphological variability and phenotypic plasticity is reported intra and inter-specifically for all species belonging to *Solanum* sect. *Petota* and their hybrids (Correll 1962; Hawkes and Hjerting 1969). Despite these constraints, *S. malmeanum* can be always differentiated from the sympatric species (*S. chacoense* and *S. commersonii*) by the following set of stable characters: stem unwinged, leaves with apex segments acute to rounded, lateral segments decreasing gradually in size from the apex to the base of leaf, base of lateral leaf segments frequently symmetric in diploids (Fig 2b) and always asymmetric in triploids (Fig 2g), corolla always white and tubers borne singly at the end of each stolon. *Solanum commersonii* presents leaves with apex segments acute, lateral segments markedly decreasing in size from the apex to the base, base of lateral leaf segments always symmetric, corolla always violet pigmented or white with lilac blush pigment, semi-rosette, decumbent to erect habit, unwinged stems. *Solanum chacoense* presents base leaf of the lateral segments asymmetric as well as pentagonal corollas that are always white, which can be confused with some triploids cytotypes of *S. malmeanum* (Fig 2g and Fig 2h), but has erect habit, unwinged stems, leaves with apex segments acute, shorter and less pointed calyx lobes.

Table 1 Stable morphological traits to differentiate *Solanum malmeanum* from the two other sympatric wild potatoes *S. commersonii* and *S. chacoense* (Solanaceae).

	Species		
Morphological traits	***S. malmeanum***	***S. commersonii***	***S. chacoense***
Habit	Semi- to rosette in diploid, semi- to erect in triploid.	Semi-rosette, decumbent.	Erect.
Stems	Unwinged.	Unwinged.	Winged or unwinged.
Leaves	Lateral leaflets decrease gradually in size towards the base of leaf.	Lateral leaflets decrease obruptly in size towards the base of leaf.	Lateral leaflets do not decrease obruptly in size towards the base of leaf
Apex of terminal leaflet	Acute.	Obtuse to acute.	Acute to acuminate.
Base leaf of lateral segments	Symmetric in diploid, and asymmetric in triploid.	Symmetric.	Asymmetric.
Corolla shape	Stellate in diploid, and pentagonal in triploid.	Stelatte	Stelatte to pentagonal.
Corolla color	White.	Purplish (not white).	White.

Some morphological traits used in previous treatments can be misleading due to the genotype environmental plasticity. The habit is generally displayed as an herbaceous rosette-forming plant with 0.15 to 0.3 m tall in sunny conditions, but in shady environments such as woodlands or in tall grasslands the plants can reach up to 1 m tall, developing a semi-rosette erect habit and more lateral interstitial segments (mostly 3-4 pairs but may be as many as 6 pairs or as few as 2 pairs) (Hawkes and Hjerting 1969; Spooner et al. 2016). In some collections realized by Hawkes and Hjerting (1969), lateral interstitial segments are present in variable forms, sometimes they are absent, but in others they are present in up to 4 pairs, whilst in others they vary from none to several pairs in different plants of a population or even on different leaves of the same plant. Leaf and stem pubescence can be also variable, from sparse to quite dense, as well as the trichomes that can develop quite long and spreading (Hawkes and Hjerting 1969). Corolla is assumed to be always white when fully developed, however, some plants were reported to show corolla color with little blush pigment when cultivated under greenhouse conditions (Mentz and Oliveira 2004). From Alelo (2021) observations in greenhouse conditions, some accessions can present a purplish hue in the very early stages of anthesis, but this weak pigmentation fades quickly as the flowers complete the development and it is unclear if this phenotype could be attributed due to soil pH or cultivation conditions, for example, not found in natural habitats until now.

Another layer of complexity added for the morphological recognition of *S. malmeanum* is the occurrence of 2n and 3n cytotypes with distinct morphologies. In general, triploid cytotypes (2n = 3x = 36) can be easily distinguished from typical diploid cytotypes (2n = 2x = 24) by an expressive increase in vigor of plant, as an increase in leaves and corolla size (Hawkes and Hjerting 1969; Tarn and Hawkes 1986). Genebank accessions observations (Alelo 2021) confirm that typical diploid *S. malmeanum* has average vigor and leaves size, stellate corollas, average berries and tubers size, while triploid *S. malmeanum* has increased vigor and leaves size, rotate corollas, reduced berries and increased tuber size (Figure 2). Due the increased vigor in habit, leaves size with asymmetrical base and rotate corolla shape, *S. malmeanum* triploids are commonly mistakenly confounded with *S. chacoense*, but the combination of rosette habit, not-winged stems, and shorter internodes, are easy to differentiate from *S. chacoense* typical erect or erect-patent habit, winged stems, and longer internodes.

Phenology

The flowering season of *S. malmeanum* is variable, occurring from April to June according to Hawkes and Hjerting (1969) and flowering and fruiting from October to July as reported by Spooner and collaborators (2016). Hawkes and Hjerting (1969) pointed out that the species behave as its frost resistance can enable growing and flowering in both the cooler Winter season as well as in the Summer.

Geographic distribution

Solanum malmeanum is distributed in Argentina (Buenos Aires, Corrientes, Chaco, Entre Ríos, Formosa, Misiones, Santa Fé), Brazil (Rio Grande do Sul, Santa Catarina), Paraguay (Boquerón, Central, Cordillera, Itapuá, Presidente Hayes) and Uruguay (Colonia, Maldonado, Montevideo, Salto, Soriano) (Fig 4) The populations are distributed at latitudes between 23°S to 34°S. This distribution partially overlaps with the distribution of *S. chacoense* and *S. commersonii*, and at some localities these species can occur in sympatry.

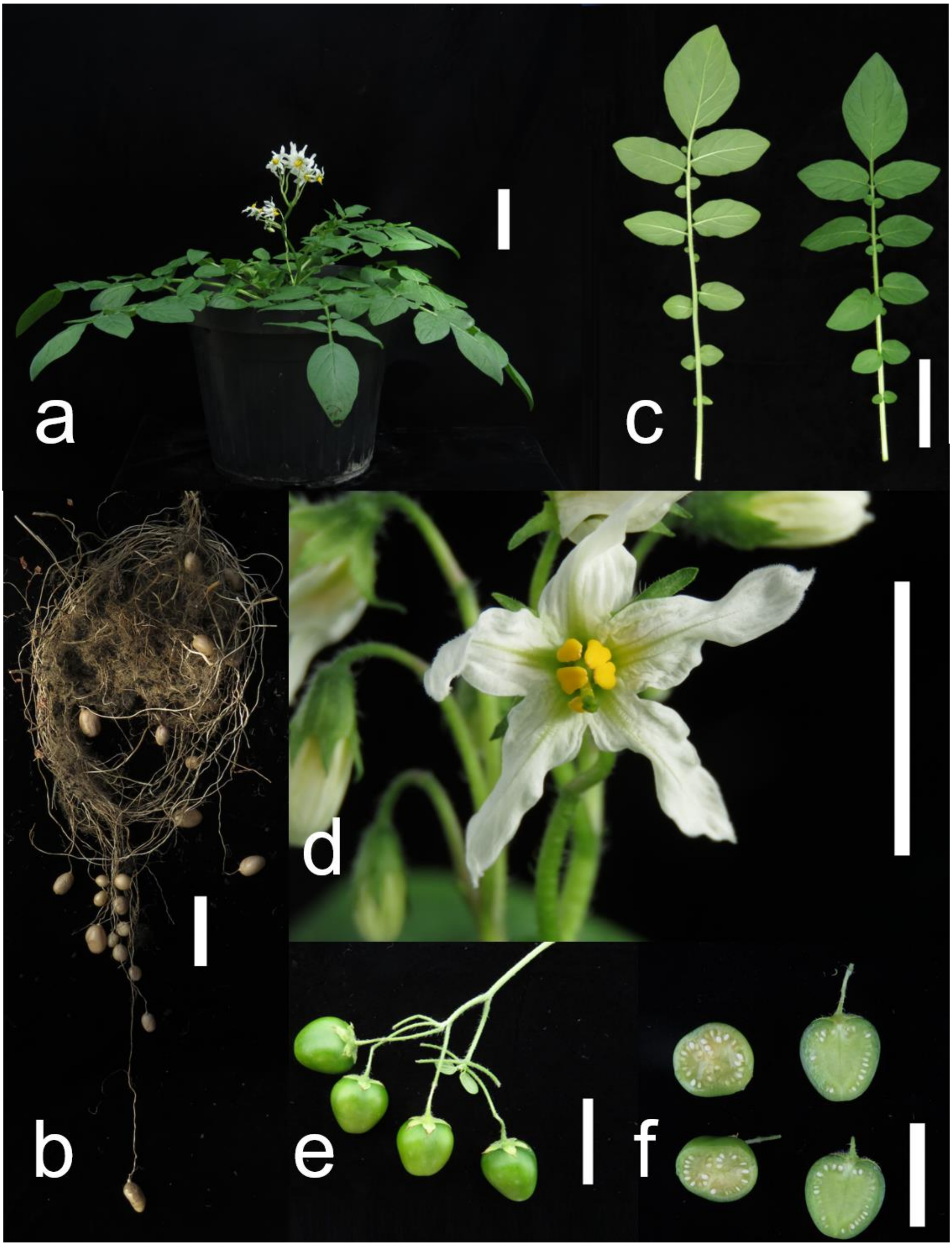

Fig 2 *Solanum malmeanum* morphology of typical diploid (2x): a) Rosette habit (bar = 10 cm), b) Stolons and tubers (bar = 5 cm), c) Leaves (bar = 5 cm) from abaxial (left) and adaxial (right) view, d) Flower (bar = 2.5 cm), e) Berries (bar = 3 cm), f) Open berries showing the seeds (bar = 3 cm). (Specimen depicted a–d) BGB471, *G. Heiden 2384*, e–f) BGB017, *D.M. Costa 57*). *Photographs by Rodrigo Nicolao*.

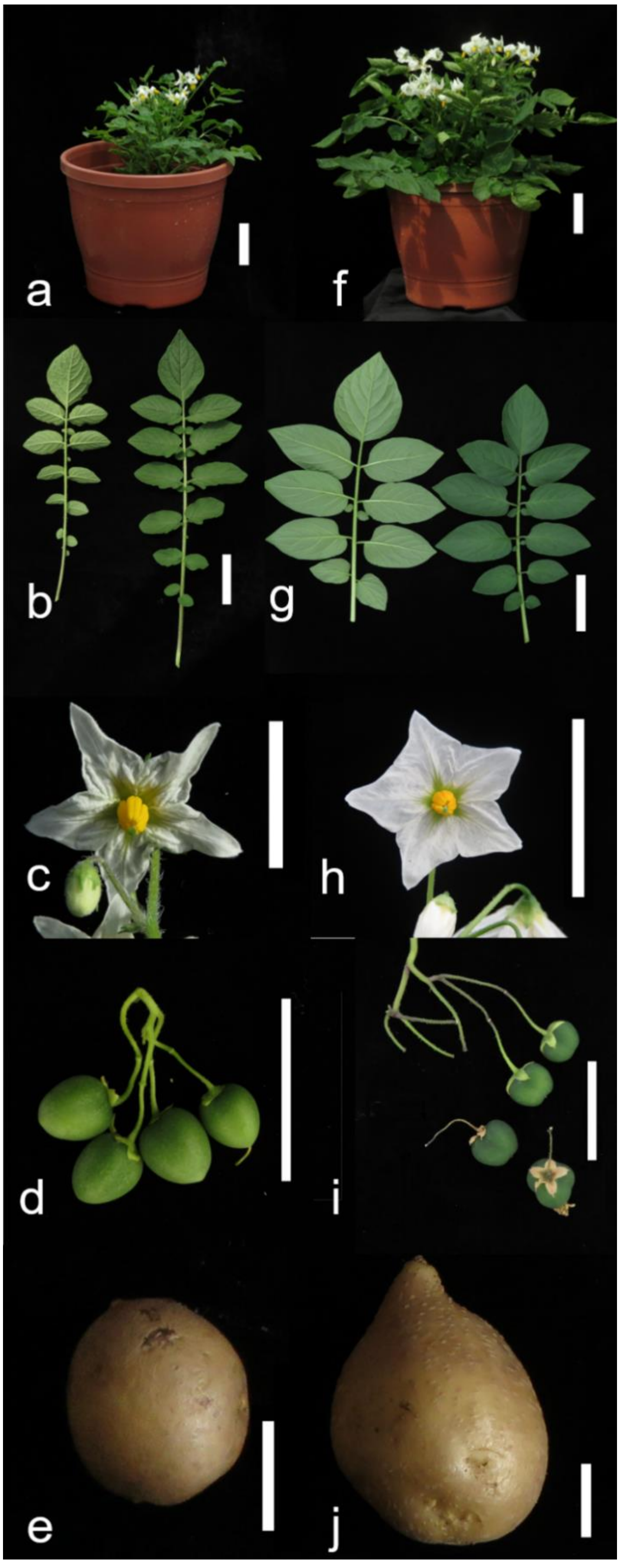

Fig 3 *Solanum malmeanum* cytotypes morphology comparison: diploid (a-e) - a) habit (bar = 10 cm), b) leaves (bar = 5 cm), c) flower (bar = 2.5 cm), d) berries (bar = 3 cm), e) tuber (bar = 1 cm); triploid (f-j) - f) habit (bar = 10 cm), g) leaves (bar = 5 cm), h) flower (bar = 2.5 cm), i) berries (bar = 3 cm), j) tuber (bar = 1 cm). Specimens depicted: a) BGB446, *D.M. Costa 26*, b) and e) BGB447, *D.M. Costa 60*, c) and d) BGB017, *D. M. Costa 57*, f-i) BGB080, *C. Kuhn & D.M. Costa 261*, j) BGB448, *D.M. Costa 63*. *Photographs by Rodrigo Nicolao.*

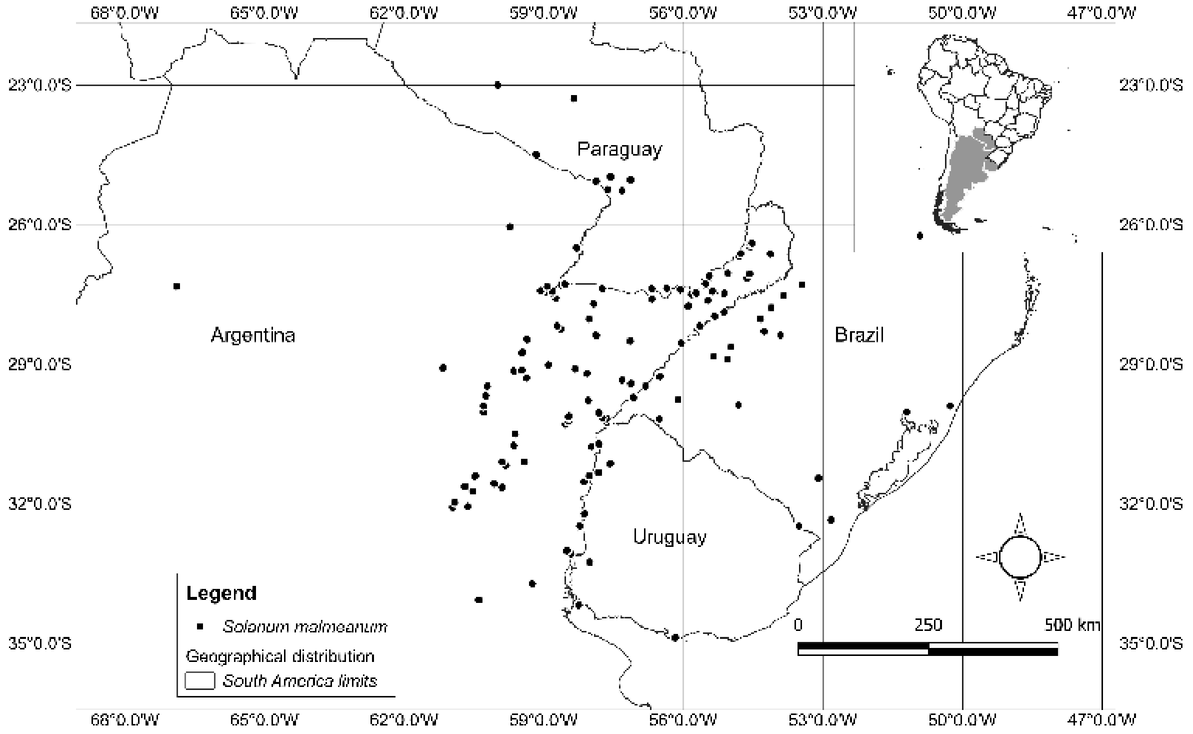

Fig 4 Geographical distribution of *Solanum malmeanum* in South America, each point refers to specimens deposited in herbaria, genebanks or referenced in literature. QGIS (v.4.0.5).

Habitats and ecological preferences

Solanum malmeanum populations are found at shady forests, grasslands, damp pastures, roadsides, stream sides, as well as weeds at crop cultivations, including potato fields. Despite no soil preference is exhibited, since it appears to grow in similar habitats as *S. commersonii*, except by dune slacks and estuarine marshes, yet more inland, apparently growing equally well in sandy riverbanks, clay soils and soils rich in humus (Hawkes and Hjerting 1969). In Argentina is found in the Eastern Subtropical Biogeographic Province at grasslands or savannas and the Mixed Forest Biogeographic Districts, Chaco Biogeographic Province by Eastern Chaco District (more humid eastern parts), and along the Espiñal Biogeographic Province by Santa Fé and Entre Ríos. In Brazil, it occurs in the *Araucaria angustifolia* subtropical Mixed Forests of the Paranense Biogeographic Province, with high annual rainfall, as well as by the Pampean Biogeographic Province with a dominant vegetation of grasses and bushes. In Paraguay it spreads through the Mixed Forest Biogeographic District into the wetter parts of the Chaco Biogeographic Province and the Chaqueño Biogeographic Domain that are characterized by xerophytic vegetation. In Uruguay it is found chiefly in the so-called 'Galería Uruguayense', that comprises the forests along the Uruguay river basin, belonging to the Mixed Forest Biogeographic District of the Eastern Subtropical Biogeographic Province, and belonging to the Pampean Biogeographic Province, with a dominant vegetation of grasses and bushes (Hawkes and Hjerting, 1969; Morrone et al. 2014; Spooner et al. 2016). Records found in the surveyed herbaria and genebanks range from the sea level up to 765 m of elevation (Alelo 2021; GBIF 2021).

Gene bank representativeness

Currently, *S. malmeanum* is represented in genebanks by a total of 52 accessions worldwide (Table 1). The U.S. National Plant Germplasm System (NPGS) contains the wider representativeness of *S. malmeanum* conserved ex-situ (25 accessions) followed by Embrapa Potato Genebank (11 accessions). The External Branch North of the Department Genebank of Germany, the Center for Genetic Resources (CGN) of the Netherlands and the James Hutton Institute (JHI) of United Kingdom account together for a total of 16 accessions.

Table 2 Representativeness of *Solanum malmeanum* accessions at genebanks.

Institution/genebank	**Country**	**Number of accessions***	**References**
Brazilian Agricultural Research Corporation (EMBRAPA)	Brazil	11	(Alelo 2021)
Centre for Genetic Resources, the Netherlands (CGN)	Netherland	06	(Genesys 2021)
External Branch North of the Department Genebank (IPK)	Germany	08	(Genesys 2021)
The James Hutton Institute (JHI)	United Kingdom	02	(Genesys 2021)
The U.S. National Plant Germplasm System (NPGS)	USA	25	(Genesys 2021)
Total		52 accessions	

*Plant genetic resources accession level data provided by: Center for Genetic Resources, the Netherlands (CGN), http://www.cgn.wur.nl/, Netherlands. Brazilian Agricultural Research Corporation (EMBRAPA), https://www.embrapa.br/, Brazil. The James Hutton Institute (JHI), United Kingdom. Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), http://www.ipk-gatersleben.de/en/dept-genebank/satellite-collections-north/, Germany. National Plant Germplasm System (NPGS), USA. All intellectual property rights (including copyright) in the Data are awned and retained by the said institutions. Data accessed through GENESYS Global Portal on Plant Genetic Resources. https://www.genesys-pgr.org/a/v223B7J8BQY and Alelo http://alelobag.cenargen.embrapa.br/AleloConsultas/Home/index.do. Accessed 31 March 2021.

From the first-time collection made by Malme in 1893 (Bitter 1913; Brücher 1999) until today, many expeditions have been realized by botanists and plant breeders interested in this species, such as Hawkes, Hjertings, Okada, and others. The specimens they gathered had their vouchers deposited in herbaria, the collection data were cited in several published works and some of them were deposited at genebanks. In Brazil, the plant breeder Delorge Mota da Costa (1919-2012) dedicated his career on potato breeding at Embrapa Clima Temperado, Pelotas, Brazil. He recognized the potential of wild potato species and conducted several expeditions in Southern Brazil from 1986 until 1992. He collected 278 samples of wild potatoes for research, from which 26 of them were *S. malmeanum* and nine of its original accessions remains conserved in the Embrapa Potato Genebank (Castro et al. 2007; Alelo 2021).

Genetic variability characterization

Lester (1965) proceeded the first study to investigate the tuber antigens of wild potatoes by immune-electrophoretic analysis, aiming to test the taxonomic treatments of the species belonging to *Solanum* ser. *Commersoniana*. One shared antigen found in *S. malmeanum* and *S. commersonii* was absent from the other species in the same series, pointing to the closer relationship between these two species.

Partial sequences of a new retrotransposon Tnt1 family member, Retrosol, were reported in several *Solanum* sect. *Petota* species, including *S. malmeanum* (Manetti et al. 2007). The accession BAL80009 presented three different fragment sequences of Tnt1 from three genotypes sized an average of 423+-(5) base pairs (bp); 0,093 πJC indicates nucleotide diversity with Jukes and Castor's (1969). However, no correlation was found between host, RNAseH and geographic origin, which suggests an early association of the Tnt1 superfamily in the evolution of Solanaceae. The RNAseH regions seem to be fixed at the host genus level while, within the genus, spreading was assured by the diversification of the U3 region. This may be due to the specific functions of these regions in the life cycle of retrotransposons, that lead to the co-evolution and adaptation of the constituent population and its host or environmental stress.

Siri et al. (2009) assessed the genetic diversity of *S. commersonii* and *S. malmeanum* from different geographical areas of Uruguay by using different PCR-based markers (three primers for AFLP, 10 primers for RAPD, and four primers for SSR). For RAPD analysis, in at least one pairwise comparison, the 10 primers selected produced a total of 179 discrete amplification products, of which 160 (89.4%) were polymorphic. The number of polymorphic bands produced by each primer was between 10 and 21, with an average frequency of 16. Using three selective primer combinations for AFLP analysis, a total of 105 bands were generated, of which 101 (96.2%) were polymorphic. The number of polymorphic bands per primer combination ranged from 23 to 41, with an average of 33.7. The primer pairs designed for the four SSR loci showed a total of 20 bands, all of which were polymorphic. Assay efficiency index (Ai), diversity index (DIavp) and genotype index (GI) were used to estimate the usefulness of each marker system. The Ai allowed them to compare techniques that detect multiple alleles and one or two bands per assay, for example, the SSR marker allowed to detect two alleles per band, and multiple bands per assay such as RAPD and AFLP markers. Furthermore, diversity index (DIavp) was higher for the SSR analysis (0.64), although Ai was higher for AFLP analysis (53.6), followed by RAPD (23.5) and SSR (4.72). The SSR had the lowest discriminatory power (0.27), followed by RAPD (0.90) and, finally, the highest by AFLP that detected many polymorphic bands per assay unit showing the best discriminatory power (0.99). The dendrogram analysis based on UPGMA, regardless of the PCR-based markers, proves that there is a highly consistency with the geographic origin of *S. malmeanum* (cluster B) from the north of Uruguay and *S. commersonii* (cluster A) from the south, previously characterized in morphology (see Siri et al. 2009). Due to representation of their respective similarity matrices (Jaccard's coefficient) followed by good cophenetic values (0.901 for RAPD, 0.945 for AFLP and 0.910 for SSR), either SSR, RAPD, and AFLP techniques, indicated that the dendrogram obtained using the three markers is an appropriate representation of their respective similarity matrix, and when associated with morphological features were able to discriminate *S. malmeanum* from *S. commersonii*.

Agronomical traits

Several interesting traits have been found in *S. malmeanum*, most of them not found in cultivated potato primary gene pool such as either resistance level against bacterial and verticillium wilt, ring rot, late and early blight, fusarium dry rot, hapla and cyst nematode, colorado potato beetle, potato leaf hopper, green peach aphid, potato aphid, and potato leafroll virus (PLRV) (Radcliffe and Laufr 1971; Flanders and Radcliffe 1992; Laferriere et al. 1999; Micheletto et al. 1999; Micheletto et al. 2000; Siri et al. 2009; USDA 2020) (Table 2). Valuable quality traits for industrial purposes such as dry matter, protein and low reducing sugar content on tubers were also reported (Rocha et al. 2000; Chalá et al. 2001; Jansen et al. 2001) (Table 3). It is especially highlighted its frost tolerance and ability to cold acclimate at field conditions (i.e., increase cold tolerance after exposure to low temperature), but also the capacity to be tolerant to heat (Vega and Bamberg 1995; USDA 2020).

Abiotic stresses

Frost and heat tolerance. *Solanum malmeanum* is particularly interesting due to its freezing tolerance and great capacity to cold acclimate (i.e., increase cold tolerance after exposure to low, non-freezing temperatures) (Hawkes 1958; Ross and Rowe 1965; Hawkes and Hjerting 1969; USDA 2020). Twenty-three accessions were reported to be frost resistant and one accession is also reported to be median resistant to heat stress (Table 2). It has been reported to be capable of surviving at extremely low temperatures, even so, this species proved to resist under negative temperatures between –0.55 °C to –5 °C, with none or a relatively small percentage (0–20%) of the leaf area damaged (Ross and Rowe 1965; Hawkes and Hjerting 1969; Vega and Bamberg 1995). Earlier observations reported that wild potatoes that have a rosette habit generally are higher resistant to cold and frost (Firbas and Ross 1962). Despite potatoes are mainly cultivated in temperate climates, freezing temperatures (–3 °C to –4 °C) causes a very adverse effect (Boydston et al. 2006). Most potatoes cultivars are sensitive to low temperatures and are unable to cold acclimate at temperatures lower than –3°C (Chen and Li 1980). Tu et al. (pre printed, 2021) successfully produced a frost-resistant somatic hybrid potato from protoplast fusion between the resistant diploid *S. malmeanum* (MLM266-2) and the susceptible dihaploid potato *S. tuberosum* (AC142), confirming the potential of this wild relative towards developing a cold resistant cultivar. Wild potatoes growing in lower elevations tended to be heat tolerant and less cold tolerant than species from higher elevations (Smillie et al. 1983), even so, tolerance to heat and cold stress can be positively correlated (Hetherington et al. 1983). Besides low temperatures and heat tolerance, drought resistance was not evaluated yet.

Biotic stresses

Bacterial.

Bacterial wilt. The range of *Solanum malmeanum* is geographically distributed in Southern South America, associated with the predominance of *R. solanacearum* phylotype II sequevars (Siri 2005; Santiago et al. 2017). The species is documented to possess interesting levels of resistance against *R. solanacearum* phylotype II sequevars 1-2 previously isolated from different potato fields in Uruguay (Siri et al. 2009). Resistance was evaluated by visual observation from three replicates under greenhouse conditions. Two accessions were classified to be resistant (i.e., absence of wilting symptoms) and three accessions demonstrate moderate resistance (i.e., incomplete, or slight wilting, delayed symptoms, or wilting symptoms not observed in all replicates). However, little is known about the resistance across the wide spectrum of distribution. Considering that each strain of bacterial wilt is present at specific geographical regions, the knowledge of its variability and diversity could be an efficient guide to identify new potential sources of resistance from different geographical populations. Resistant accessions are summarized in Table 3.

Bacterial ring rot. Two accessions (PI 458318 and PI 472841) proved to be resistant against bacterial ring rot of potato (Table 3). Ring rot is caused by the *Corynebacterium michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff) (USDA 2020) (Table 3).

Fungal.

Early blight: One accession was reported to be early blight resistant (Table 3). Accession PI 472840 from Argentina was evaluated and has been reported to be resistant against potato early blight (Table 3) (USDA 2020). The disease is caused by *Alternaria solani* (Ellis. & G. Martin) Ser., a leaf-spotting and defoliation agent that is responsible for significantly reducing yields in many tropical and subtropical producing regions.

Potato late blight. In total thirteen accession were reported to be late blight resistant (Table 3). Four genotypes (OKA 7310.01, OKA 7256.08, OKA 7256.07, OKA 7256.01) showed incompatible reaction after inoculation with *R0* in trials under greenhouse conditions. The second experiment aimed to reconfirm the presence of *R*-genes and the offspring produced by the cross between OKA 7310.01 (resistant to *R0*) and OKA 7282.06 (susceptible to *R0*) was inoculated with a complex race of late blight. Among the offspring of 300 plants, 70% (210) showed incompatible (resistance) responses, confirming the presence of the *R* genes in *S. malmeanum* (Micheletto et al. 1999). The disease caused by the oomycete like-fungi *Phytophthora infestans* (Mont.) de Bary is considered the most important disease of potato crop in all producing regions worldwide (Santana et al. 2013; Göre et al. 2019). Micheletto and collaborators (2000) screened four Argentinean diploid accessions for quantitative resistance to late blight *P. infestans* virulent (R1, R3, R4, R5, R7, R10, R11) and non-virulent (*R0*) complex races under both greenhouse and field conditions during two seasons (1996/1997 and 1997/98) to assess the year × genotype interaction. Results of statistical analysis ($p=0.01$) measuring the area under the disease progress curve (AUDPC) showed high variability reaction of *P. infestans* among genotypes. Two accessions were susceptible (OKA 7282, OKA 7291), while two resistant accessions segregate for resistance (Table 2). OKA 7292 genotypes 5 and 9 and OKA 7256 genotypes 2, 7 and 9 performed as resistant. Furthermore, no genotype × year interaction was detected, and the behavior was consistent across years. Despite Micheletto and collaborators (1999; 2000) treat each accession conserved at the genebank of Instituto Nacional de Tecnologia Agropecuaria (INTA), Balcarce, Argentina based on an outdated taxonomic approach, here we confirm that accessions OKA 7281, OKA 7282, OKA 7291, OKA 7304, OKA 7310, OKA 7313 reported as "*S. commersonii*", are currently to be considered as *S. malmeanum* (Spooner et al. 2014; Spooner et al. 2016; USDA 2020). Furthermore, *S. malmeanum* (accession OCL 7256) was probably documented with a mistaken collector name OCL 7256, referring to OKA 7256 (Micheletto 1999; Micheletto 2000).

Verticillium wilt. The accession PI 472850 is reported to be immune against *Verticillium. dahliae* (Table 2) (USDA 2020). Verticillium wilt is one of the most important soilborne fungal diseases on potato, also known as potato early dying disease, caused by *V. dahliae* Klebahn and *V. albo-atrum* Reinke & Berthold (Frost et al. 2007; Simko and Haynes 2017; Li et al. 2019).

Table 3 Resistance against abiotic and biotic stresses reported for the potato wild relative *Solanum malmeanum* (Solanaceae).

Trait	Causal agent	Accession[1]	Source
Abiotic stress	Frost	PI 320269; PI 414154; PI 472840; PI 472841; PI 472841; PI 472843; PI 472844; PI 472845; PI 472846; PI 472847; PI 472848; PI 472849; PI 472851; PI 472852; PI 472853; PI 472854; PI 472855; PI 498408; PI 498416; PI 498417; PI 498418; PI 498419; MLM266-2	(USDA 2020; Tu et al.2021)
	Heat	PI 498416	(USDA 2020)
Biotic stress	Bacterial wilt (*R. solanacearum)*	*	(Siri et al. 2009)
	Ring rot (*C. michiganensis* subsp. *sepedonicus*)	PI 458318; PI 472841	(USDA 2020)
	Early blight (*A. solanii)*	PI 472840	(USDA 2020)
	Late blight (*P. infestans)*	OKA 7256; OKA 7256.01; OKA 7256.07; OKA 7256.08; OKA 7292; OKA 7310; OKA 7310.01	(Micheletto et al. 1999; Micheletto et al. 2000)
		PI 472844; PI 472846; PI 472847; PI 472850; PI 472853; PI 472855	(USDA 2020)
	Verticillium wilt (*V. dahlia)*	PI 472851	(USDA 2020)
	Green peach aphid (*M. persicae*)	PI 320269; PI 458318; PI 472840	(Radcliffe and Laufr 1971; USDA 2020)
	Potato aphid (*M. euphorbiae*)	PI 320269	(Radcliffe and Laufr 1971)
	Colorado potato beetle (*L. decemlineata*)	PI 458318	(Flanders and Radcliffe 1992)
	Potato leafhopper (*E. fabae*)	PI 472851; PI 472843	(Flanders and Radcliffe 1992)
	Potato cyst nematode (*G. rostochiensis)*	*	(Castelli et al. 2003)
	Root-knot nematode (*M. hapla)*	PI 472841	(USDA 2020)
	Potato leaf roll virus (*Polerovirus* sp.)	PI 458318	(USDA 2020)

[1] Accessions as: (PI) Plant introduction, (CN) Collector number, or (CL) Clone number. (*) No data provided.

Fusarium dry rot. Resistant accessions are presented in Table 2. One accession (PI 414154) was screened in tuber disk essay for resistance to *Fusarium sambucinum*, but the results are inconclusive (Lynch et al. 2003). Fusarium dry rot of potato tubers is caused by several species such as *F. avenaceum, F. culmorum, F. equiseti, F. oxysporum, F. sambucinum, F. solani,* among others. It is a severe disease responsible for causing significant economic losses principally during the post-harvest and storage periods (Peters et al. 2008; Du et al. 2012; Gachango et al. 2012).

Nematodes. *Solanum malmeanum* showed full resistance to potato cyst nematode *Globodera rostochiensis* (average cyst mature female count of => 5) and susceptibility to *G. pallida*, but the inheritance of resistance was not investigated (Castelli et al. 2003). Furthermore, it has also been reported to be resistant against root-knot nematode (*Meloidogyne hapla*) (Table 2) (USDA 2020). Nematodes, when the population density is high, negatively affect potato production from the field, restricting plant growth, to the post-harvest period, rendering tubers unmarketable (Contina et al. 2019; Koirala et al. 2020).

Insect Pest. *Solanum malmeanum* was intermediary resistant against potato leafhopper (*Empoasca fabae* Harris), although the defense mechanisms have not been elucidated, fully resistant to potato aphid *Macrosiphum euphorbiae* (Thomas) and potato green peach aphid *Myzus persicae* (Sulzer) (Table 2) (Radcliffe and Laufr 1971; USDA 2020). Insect resistance in potato is mainly associated to morphological features, commonly glandular trichomes (type A and type B) with high trichome densities on leaves protecting from herbivory (Flanders et al. 1992; Jansky et al. 2009).

Viruses. Accession PI 458318 from Argentina has been reported to be resistant against potato leaf roll virus (*Polerovirus* sp.) (Table 2) (USDA 2020). However, *S. malmeanum* has also been reported to be susceptible and spontaneously infected by the systematic ringspot virus (TMV tobacco mosaic virus) that is transmitted from generation to generation via tubers (Hansen 1960).

Quality traits for production and industry

Tuber dry matter, starch, and protein contents. *Solanum malmeanum* has been reported (Table 3) to contain a range from 28.6% to 34.62% of dry matter content (Rocha et al. 2000; Jansen et al. 2001), 15.4 to 23% starch content (Jansen 2001), 4.5% of protein in tuber dry matter content, and 32.1% of amylose content in starch, even so the mean particle diameter of starch granules measured 45.8 μm (CV%=9.2) (Jansen et al. 2001). In addition, reducing sugars on its tubers have been evaluated from two different growing seasons, with an average of 0.22% in spring, and 0.33% in autumn, far below the levels presented in the reference cultivar 'Baronesa' (*S. tuberosum*) with 0.75% in autumn and 0.63% in spring (Chalá et al. 2001). A strong association in diploid *S. malmeanum* clones was verified between glutamate oxaloacetate transaminase (GOT) at relative mobility band 1.00 of electrophoretic standards for aspartate transaminase and the high dry matter content (average of 34.62 of dry matter content on tubers). Conversely, Andreu and Pereira (2004) did not find a significative association between the band 1.00 of GOT with the dry matter content of the *S. tuberosum* tetraploid potato. GOT may be efficient in more accurately predicting this character in the early stages of selection in diploid than tetraploid species (Andreu and Pereira 2004).

Table 4 Quality traits found in potato wild relative *Solanum malmeanum* accessions. Data provided from references as follows. ^{a}(CL) Clone number (-) No data provided.

Organ	**Specificities**	**Accession^{a}**	**References**
Tubers	Dry matter content 28.6% - 34.62%	(CL55; CL57; CL60; CL63; CL65)	(Rocha et al. 2000; Jansen 2001)
	Tuber starch 15.4- 23%	*	(Jansen et al. 2001)
	Starch granules size 45.8 um	*	(Jansen et al. 2001)
	Reducing sugar 0.19-0.33%	(CL63; CL65)	(Chalá et al. 2001)
	Protein 4.5% of dry matter	*	(Jansen et al. 2001)
	Amylose 32.1% of amylose content in starch	*	(Jansen et al. 2001)
Leaves	Glycoalkaloid (dehydrocommersonine) 23%	PI 320269	(Gregory et al. (1981)

Glycoalkaloids. *Solanum* ser. *Commersoniana* species mainly produces α-solanine and β-chaconine (Schreiber 1963). *Solanum malmeanum* accession PI 320269 (cited as *S. commersonii*) leaves have been reported (Table 3) to contain 23% (mg/g dry leaves) of glycoalkaloid content, whereas 100% is dehydrocommersonine, despite no level detected for α-solanine or β-chaconine, or these later coumpounds were at too low content to be detectable (<3%) (Gregory et al. 1981) (Table 3). Dehydrocommersonine present in *S. oplocense* was associated with host resistance against *Leptinotarsa decemlineata* (Tai et al. 2014; Tai et al. 2015), then *S. malmeanum* could be predicted as a potential source of resistance against this pest. Steroidal glycoalkaloids are secondary metabolites toxic for human consumption, although they may also have advantages to improve resistance against viral, insect-pests or micro-organisms diseases. They are controlled by genetic factors (Mensinga et al. 2005) and even though the increasing foliar steroidal glycoalkaloids are frequently associated with high contents in tuber (Sanford et al. 1992), they are accumulated during the development of each organ (Krits et al. 2007; Mweetwa et al. 2012; Paudel et al. 2019; Zhen et al. 2019). In addition, this different accumulation behavior allows to change the expression and the distribution of accumulation in specific plant tissues across some generations of selection (Sanford et al. 1992; Umemoto et al. 2016; Nahar et al. 2017; Zhen et al. 2019).

Cytogenetics

The basic chromosome number of *S. malmeanum* is x=12 (2n), with occasional triploid plants (Hawkes and Hjerting 1969; Tarn and Hawkes 1986; Spooner et al. 2016). Diploid plants usually produce higher frequency of stainable pollen than triploids. The mean pollen diameter is 23 µm in diploid plants and varies from 18.5 to 24.9 µm in triploids (Tarn and Hawkes 1986).

This species generally showed regular meiotic abnormalities observed at microsporogenesis, including chromosomes out of the equatorial plate in metaphases I and II (MI and MII), and lagging chromosomes in anaphases I and II, and telophases I and II (Pandolfi 1998; Tomé et al. 2007; Tomé et al. 2009). Furthermore, it was observed a range of 3.7% to 50.8% frequency of pollen mother cells (PMC) with meiosis abnormalities. The metaphase I showed 42% of abnormal PMC, differing significantly from anaphase I and telophase I with 18%, metaphase II with 21% and anaphase II and telophase II with 13% of abnormal PMC. Such decrease in the frequency of abnormal PMC and the average good pollen viability (60.33%) suggests the existence of selection against these abnormal PMC cells during the microsporogenesis process with implications for the choice of pollen donors for crosses. In both techniques, the pollen viability was significantly higher in *S. malmeanum* than in cultivated potatoes (Pandolfi 1998; Tomé et al. 2007). *Solanum malmeanum* is known to produce high pollen amount, ranging from 454.20 to 476.20 pollen grains per field on the glass slide, exceeding the means of *S. tuberosum* clones that produce less than 307.4 pollen grains per field on the glass slide (Tomé et al. 2007). A high pollen viability for *S. malmeanum* was verified by both techniques such as acetocarmine staining 2% (60.33–96.5% viability) and in vitro germination (78.5–75% viability) (Pandolfi 1998; Tomé et al. 2007). Although the genotypes SCM57 and SCM60 did not show non-reduced pollen grain, they had meiotic mechanisms (parallel spindles) that possibly can lead to the formation of non-reduced pollen, presenting even the highest percentages of cells with parallel spindles (27% and 22%). Furthermore, pollen grain size of *S. commersonii* (13.68–35.52 µm) tends to be larger than of *S. malmeanum* (11.4 to 23.9 µm) (Tomé et al. 2009). This approach put forward by Tomé and collaborators (2009) raises the hypothesis that *S. malmeanum* has no pollen grain diameters satisfying the value of 25 µm suggested by Quinn et al. (1974) and Ramanna (1974) and would imply on the proposition of a new limit for discrimination of unreduced pollen grains. However, the selection of 2n pollen based only on the size and shape of the grain is feasible only in species with known differences between haploid and diploid pollen grains (Parrott and Smith 1984; Parrott and Smith 1986). Two of 12 clones of *S. malmeanum* (PI 414154, cited as "*S. commersonii*") were reported to produce 2n pollen (den Nijs and Peloquin 1977a). Therefore, it is expected that in natural populations, functional 2n gametes can be found.

Reproductive biology, breeding system and Endosperm Balance Number (EBN)

Solanum malmeanum reproduces sexually and asexually, as most of the potato wild relatives. It is a tertiary gene pool (1EBN) potato relative and is considered reproductively isolated from other tuber-bearing 2EBN and 4EBN species, including the cultivated potatoes (*S. tuberosum*, 4x 4EBN), by different crossability groups (Hanneman 1994; Spooner et al. 2016). Data on its self-compatibility (SC) or self-incompatibility (SI) behavior are lacking.

Hanneman (1994), used a diploid female plant of *S. malmeanum* for the assignment of the EBN on crossing tests. The male parent species with standard EBN selected were: *S. cardiophyllum* 2x (1EBN), *S. commersonii* 2x (1EBN), *S. chacoense* 2x (2EBN), *S. tuberosum* Group Phureja 2x (2EBN), *S. chacoense* 4x (4, 7 and 8EBN by colchicine doubling), and *S. tuberosum* Group Andigena 4x (4EBN). Although only one accession of *S. malmeanum* was used as female to determine its EBN, it produced an average of six seeds per fruit from any crosses with male standard species 2x (1EBN), less than one seed per fruit from 2x (2EBN) species, and no seed set from 4x (4EBN) crosses. Therefore, it was designated as 1EBN.

Crosses between different potato species with the same EBN number, regardless of ploidy, are usually successful (Johnston and Hanneman 1980; Johnston and Hanneman 1982). Theoretically, species with the same EBN may be intercrossed in nature if sympatry and synchronized flowering occur and pre-zygotic barriers are absent. Crossability essays with both species that can be sympatric with *S. malmeanum* were tested and *S. malmeanum* (1 EBN) was reported to be cross-incompatible with *S. chacoense* (2EBN) (Summers and Grun 1981) and assumed as easily crossable with *S. commersonii* (1EBN) (Tarn and Hawkes 1986).

Tarn and Hawkes (1986) obtained vigorous and fertile hybrids progenies from reciprocal crosses between 2x *S. malmeanum* and 2x *S. commersonii*. The seed production per berry and mean weight per 100 seeds were equally variable. *Solanum commersonii* produces fewer (66.5), but heavier seeds (52.8 mg per 100 seeds) per berry than *S. malmeanum* (128.4 seeds per berry, with 12.8 mg per 100 seeds) (Tarn and Hawkes 1986). Furthermore, the seedlings grown from reciprocal crosses between *S. commersonii* and *S. malmeanum* usually were vigorous, fertile, highly variable and generally displayed intermediate morphology between the two parental species (Tarn and Hawkes 1986). It is interesting to note that parental traits as flower pigmentation (typically purple-pigmented corolla in *S. commersonii* and white (not-pigmented) corolla in *S. malmeanum*), always segregate in F1 progenies with intermediate phenotypes.

Despite Hawkes and Hjerting (1969) made significant efforts to perform reciprocal crosses with *S. chacoense*, they obtained only one seed that germinated and generated a vigorous plant. By the other hand, Ehlenfeldt and Hanneman (1988) produced a small number of seeds from many hand pollinations between *S. malmeanum* (accession PI 320269, cited as "*S. commersonii*") (1EBN) and *S. chacoense* (2EBN) (see Ehlenfeldt and Hanneman 1988). As a result, the F1 hybrids were heterozygous (assigned as 1 ½ EBN) for those alleles determining the different EBN of the parental species. In addition, the meiotic products of the hybrids may hold different combinations of parental alleles. F1 Hybrid offspring produce neither consistent development nor consistent endosperm failure, but the ratio of each type of endosperm was variable. In backcrosses of the F1 hybrids as male parent with *S. chacoense* (2EBN) as female parent, about 50% of seeds aborted and the viable seeds were small to medium in size. In other way, backcrossing *S. chacoense* as male parent with F1 hybrids as female parent, most seeds were inviable, but the small number of viable seeds were medium to large in size. In crosses of F1 hybrids with *S. malmeanum* (1EBN), the outcomes were reversed: viable seeds were medium to large with the F1 hybrid as male parent, but small to medium in the reciprocal cross. Therefore, the results are consistent with a positive relation between seed size and relative paternal activity, except that seeds are aborted when paternal activity is either extremely high or extremely low. The results of the hybrid offspring of *S. malmeanum* × *S. chacoense* are satisfactory, but the endosperm barrier between these two species greatly limits viable seed development. The difference in the ratio of viable seeds to aborted seeds seems to be a directional effect, related to which parent is male and which parent is female in any given cross, but it does not seem to be a cytoplasmic effect, because the same female may produce different results depending on pollen source. This effect is most likely due to the dose-effect in the endosperm (Ehlenfeldt and Hanneman 1988).

Crosses between 4x (2EBN) *S. hjertingii* and *S. papita* as female parent with parental pollinator 2x 1(EBN) *S. malmeanum* (PI 320269, cited as "*S. commersonii*") were done to generate haploid-plants. A total of 303 pollinations between *S. hjertingii* × *S. malmeanum* produced 29 berries with only 2 seeds in total, generating only one triploid offspring plant. From 263 pollinations between *S. papita* × *S. malmeanum*, a total of 65 berries with 70 seeds were produced in total originating 17 tetraploid plants and one triploid plant (Singsit and Hanneman 1987; Singsit and Hanneman1991).

Crossability barriers between 2x 2EBN *S. chacoense* and 2x 1EBN *S. malmeanum* are well-established, but no sexual barriers were observed between 2x 1EBN *S. commersonii* and 2x 1EBN *S. malmeanum*, elucidating that EBN is a strong internal barrier (post-zygotic) (Johnston et al. 1980; Summers and Grun 1981; Hanneman 1994). Thus, it may predict that *S. malmeanum* could freely cross with others 1EBN sympatric wild potatoes such as 2x 1EBN *S. commersonii*, but not with 2EBN such as *S. chacoense*, except when 2n gametes are present (Hawkes and Hjerting 1969; Hanneman 1994).

Strategies to use wild potatoes in the breeding of potatoes for the 21st century

Breeding potatoes with wild species is challenging. *Solanum malmeanum* is cross-incompatible with cultivated potatoes due to the strong post-zygotic barrier of EBN (Hanneman 1994). To overcome the barrier of hybridization residing on EBN, breeders usually use di-haploids (2x 2EBN) derived from tetraploid *S. tuberosum* (Group Tuberosum or Group Phureja) or wild relatives (2x 2EBN) for genetic studies (Hermundstad and Peloquin 1985a; Peloquin et al. 1989b; Jansky et al. 1990; Carputo et al. 2003) or bridge crosses to transmit their genetic diversity and generate 4x progeny (Peloquin et al. 1989a; Peloquin et al. 1989b; Werner and Peloquin 1991a; Masuelli et al. 1992; Carputo et al. 2003; Jansky and Hamernik 2009). Because haploids are male sterile lineages derived from tetraploids *S. tuberosum* (2n = 2x = 24), they can be used in the crosses as female parental to most of the diploids (2n = 2x = 24) (Hanneman 1999). However, the efficiency of this technique is determined either by the production ability of seed set from the female haploid genitor (*S. tuberosum*), and the performance of the wild pollinator genitor (Hermsen and Verdenius 1973; Werner and Peloquin 1991d).

Once the 2n gametes are absent in *S. malmeanum*, it is possible to direct crossing it with di-haploids of *S. tuberosum* by chromosome duplication via ploidy manipulation, to convert *S. malmeanum* 2x (1EBN) to 4x (2EBN) or 6x (4EBN). Diploid F1 offspring that produces 2n gametes could be an efficient method in capturing allelic diversity from most of the diploid wild potatoes (Hermundstad and Peloquin 1985a; Peloquin et al. 1989b; Jansky et al. 1990; Jansky and Peloquin 2006). Superior 2x *S. tuberosum* × wild species hybrids can be screened for desirable agronomical traits coupled with 2n gametes (2n pollen and 2n eggs) and male fertility (Hermundstad and Peloquin 1985b; Werner and Peloquin 1991a; Serquén and Peloquin 1996; Jansky and Peloquin 2006; Jansky 2011). Furthermore, crossing 2x species via 2n eggs or 2n pollen, or colchicine doubling the 2x hybrid parents, can generate 4x progeny (Jansky 2006). Tetraploid progeny can be selected by the maximum intra- and inter-loci interactions (Peloquin et al. 1989b; Werner and Peloquin 1991b; Werner and Peloquin 1991c), as depicted in the scheme of Figure 4, adapted from Peloquin et al. (1989) and Carputo et al. (2003).

A successful example of introgressive hybridization with 2x 1EBN *S. commersonii* was performed by the mean of bridge-cross with a *S. tuberosum* Group Phureja species along with non-reduced gametes (2n gametes) to overcome sexual barriers of hybridization and improve resistance to bacterial wilt in tetraploid potatoes (4x 4EBN) *S. tuberosum* Group Tuberosum (González 2010; Gaiero et al. 2017). Given the similarity of *S. commersonii* and *S. malmeanum*, it is expected the same approach could be followed for successful introgressions of the later species genes into the *S. tuberosum* primary genepool. These approaches elucidate the utility of the EBN concept coupled with 2n gametes, as means to transmit allelic diversity from distant species of 1EBN group (Ehlenfeldt and Hanneman 1984; Peloquin et al. 1989a; Peloquin et al. 1989b). Furthermore, the evidence on 2n pollen production in *S. malmeanum* previously reported (Pandolfi 1998; Tomé et al. 2007; Tomé et al. 2009) enhance the possibilities to introgress its genetic diversity and desirable traits into the cultivated potato genepool.

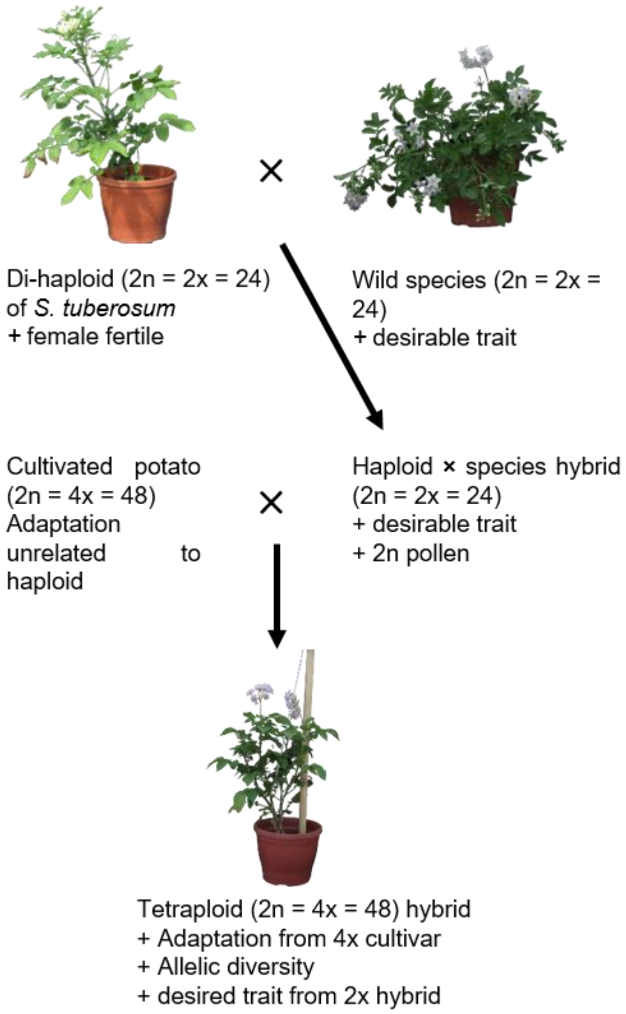

Fig 5 Potato breeding scheme using wild species, haploids, and 2n pollen to obtain 4x progeny from 4x × 2x crosses. Adapted by Rodrigo Nicolao based on Peloquin et al. (1989) and Carputo et al. (2003).

Diploid hybrid breeding

The transformation of vegetatively propagated potato crop into a diploid potato hybrid crop has an increasing interest worldwide. The potato crop propagated via true potato seeds (TPS) has been enlighted as a modern alternative wayof the traditional breeding and cultivation. The main advantages of breeding at diploid level are to reduce transportation and storage costs associated with the potential for increase genetic gains in yield, facilitates the introgression of pests and diseases, to reduce virus transmission, as to reduces the pests and diseases spread (Lindhout et al. 2011a; Lindhout et al. 2011b; Endelman and Jansky 2016; Jansky et al. 2016). Homozygous diploids are more efficient to generate than homozygous tetraploid potatoes. Seven generations of selfing are required to obtain 50% homozygous loci starting from a tetragenic tetraploid heterozygote (carrying four different alleles). The same homozygosity level can be reach starting from a heterozygous diploid by only one generation of selfing. However, self-incompatibility and inbreeding depression have for long time been hampered the advance of hybrid potato breeding at diploid level (De Jong and Rowe 1971; Charlesworth and Willis 2009; Jansky et al 2016). The introduction of the S-locus inhibitor (*Sli*) gene, previously identified from diploid *S. chacoense* (Hosaka and Hanneman 1998a; Hosaka and Hanneman 1998b) and by an extensive and consistent breeding program (Lindhout et al. 2011a), made it possible to create the first diploid inbred-potato 'clone M6' of *S. chacoense* carrying the dominant self-incompatibility inhibitor gene *Sli* (Jansky et al. 2014). These recent developments are opening new ways for introgression of diploid wild potatoes desirable traits and the quest for self-compatibility in *S. malmeanum* genotypes is advisable.

Tools to aid the improvement of new potato cultivars

The natural genetic variability of *S. malmeanum* is mainly ex-situ conserved at several gene banks worldwide by clonal manners (i.e., vegetative tuber, in vitro) or by botanical seeds (Salas 2008). Even so, gene banks are lacking basic information of this species related to the taxonomy, breeding system, crossability, and agronomical traits such as biotic and abiotic resistance. Most wild and cultivated potato introductions have been commonly characterized for qualitative and quantitative traits adopting the potato descriptors such as the morphological ones proposed by Huamán et al. (1998). Furthermore, one useful kit of simple sequence repeats (SSR) by Ghislain and collaborators (2004; 2009) made available markers for use in characterization of potato genetic resources. Recently, the genome-wide SNP markers on the Infinium 12K V2 Potato Array enabled a better understanding of potato genetic diversity, population structure and genetic relationship, and is considered an important tool for the efficient maintenance, conservation and use of potato genetic resources (Ellis et al. 2019).

Genebanks carry out pre-breeding efforts to introduce valuable traits from germplasm into the cultivated gene pool (Sharma et al. 2013; Christensen et al. 2017; Rakosy-Tican et al. 2020). Potato breeders require genetic variability for the continuous improvement process, especially for genes that encode to agronomic traits for more resilient and productive new cultivars. If systematically characterized, any potato wild relatives can be efficiently used in breeding programs. The challenges may be overcome by plant breeding programs from carrying out pre-breeding efforts associated with multidisciplinary collaboration (i.e., agronomists, botanists, phytopathologists, plant physiologists, geneticist, bioinformaticians, statisticians...) (Warschefsky et al. 2014; Yu et al. 2016; Masher et al. 2019; Bruning et al. 2020).

While primary and secondary CWRs are of high conservation priority, we do not deny the need to assign conservation priority to taxa in tertiary wild relatives. In this regard, there are two specific considerations when applying Criterion 2 in CWR conservation planning. Taxa that have already been used in plant breeding or that are known to contain traits of interest for crop improvement mainly due to climate change scenarios (increasing the likelihood of them being used in the future) should be given high priority status (Maxted and Kell 2009). The most primitive potato species located into the tertiary gene pool (1EBN) are a valuable source of those genes of resistance to combat biotic (Castelli et al. 2003; Lynch et al. 2003; Siri et al. 2009) and abiotic stressors (Esposito et al. 2017; Esposito et al. 2020) as well as many interesting agronomic traits (Kuhl et al. 2001; Jansen et al. 2001; Jansky and Hamernik 2009), including processing quality traits for industry such as starch and chip quality (i.e., reducing sugar content) (Hanneman 1993; Hanneman 1996; Chalá et al. 2001; Jansen et al. 2001). The increased use of the genetic diversity sources from wild potatoes germplasm may demand an extensive multidisciplinary work associating many tools. Fortunately, several approaches are available to allow the capture of desirable alleles, such as resistance to pathogens, as well as to expand the genetic variability of the germplasm of the cultivated potato (Li et al. 2018; Masher et al., 2019; Tu et al. 2021).

Concluding remarks

The confuse taxonomic history of *S. malmeanum* hindered its true potential for potato breeding turning it into a neglected wild relative shaded by *S. commersonii*. The reinstatement at species level brings new light to a comeback and promising future for the applied uses of this wild potato in classic and avant-garde new techniques for potato breeding. Towards the truly exploitation of this promising potato genetic resource, the current taxonomy resulting from the last treatment must be updated in all gene banks and reference collections to allow better use of the conserved germplasm and enhance its applied uses.

The morphological variability, wide geographic distribution, diverse array of habitats occupied and ecological preferences from this species are not fully represented in current genebanks. Limited studies were performed to assess the genetic variability and the few available ones not fully covered these aspects.

Important agronomical traits were documented, although comprehensive studies on this species are lacking and most of the references available for response to abiotic and biotic stresses, and quality traits for production and industry are from occasional works or have evaluated *S. malmeanum* as “*S. commersonii*”. Assessments of all the germplasm available for desirable traits targeting *S. malmeanum* are needed to highlight the best performing genotypes.

Sequencing approaches may allow a better understanding of the genetics of *S. malmeanum* linking molecular markers with the target traits. Plant breeders require genetic variability information to speed up the search of each desirable agronomic trait, particularly resistance (*R*) genes, tolerance against stresses, as well as for the genes encoding for quality agronomical traits. The combination of resistance QTLs and complementary modes are interesting strategies for potentially durable resistance. Furthermore, marker assisted selection may be efficient to guide the introgression of desirable traits as well as to pyramiding of multiple resistance to achieve field resistance.

As a final remark, we highlight that *S. malmeanum* potential for potato breeding have been neglected, especially when compared to other closer potato wild relatives, and must be properly addressed with contemporary molecular tools to unlock its applied use as a promising potato wild relative to face the challenges imposed to the potato crop in the 21st century.

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Chapter 2. How to unlock the reproductive isolation of *Solanum malmeanum*, a promising wild relative to broaden the potato crop genetic basis?*

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Abstract. *Solanum malmeanum* is a South American wild potato which harbors agronomical traits desirable to broad the genetic basis of the potato. This species is in the *S. tuberosum* tertiary gene pool, assigned as 1EBN (Endosperm Balance Number). So, it is hypothesized as cross-incompatible with 2EBN and 4EBN species, the later including the potato crop. For an efficient use of genetic resources, it is necessary to characterize the germplasm according to the breeding system behavior. Then, data on its mode of reproduction could help to find pathways to overcome reproductive isolation, allowing hybridizations to introgress desirable traits. To drive genotypes towards pre-breeding efforts, this study characterizes 10 accessions (BGB015, BGB017, BGB021, BGB080, BGB081, BGB084, BGB446, BGB447, BGB448, BGB471) of *S. malmeanum* from Embrapa Clima Temperado Potato Genebank to evaluate pollen production and test the occurrence of parthenocarpy, apomixis, autogamy, allogamy, and the response to reciprocal crossings with 2x 1EBN *S. commersonii* (BGB001), 2x 2EBN *S. chacoense* (BGB444), and the cultivated 4x 4EBN *S. tuberosum* (cultivar BRS ANA and advanced breeding line C1750-15-95). The five *S. malmeanum* diploid accessions (BGB015, BGB017, BGB446, BGB447, BGB471) and three triploid accessions (BGB021, BGB080, BGB448) produce pollen and are male fertile, meanwhile two triploid accessions (BGB081, BGB084) do not produce pollen and are male sterile. Two triploid accessions (BGB021, BGB448) produce induced or spontaneous parthenocarpic fruits lacking seeds. Self-pollination and apomixis are absent in all evaluated accessions. There are no autogamous self-pollinated accessions, however, one self-compatible accession (BGB447) was identified through mediated self-pollination treatment (geitonogamy). The five diploids (BGB015, BGB017, BGB446, BGB447, BGB471) are allogamous and fertile and two triploids (BGB080, BGB448) are allogamous and partially fertile due to below average fruit and seed set and below average germination, while three triploids (BGB021, BGB081, BGB084) do not produce seeds and reproduce only vegetatively. The diploid accessions easily hybridize with 2x (1EBN) *S. commersonii* and produce fruits with normal seeds and germination rate. Average number of seeds was 116.3 and germination was 33%, when *S. malmeanum* is crossed as female parent with *S. commersonii*. Average number of seeds was 114.7 and germination was 41% when crossed as male parent with *S. commersonii*. When diploid *S. malmeanum* accessions were crossed as female parent with 2x (2EBN) *S. chacoense*, the seeds were malformed (average number of seeds of 8.8), and had low germination percentage (13.6%), conversely, reciprocal crosses produced lower average of seeds per berry (0.11), and no seed germinated. The direct crosses between *S. malmeanum* (1EBN) and *S. tuberosum* (4EBN) confirmed the expected cross-incompatibility. However, the partially successful results when crossed with *S. chacoense* (2EBN) suggests that other ways of introgression through indirect crosses mediated by bridge species are possible and could lead to alternative pathways to unlock *S. malmeanum* reproductive barriers.

Key words Crop wild relatives (CWR) · Crossability · Endosperm Balance Number (EBN) · Mode of reproduction · Interspecific crosses · Solanaceae

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Introduction

Potato wild relatives (*Solanum* sect. *Petota* Dumort., Solanaceae) represent a large genetic reservoir for potato (*Solanum tuberosum* L.) breeding (Hawkes 1958; Hanneman 1989; Bradshaw et al. 2006; Birch et al. 2012; Jansky et al. 2013; Hardigan et al. 2017), while reproductive barriers of hybridization maintain the substantial diversity presented in each population of wild potato. Then, understanding these barriers and developing techniques to overcome them is primordial to exploiting this genetic variability for breeding. Screening of germplasm for desirable traits are largely documented and some traits have been successfully introgressed into modern potatoes, meanwhile countless genes for novel traits, and resistances for abiotic and biotic stresses not found in commercial cultivars are still to be assessed and introgressed (Bashir et al. 2021).

During many years, the plant sciences focused on the collection of as much as possible samples of crop wild relatives (CWR). Nowadays, such efforts are still underway in a global and multinational task to collect, conserve, characterize and pre-breed plant genetic resources for food and agriculture (FAO, 2009). Samples of wild potatoes are worldwide conserved at genebanks and many endeavors started to adapt agriculture to climate change using CWR for broadening the gene pool of crops (Dempewolf et al. 2014; Koury et al. 2019).

Potato and its wild relatives comprise a phylogenetic lineage represented by 107 wild species, four domesticated species and their natural and artificial hybrids (Spooner et al. 2014). Wild potatoes reproduce by two ways: sexually (e.g., botanical seeds, also called “True Potato Seeds”) and vegetatively (e.g., stolons and tubers) (Hawkes 1958; Correll 1962). Sexual reproduction favors for the continuous genetic flow by the inter- and intraspecific hybridization, and permits generating genetic variability and new traits expression, whereas the vegetative reproduction could guarantee the genetic fixation of individuals or populations in unfavorable environmental conditions (Hawkes and Hjerting 1969). Wild potatoes are allogamous, with remarkably similar floral structures (Howard and Swaminathan 1952). In nature, the main pollinator of wild potatoes are the bumblebees (*Bombus* spp.), which can fly distances varying from 100 to 1750 meters (Batra 1993; Walther-Hellwig and Frankl 2000), facilitating geneflow and interspecific hybridization between compatible and sympatric populations, while rivers and animals that feed on the berries are considered the most likely potential dispersers.

Around 70% of the wild potato species are diploid (Hawkes 1990; Spooner et al. 2014), and are allogamous due to the action of gametophytic self-incompatibility system governed by multiallelic *S*-locus, which act at the pollen-stigma or pollen-style level (Grun and Aubertin 1966; Camadro and Peloquin 1981; Camadro et al. 2004). The *S* alleles are codominantly expressed in the style (Pushkharnath 1942; Pandey 1962; Cipar et al. 1964). During the process between pollination and fertilization, when there are one or more alleles present in both the pollen grain and the style, the pollen cannot germinate or penetrate through the stigma due the *S*-RNase produced by the style presents a protein cytotoxic action (Kao and Mccubbin 1996) that inhibits the growth of genetically matching pollen tubes (Luu et al. 2000). On the other hand, polyploids are allogamous with variable autogamy, then, self-fertilization can occur due to a phenomenon known as ‘competitive interaction’ in pollen grains that are heterozygous at the *S* locus (Frankel and Galun 1977).

The post-zygotic barrier of hybridization act in the embryo-endosperm development level, leading to aberrations in chromosome pairing, and is observed through embryonic abortion, male sterility, and breakdown of F1 or F2 hybrid offspring (Camadro et al. 2004; Camadro et al. 2012). According to Johnston et al. (1980), this post-zygotic barrier is explained under the hypothesis of the Endosperm Balance Number (EBN) in which *Solanum* sect. *Petota* species have a true ploidy number (determined by their actual number of chromosomes), and an "effective" ploidy generated by the action of hypothetical genetic factors (called EBN) (Johnston et al. 1980). This system requires a 2:1 maternal to paternal EBN dosage for a normal endosperm development, which could predict the success of hybridization of some specific crosses. The EBNs have been determined empirically for wild potato species by crossing each one with a standard species of known EBN (Hijmans and Spooner 2001; Hanneman 1994; Mansuelli and Camadro, 1997), furthermore, EBNs are also under genetic control (Ehlenfeldt and Hanneman, 1988; Camadro and Mansuelli 1995; Johnston and Hanneman 1996).

Besides, potato species are polyploid forming series, and its ploidy and EBN values combinations follow 2x(1EBN), 2x(2EBN), 4x(2EBN), 4x(4EBN) and 6x(4EBN) (Spooner et al. 2008; Rodríguez and Spooner 2009; Cai et al. 2012; Spooner et al. 2014). Bradeen et al. (2011) explained the use of the Gene Pool concept with the knowledge of the EBN to bridge the gaps and elucidate the cross-ability of wild potato species with the cultivated crop. Spooner et al. (2014) proposed five cross-ability groups to guide the potato breeders for an efficient use of wild potato species germplasm. This crossing scheme follows the endosperm balance number (EBN) along the ploidy, and self-compatible (SC) / self-incompatible (SI) systems of each potato species. The first group comprises the cultivated potatoes, landraces, and its wild relatives designated as 4EBN (4x, 6x), the second group is the most representative in number of members, and includes the 2EBN (2x, 4x) species, and the third group includes the 1EBN (2x) species. Within the 1EBN and 2EBN groups, SC species are isolated from the SI ones. The cross-incompatibility (CI) system of hybridization in potatoes can be either unilateral (UI) or bilateral (BI) (Camadro et al. 2004). Hybridization between SC species (as female) with the SI (as male) are frequently successful, but on the other hand, the reciprocal crosses usually fail, a phenomenon known as unilateral incompatibility. All 4EBN potato species are SC (Spooner et al. 2014). However, hybridization can occur inter-EBN groups if facilitated by 2n gametes when they are present (Mendiburu and Peloquin, 1977; Hanneman 1999; Iwanaga et al. 1989).

Solanum malmeanum Bitter is a South American wild potato that has been neglected in breeding programs. This species is known to be a 2x (1EBN) wild potato with occasional triploid (3x) cytotypes and is in the *S. tuberosum* L. tertiary gene pool (Hawkes and Hjerting 1969; Tarn and Hawkes, 1986). So, it is considered as CI with the 2EBN and 4EBN species groups (Hanneman 1994; Spooner et al. 2014; Clausen et al. 2018). However, variability in the mode of reproduction within a given species can occur. For example, self-compatible plants of *S. chacoense* have been identified (Hosaka and Hanneman 1998a) and the *Sli* gene (S-locus inhibitor) that allows diploid potatoes to self-fertilize was mapped at the distal end of chromosome 12 (Hosaka and Hanneman 1998b). Thus, besides rare within diploid potatoes species, self-compatible genotypes bearing the *Sli* gene are very desirable for breeders that aim to develop homozygous inbred lines of diploid potatoes (Alsahlany et al. 2021; Clot et al. 2020; Jansky et al. 2014). In addition, the prediction of the presence of 2n gametes in crosses with 2EBN species could allow the introgression of traits via bridge crossings.

Not all wild potatoes accessions conserved at gene banks have their breeding system well characterized, according to its pollen production, self- and cross-compatibility fertilization, 2n gametes production, male sterility, and so on. According to Hawkes and Hjerting (1969) the lack of knowledge in relation to the mode of reproduction of wild potatoes, especially the information about the breeding system, make it difficult to understand the pattern of variability encountered in the tuber-bearing *Solanum* species along their geographical distribution. Then, the characterization of the breeding system and the mode of reproduction of wild potatoes is a fundamental step to modulate specific strategies to overcome barriers of hybridization as well to test and design breeding schemes (Hanneman 1999; Lindhout et al. 2011a, 2011b). Therefore, for an efficient use of *S. malmeanum* genetic resources, it is necessary to characterize the germplasm according to the breeding system behavior. Then, data on the mode of reproduction of each accession could help to find pathways to overcome reproductive isolation, allowing hybridizations to introgress desirable traits via bridge crosses. Thus, this research aims to find how to unlock the reproductive isolation of *Solanum malmeanum*, a promising wild relative, to broaden the potato crop genetic basis based on two specific goals:

1. Characterization of the breeding system of 2x and 3x (1EBN) *Solanum malmeanum* accessions conserved at Embrapa Clima Temperado Potato Gene Bank for pollen production, self-pollination, apomixis, parthenocarpy, autogamy (self-compatibility/self-incompatibility), and allogamy.

2. Evaluation of the cross-ability relationships of 2x 1EBN *S. malmeanum* with 2x 1EBN *S. commersonii* Dunal, 2x 2EBN *S. chacoense* Bitter, and two cultivated potatoes 4x 4EBN *S. tuberosum*.

Material and Methods

Plant material

Fourteen accessions from four potato species were included in this study (Table 1): ten accessions of *S. malmeanum*, one accession of *S. chacoense,* one accession of *S. commersonii*, and two accessions of *S. tuberosum*, corresponding to one commercial cultivar (BRS ANA regarded as a good pollen receptor) and one clonal breeding line (C1550-15-95, considered a good pollen donor). The plants were cultivated from tubers under greenhouse conditions at Embrapa Clima Temperado in Pelotas, Rio Grande do Sul (32° 45’S, 52° 30’W and 50 m.a.s.l.) (Fig. 1). The treatments and crossing tests were done in the semesters 2019/2 and 2020/2, while the germination essays were carried out in 2021/1.

Table 1 Accessions of wild (*Solanum malmeanum*, *S. commersonii*, *S. chacoense*) and cultivated potatoes (*S. tuberosum*) from Embrapa Clima Temperado Potato gene Bank (Pelotas, RS, Brazil) evaluated to characterize the breeding system and cross-ability relationships.

Accession	Species	Ploidy	EBN	Origin	Lat	Long
BGB001	*S. commersonii*	2	1	Ijuí, RS, Brazil	-28.388	-53.915
BGB015	*S. malmeanum*	2	1	Augusto Pestana, RS, Brazil	-28.517	-53.992
BGB017	*S. malmeanum*	2	1	Santo Ângelo, RS, Brazil	-28.299	-54.263
BGB021	*S. malmeanum*	3	1	Horizontina, RS, Brazil	-27.626	-54.308
BGB080	*S. malmeanum*	3	1	São Miguel das Missões, RS, Brazil	-28.563	-54.554
BGB081	*S. malmeanum*	3	1	São Luiz Gonzaga, RS, Brazil	-28.408	-54.961
BGB084	*S. malmeanum*	3	1	Pelotas, RS, Brazil	-31.772	-52.343
BGB444	*S. chacoense*	2	2	Mafra, SC, Brazil	-26.116	-49.810
BGB446	*S. malmeanum*	2	1	Pelotas, RS, Brazil	-31.772	-52.342
BGB447	*S. malmeanum*	2	1	Porto Lucena, RS, Brazil	-27.856	-55.016
BGB448	*S. malmeanum*	3	1	Porto Lucena, RS, Brazil	-27.856	-55.016
BGB471	*S. malmeanum*	2	1	Canoinhas, SC, Brazil	-26.187	-50.368
BRSANA	*S. tuberosum*	4	4	Cultivar	-	-
C1750-15-95	*S. tuberosum*	4	4	Breeding line	-	-

Fig. 6 Experiment of *Solanum malmeanum* breeding system characterization and cross-ability relationships with wild and cultivated potatoes from the same and distinct Endosperm Balance Number (EBN) groups at greenhouse in the second semester of 2019 at Embrapa Clima Temperado, Pelotas, RS, Brazil.

Samples preparation for treatments and crosses

The emasculation was performed in flower buds in the pre-anthesis stage, one day prior to pollination, by extracting gently the anthers one by one with the aid of a forceps sterilized in 70º ethylic alcohol. Pollen was collected separately from all accessions, from mature flowers in anthesis stage. The anthers from flowers in anthesis were collected in an identified plastic container and later packed in waxed paper envelopes identified with the respective BGB and stored in a BOD chamber at 25°C for 24 hours. After 24 hours, the envelopes were removed from the BOD chamber and the pollen extracted from the anthers was transferred to test tubes of 1.5 mL identified with the respective BGB, and later stored in freezer (0°C) for medium term conservation. The pollinations were done according to each treatment or crossing scheme in the morning, period of greatest stigma receptivity, in pistils from buds emasculated one day before anthesis. The process was carried out by lightly touching the stigma of the pistil with the pollen. After the procedure, the inflorescences were bagged to avoid pollen contamination from other sources.

Breeding system essay treatments

Pollen production (pollen sampling: male fertility/male sterility): to test if an accession produces pollen and if the pollen is successful to fertilize other accessions. The information is assessed through the collection of anthers and pollen for samples preparation as described in the previous section. Male fertile accessions are the ones who yield pollen that result in successful crosses attested by fruit setting containing viable seeds.

Control (spontaneous self-pollination; spontaneous parthenocarpy): to test if an accession is able to self-pollinate and self-fertilize the flowers are untreated. The inflorescences with flower buds in normal conditions and in pre-anthesis (closed floral bud) are bagged with voile bags and remain so throughout blooming and until the end of the cycle. The number of flowers treated (bagged) is recorded. At the end of the cycle, it is observed whether there are fruit and seed formation to determine the probability of occurrence of natural self-pollination, without induction from manual pollination.

Apomixis and parthenocarpy (emasculation; no pollination): to test if an accession is able to set fruit and seeds without fertilization the flowers are emasculated prior to anthesis. Emasculation is performed on flower buds in the pre-anthesis stage. The emasculation process occurs by extracting the anthers one by one with the aid of sterilized forceps in 70º alcohol. After flowers emasculation, inflorescences are bagged with voile bags that remain closed until the end of the cycle to avoid pollen contamination from other sources. The number of flowers treated (emasculated) was recorded. At the end of the cycle, it is observed whether there was fruit formation with non-fertilized seeds (parthenocarpy and apomixis) or without any seeds (only parthenocarpy).

Autogamy (manual self-pollination: self-compatibility-SC/self-incompatibility-SI; induced parthenocarpy): to test if an accession is able to set fruit and seeds when pollinated with its own pollen. Induced self-pollinations are carried out in the morning after emasculation, in the same emasculated flowers of each accession, with their own pollen. After manual self-pollination, the inflorescences are bagged to avoid pollen contamination from other sources. The number of self-pollinated flowers is recorded. At the end of the cycle, it is observed whether there are fruit and seeds formation. The number of fruits and seeds per fruit was recorded. If there were no fruit and seeds development SI was confirmed. If there was fruit formation without seeds, induced parthenocarpy was confirmed. If, there was fruit and seed formation and the same accession did not produce fruit and seeds in the apomixis/parthenocarpy emasculation and no pollination treatment, then autogamy/SC was confirmed.

Allogamy (manual cross-pollination: female fertility/female sterility; induced parthenocarpy): to test if an accession is female infertile or female fertile and allogamous. A pollen admixture (bulk) composed of all *S. malmeanum* accessions able to produce pollen was prepared in a single 1.5 mL tube. Emasculation was performed in flower buds in the pre-anthesis stage. The emasculation process occured by extracting the anthers one by one with the aid of a sterilized forceps in 70º ethylic alcohol. Induced cross-pollinations with the bulk were carried out in the morning after emasculation. After manual cross-pollinations, the inflorescences were bagged to avoid pollen contamination from other sources. The number of cross-pollinated flowers was recorded. At the end of the cycle, it was observed whether there was fruit and seeds formation. The number of fruits and seeds per fruit was recorded. If no fruits nor seeds formation occurred, the accession was considered female sterile. If there was fruit formation without seeds the accession presented induced parthenocarpy. If there were fruit and seeds formation, the number of fruits and seeds per fruit was recorded and female fertility and allogamy were confirmed.

Cross-ability evaluation

Interspecific crosses were performed with three species belonging to variable ploidy levels, from the same or distinct EBN groups and belonging to the same or distinct gene pools in relation to *S. tuberosum* to test ways to overcome the reproductive isolation of *S. malmeanum* and to find pathways allowing hybridization with other species, development of segregant offspring and introgression of desirable traits for pre-breeding efforts. Interspecific crosses as diallel crosses were performed between *S. malmeanum* 2x and 3x (1EBN) and the wild potatoes species *S. commersonii* 2x (1EBN), *S. chacoense* 2x (2EBN), and two cultivated potatoes belonging to *S. tuberosum* 4x (4EBN) (Table 1). Emasculation, pollen collection and preparation, and pollinations were carried out as previously described. After the controlled pollinations, inflorescences were labeled and bagged with voile bags that remained closed until the end of the cycle to avoid pollen contamination from other sources. The number of cross-pollinated flowers was recorded for every cross. At the end of the cycle, it was observed whether there was fruit and seeds formation. The number of fruits and seeds per fruit was recorded. Berries were harvested one month after pollination. Seeds were extracted, counted, and sorted according to size and plumpness in the following types: A, plump; B, smaller than A with a less developed endosperm; C, shrunken. Seed weight was determined by weighting 100 seeds on analytical balance. The absence of fruit was considered testimonials of the existence of pre-zygotic barriers. The proper formation of well-developed seeds was the criterion used to attest the success in cross from each treatment and the absence of pre-zygotic barriers. The formation of fruit without seeds or the production of fruit bearing only malformed seeds were attributed to the post-zygotic barriers. Germination of seeds confirmed success of hybridization. Germination test was made starting from the inoculation of the seeds into 1500 ppm of GA_3 for 24 h. The seeds were then sown under greenhouse condition in plastic boxes filled with TurfaFértil® substrate and were manually irrigated every two days. The germination was evaluated weekly for up to 30 days after sowing. The number of seedlings was recorded to attest the absence of post-zygotic barriers and the success of different compatibility groups according to the ploidy, EBN and genepool classification.

Total number of seeds (TNS) was determined indirectly using the following equation:

$$\text{TNS} = \frac{\text{Total seed weight}}{\text{Weight of 100 seeds}} \times 100$$

Cross efficiency (CE) from interspecific crosses was determined by:

$$\text{Cross efficiency (CE)} = \frac{\text{Number of seeds per berry}}{\text{Number of pollinations}}$$

Percentage of germinated seeds (%) was determined following the equation:

$$\text{Germination (\%)} = \frac{\text{Number of seeds sown}}{\text{Number of germinated seeds}} \times 100$$

Statistical analyses to determine average of cross efficiency (CE) and germination (%) from interspecific crosses were performed in Rstudio environment (v4.0.5) with packages “TukeyC” and “ExpDes.pt” (Rstudio 2021).

Results

Breeding system behavior

A total of 3263 flowers of 10 *S. malmeanum* accessions were evaluated, from which 2663 were hand pollinated. The behavior per accession is summarized in Table 2. The number of treated flowers (NTF), number of fruit set (NFS), total number of seeds (NTS), average number of seeds per fruit (NSF), and seed germination (G) per accession × treatment is detailed in Table 3.

Table 2 Breeding system behavior of *Solanum malmeanum* accessions from Embrapa Clima Temperado Potato Gene Bank (Pelotas, RS, Brazil).

Accession	**Pollen production (male fertility)**	**Control (self-pollination)**	**Parthenocarpy**	**Apomixis**	**Autogamy* (manual self-pollination)**	**Allogamy (manual bulk pollination, female fertility)**
BGB015	Present	Absent	Absent	Absent	Self-incompatible	Fertile
BGB017	Present	Absent	Absent	Absent	Self-incompatible	Fertile
BGB021	Present	Absent	Induced	Absent	Self-incompatible	Infertile
BGB080	Present	Absent	Absent	Absent	Self-incompatible	Partially fertile
BGB081	Absent	Absent	Absent	Absent	Not assessed	Infertile
BGB084	Absent	Absent	Absent	Absent	Not assessed	Infertile
BGB446	Present	Absent	Absent	Absent	Self-incompatible	Fertile
BGB447	Present	Absent	Absent	Absent	Self-compatible	Fertile
BGB448	Present	Absent	Spontaneous	Absent	Self-incompatible	Partially fertile
BGB471	Present	Absent	Absent	Absent	Self-incompatible	Fertile

Summary of reproductive behavior

Pollen production (male fertility/male sterility): All diploid accessions (BGB015, BGB17, BGB446, BGB447, BGB471) and three triploid accession (BGB021, BGB080, BGB448) produced pollen and are male fertile, while other two triploid accessions (BGB081, BGB084) did not produce pollen and are male sterile.

Control (spontaneous self-pollination; parthenocarpy): There was no development of fruit in most accessions (BGB015, BGB017, BGB021, BGB080, BGB081, BGB084, BGB446, BGB447, BGB448, BGB471). BGB448 spontaneously produced five parthenocarpic fruits without seeds. The results confirmed the absence of self-pollination.

Apomixis and parthenocarpy: There was no development of apomictic seeds in any accession (BGB015, BGB017, BGB021, BGB080, BGB081, BGB084, BGB446, BGB447, BGB471). BGB448 spontaneously produced four parthenocarpic fruits with no seeds, confirming again the occurrence of parthenocarpy.

Autogamy (geitonogamy; self-compatibility-SC/self-incompatibility-SI): Four diploid accessions (BGB015, BGB017, BGB446, BGB471) and three triploids (BGB021, BGB080, BGB448) were self-incompatible (SI). One diploid accession (BGB447) behaved as self-compatible (SC), producing three fruits from 14 manual self-pollinations, with 51 seeds in total, average 17 seeds per fruit and 37.1% germination of the endogamous seeds. Two triploid accessions (BGB081, BGB084) could not be assessed for autogamy since they do not produce pollen.

Allogamy (female fertility/female sterility; induced parthenocarpy): Five diploid accessions (BGB015, BGB017, BGB446, BGB447, BGB471) are allogamous and female fertile. BGB015 produced four fruits from 11 pollinations, 103 seeds in total, average 25.7 seeds per fruit and 75% seed germination. BGB017 produced 13 fruits from 16 pollinations, 1542 seeds in total, average 118.6 seeds per fruit and 50% seed germination. BGB446 produced 25 fruits from 28 pollination, 1743 seeds in total, average 69.7 seeds per fruit and 61.1% seed germination. BGB447 produced seven fruits from 15 pollinations, 425 seeds in total, average 60.7 seeds per fruit and 100% seed germination. BGB471 produced 20 fruits from 30 pollinations, 1625 seeds in total, average 81.2 seeds per fruit and 100% seed germination. Two triploid accessions (BGB080, BGB448) are allogamous and partially female fertile. BGB080 produced 22 fruits from 30 pollinations, 78 seeds in total, average 3.5 seeds per fruit and 50% seed germination. BGB448 produced 11 fruits from 30 pollinations, three seeds in total, average 0.3 seed per fruit and 66,7% seed germination. Three triploid accessions had 30 flowers pollinated each one (BGB021, BGB081 and BGB084) and behave as a female sterile. BGB021 produced 10 induced parthenocarpic fruits, with no seeds.

Characterization of the accessions' mode of reproduction

BGB015 produces pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent, is self-incompatible and allogamous, thus it reproduces sexually through seeds and asexually through stolons and tubers.

BGB017 produces pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent, is self-incompatible and allogamous, thus it reproduces sexually through seeds and asexually through stolons and tubers.

BGB021 produces pollen, does not self-pollinate, presents induced parthenocarpy, apomixis is absent, is self-incompatible and female sterile, thus it reproduces asexually through stolons and tubers, although allow gene flow as pollen donor.

BGB080: produces pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent, is self-incompatible and allogamous, thus it reproduces sexually through seeds and asexually through stolons and tubers.

BGB081 does not produce pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent and is female sterile, thus it reproduces only asexually through stolons and tubers.

BGB084 does not produce pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent and is female sterile, thus it reproduces only asexually through stolons and tubers.

BGB446 produces pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent, is self-incompatible and allogamous, thus it reproduces sexually through seeds and asexually through stolons and tubers.

BGB447 produces pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent, is self-compatible and allogamous, thus it reproduces sexually through seeds and asexually through stolons and tubers.

BGB448 produces pollen, does not self-pollinate, presents spontaneous parthenocarpy, apomixis is absent, is self-incompatible and allogamous, thus it reproduces sexually through seeds and asexually through stolons and tubers.

BGB471 produces pollen, does not self-pollinate, parthenocarpy is absent, apomixis is absent, is self-incompatible and allogamous, thus it reproduces sexually through seeds and asexually through stolons and tubers.

Based on the compilation of the breeding system behavior results of ten evaluated accessions, *Solanum malmeanum* is a mostly allogamous species with the rare occurrence of autogamous (self-compatible) genotypes, although self-pollination did not occur in controlled conditions it may happens mediated by pollinators in nature (geitonogamy – functionally cross-pollination, but genetically autogamy, since the gametes involved come from the same genotype). Diploid plants always produce pollen and are male fertile. Some triploids produce pollen and are male fertile and others do not produce pollen and are male sterile. Diploids always produce seeds and are female fertile. Some triploids produce few viable seeds and are partially female fertile, while others do not produce seeds at all and are female sterile.

Table 3 Fruit and seed production of ten *Solanum malmeanum* accessions from Embrapa Clima Temperado Potato Genebank under different treatments to characterize the breeding system. Number of Treated Flowers (NTF), Number of Fruit Set (NFS), Total Number of Seeds (NTS), average Number of Seeds per Fruit (NSF), and seed Germination (G) (%). (-) not evaluated.

Accession	**Treatment**	**NTF**	**NFS**	**TNS**	**NSF**	**G (%)**
BGB015	Control	30	0	0	0	-
BGB017	Control	30	0	0	0	-
BGB021	Control	30	0	0	0	-
BGB080	Control	30	0	0	0	-
BGB081	Control	30	0	0	0	-
BGB084	Control	30	0	0	0	-
BGB446	Control	30	0	0	0	-
BGB447	Control	30	0	0	0	-
BGB448	Control	30	5	0	0	-
BGB471	Control	30	0	0	0	-
BGB015	Apomixis	30	0	0	0	-
BGB017	Apomixis	30	0	0	0	-
BGB021	Apomixis	30	0	0	0	-
BGB080	Apomixis	30	0	0	0	-
BGB081	Apomixis	30	0	0	0	-
BGB084	Apomixis	30	0	0	0	-
BGB446	Apomixis	30	0	0	0	-
BGB447	Apomixis	30	0	0	0	-
BGB448	Apomixis	30	4	0	0	-
BGB471	Apomixis	30	0	0	0	-
BGB015	Autogamy	14	0	0	0	-
BGB017	Autogamy	12	0	0	0	-
BGB021	Autogamy	23	0	0	0	-
BGB080	Autogamy	20	0	0	0	-
BGB081	Autogamy	0	0	0	0	-
BGB084	Autogamy	0	0	0	0	-
BGB446	Autogamy	22	0	0	0	-
BGB447	Autogamy	14	3	51	17	37.1
BGB448	Autogamy	30	1	0	0	-
BGB471	Autogamy	30	0	0	0	-
BGB015	Allogamy	11	4	103	25.7	75
BGB017	Allogamy	16	13	1542	118.6	50
BGB021	Allogamy	30	10	0	0	-
BGB080	Allogamy	30	22	78	3.5	50
BGB081	Allogamy	30	0	0	0	-
BGB084	Allogamy	30	0	0	0	-
BGB446	Allogamy	28	25	1743	69.7	61.1
BGB447	Allogamy	15	7	425	60.7	50
BGB448	Allogamy	30	11	3	0.3	50
BGB471	Allogamy	30	20	1625	81.2	100

Cross-ability

Solanum malmeanum freely crosses with the 2x (1EBN) *S. commersonii* with high seed production and seed germination (%), however, crossing *S. malmeanum* with the 2x (2EBN) *S. chacoense* resulted in the reduction on number of fruits and seeds produced, and seed germination. *Solanum malmeanum* crosses with the 2EBN *S. chacoense* is possible but limited due to the post-zygotic barrier of hybridization. Despite the lower seed production and germination, crosses occur probably for the presence of 2n gametes. *S. malmeanum* is cross incompatible with the crop 4EBN *S. tuberosum*. Results of fruit and seed production, average of seeds per fruit, and seed germination from interspecific crosses among *S. malmeanum* accessions with *S. commersonii*¸ *S. chacoense* and *S. tuberosum* accessions from Embrapa Clima Temperado Potato Gene Bank are presented in Table 4. Average of number of seeds per berry involving *S. malmeanum, S. chacoense* and *S. commersonii* is presented in Table 5. Average of seed germination (%) from interspecific *crossings between S. malmeanum, S. chacoense*, and *S commersonii* is presented in Table 6.

When *S. malmeanum* accession were crossed as female parent with male *S. commersonii* (accession BGB001), BGB015 produced 13 fruits from 23 flowers pollinated, with 3500 seeds in total and average of 270 seeds per fruit, and seed germination was 55.6%. BGB017 produced eight fruits from eight flowers pollinated, with 2500 seeds in total and average of 313 seeds per fruit, and seed germination was 38.9%. BGB021 do not produced fruits from 31 flowers pollinated. BGB080 produced six parthenocarpic fruits from 31 flowers pollinated. BGB081 do not produced fruits from 23 flowers pollinated. BGB084 do not produces fruit from 32 flowers pollinated. BGB446 produces eight fruits from eight flowers pollinated, with 2500 seeds in total and average of 313 seeds per fruit, and seed germination was 83.3%. BGB447 produced eight fruits from 18 pollinations, with 700 seeds in total and average of 87.5 seeds per fruit, and seed germination was 61.1%. BGB448 produced 13 parthenocarpic fruits from 30 pollinations. BGB471 produced 19 fruits from 30 pollinations, with 3400 seeds in total and average of 179 seeds per fruit, and seed germination was 88.9%.

Solanum commersonii (accession BGB001) was crossed as female parent with the male *S. malmeanum* accession. From BGB015, BGB001 produced 20 fruits from 20 flowers pollinated, with 3300 seeds in total and average of 165 seeds per fruit, and seed germination was 38.9%. BGB001 pollinated by BGB017 produced 13 fruits from 18 pollinations, with 1525 seeds in total and average of 117 seeds per fruit, and seed germination was 88.9%. *Solanum commersonii* (BGB001) do not produced fruits with seeds when crossed with BGB021, BGB080 and BGB448, from 17, 14 and 14 flowers pollinated, respectively. From BGB446, BGB001 produced nine fruits from 20 pollinations, with 2100 seeds in total and average of 233 seeds per fruit, and seed germination was 66.7%. From BGB447, BGB001 produced 12 fruits from 20 pollinations, with 1550 seeds in total and average of 129 seeds per fruit, and seed germination was 38.9%. From BGB471, BGB001 produced 15 fruits from 22 pollinations, with 4100 seeds in total and average of 273 seeds per fruit, and seed germination was 94.4%.

When *S. malmeanum* accessions were used as female at crosses with male *S. chacoense* genotype (BGB444), BGB015 produced nine fruits from 23 flowers pollinated, with 53 seeds in total and average of six seed per fruit, and seed germination was 0%. BGB017 produced six fruits from 10 pollinations, with 32 seeds in total and average of five seeds per fruit, and seed germination was 5.6%. BGB021 produced five fruits from 30 pollinations, with two seeds in total and average of 0.4 seed per fruit, and seed germination was 50%. BGB080 produced 20 fruits from 30 pollinations, with 59 seeds in total and average of three seeds per fruit, and seed germination was 13.6%. Both BGB081 and BGB084 do not produced fruit from 23 flowers pollinated each accession. BGB446 produced six fruits from six pollinations, with 160 seeds in total and average of 27 seeds per fruit, and seed germination was 6.3%. BGB447 produced three fruits from 24 pollinations, with 96 seeds in total and average of 32 seeds per fruit, and seed germination was 10%. BGB448 produced 16 fruits from 31 pollinations, with six seeds in total and average of 0.38 seed per fruit, and seed germination was 50%. BGB471 produced 24 fruits from 30 pollinations, with 334 seeds in total and average of 14 seeds per fruit, and seed germination was 0%.

Solanum chacoense (accession BGB444) were crossed as female parent with the male *S. malmeanum* accessions. From BGB015, BGB444 produced six parthenocarpic fruits (no seed formation). From BGB017, BGB444 produced 21 fruits from 28 pollinations, with 12 seeds in total and average of 0.57 seed per fruit, and no seed germinated (0%). BGB444 do not produced fruit from 31 pollinations of BGB021, 33 pollinations of BGB080 and 32 pollinations of BGB448. From BGB446, BGB444 produced 16 fruits from 32 flowers pollinated, with three seeds in total and average of 0.19 seed per fruit, and no seed germinated (0%). From BGB471, BGB444 produced nine parthenocarpic fruits from 35 flowers pollinated. No germination of seeds from 1EBN *S. malmeanum* and 2EBN *S. chacoense* can be explained due to the post-zygotic barriers of EBN system (Johnston et al. 1980). Once the seeds germinated, there is evidence of presence of 2n gametes in *S. malmeanum* accessions.

Solanum malmeanum accessions were cross-incompatible with the 4x (4EBN) *S. tuberosum* (BRSANA and C1750-15-95).

Table 5 Interspecific crosses among *Solanum malmeanum* accessions with *S. commersonii*¸ *S. chacoense* and *S. tuberosum* accessions from Embrapa Clima Temperado Potato Gene Bank. Heading is follows by genotypic combination, number of treated flowers (NTF), number of fruit set (NFS), total number of seeds (TNS), number of seeds per fruit (NSF), and seed germination (G) (%). *(-) not evaluated due to non-seed production.

Genotypic combination	**NTF**	**NFS**	**TNS**	**NSF**	**G (%)**
S. malmeanum ♀ × ♂ *S. commersonii*					
BGB015 × BGB001	23	13	3500	270	55.6
BGB017 × BGB001	8	8	2500	313	38.9
BGB021 × BGB001	31	0	0	0	-
BGB080 × BGB001	31	6	0	0	-
BGB081 × BGB001	23	0	0	0	-
BGB084 × BGB001	32	0	0	0	-
BGB446 × BGB001	8	8	2500	313	83.3
BGB447 × BGB001	18	8	700	88	61.1
BGB448 × BGB001	30	13	0	0	-
BGB471 × BGB001	30	19	3400	179	88.9
S. commersonii ♀ × ♂ *S. malmeanum*					
BGB001 × BGB015	20	20	3300	165	38.9
BGB001 × BGB017	18	13	1525	117	88.9
BGB001 × BGB021	17	0	0	0	-
BGB001 × BGB080	14	0	0	0	-
BGB001 × BGB446	20	9	2100	233	66.7
BGB001 × BGB447	20	12	1550	129	38.9
BGB001 × BGB448	14	0	0	0	-
BGB001 × BGB471	22	15	4100	273.33	94.4

Table 4 (continued)

aGenotypic combination	NTF	NFS	TNS	NSF	G (%)
S. malmeanum ♀ × ♂ *S. chacoense*					
BGB015 × BGB444	24	9	53	6	0
BGB017 × BGB444	10	6	32	5	5.6
BGB021 × BGB444	30	5	2	0.4	50
BGB080 × BGB444	30	20	59	3	13.6
BGB081 × BGB444	23	0	0	0	-
BGB084 × BGB444	23	0	0	0	-
BGB446 × BGB444	6	6	160	27	6.3
BGB447 × BGB444	24	3	96	32	10
BGB448 × BGB444	31	16	6	0.38	50
BGB471 × BGB444	30	24	334	14	0
S. chacoense ♀ × ♂ *S. malmeanum*					
BGB444 × BGB015	35	6	0	0	-
BGB444 × BGB017	28	21	12	0.57	0
BGB444 × BGB021	31	0	0	0	-
BGB444 × BGB080	33	0	0	0	-
BGB444 × BGB446	32	16	3	0.19	0
BGB444 × BGB447	33	24	3	0.13	0
BGB444 × BGB448	32	0	0	0	-
BGB444 × BGB471	35	9	0	0	-

Table 4 (continued)

Genotypic combination	**NTF**	**NFS**	**TNS**	**NSF**	**G (%)**
S. malmeanum ♀ × ♂ *S. tuberosum*					
BGB015 × BRS ANA	30	0	0	0	-
BGB017 × BRS ANA	30	0	0	0	-
BGB021 × BRS ANA	30	0	0	0	-
BGB080 × BRS ANA	30	0	0	0	-
BGB081 × BRS ANA	30	0	0	0	-
BGB084 × BRS ANA	30	0	0	0	-
BGB446 × BRS ANA	11	0	0	0	-
BGB447 × BRS ANA	26	0	0	0	-
BGB448 × BRS ANA	36	8	0	0	-
BGB471 × BRS ANA	30	0	0	0	-
BGB015 × C1750-15-95	7	0	0	0	-
BGB017 × C1750-15-95	16	0	0	0	-
BGB021 × C1750-15-95	30	0	0	0	-
BGB080 × C1750-15-95	30	0	0	0	-
BGB081 × C1750-15-95	30	0	0	0	-
BGB084 × C1750-15-95	30	0	0	0	-
BGB446 × C1750-15-95	7	0	0	0	-
BGB447 × C1750-15-95	6	0	0	0	-
BGB448 × C1750-15-95	31	9	0	0	-
BGB471 × C1750-15-95	30	0	0	0	-

Table 4 (continued)

Genotypic combination	**NTF**	**NFS**	**TNS**	**NSF**	**G (%)**
S. tuberosum ♀ × ♂ *S. malmeanum*					
BRS ANA × BGB015	10	0	0	0	-
BRS ANA × BGB017	12	0	0	0	-
BRS ANA × BGB021	30	0	0	0	-
BRS ANA × BGB080	30	0	0	0	-
BRS ANA × BGB446	22	0	0	0	-
BRS ANA × BGB447	15	0	0	0	-
BRS ANA × BGB448	16	0	0	0	-
BRS ANA × BGB471	36	0	0	0	-
BRS ANA × Bulk *S. malmeanum*	30	0	0	0	-
C1750-15-95 × BGB015	13	0	0	0	-
C1750-15-95 × BGB017	16	0	0	0	-
C1750-15-95 × BGB021	30	0	0	0	-
C1750-15-95 × BGB080	30	0	0	0	-
C1750-15-95 × BGB446	12	0	0	0	-
C1750-15-95 × BGB447	10	0	0	0	-
C1750-15-95 × BGB448	7	0	0	0	-
C1750-15-95 × BGB471	30	0	0	0	-
C1750-15-95 × BULK *S. malmeanum*	30	0	0	0	-

Discussion

Solanum malmeanum demonstrated to be a potential genetic resource to improve potatoes (*S. tuberosum*). This wild potato can freely cross with the same group of 1EBN species *S. commersonii*, and is cross-incompatible with (4EBN) *S. tuberosum*. Barriers of hybridization between *S. malmeanum* (1EBN) and *S. chacoense* (2EBN) is not complete, enlightening the possibility to transmit genes to upper groups (4EBN) of cultivated potato via bridge crosses with species of 2EBN group. The discovery of self-compatible plants of *S. malmeanum* provides additional sources to promote diploid breeding at 1EBN level.

At the open pollination treatment (allogamy) all 2x *S. malmeanum* genotypes produced fruit with normal seeds formation and the seeds germinated well (Fig. 2), however, two 3x genotypes BGB081 and BGB084 did not produce seeds. *Solanum malmeanum* accessions, except BGB447, are self-incompatible due to a genetically based gametophytic self-incompatibility system (Pushkarnath 1942; Pandey 1962). Only the 2x S. *malmeanum* genotype BGB447 behaved as a self-compatible, produced fruits with normal and plump seeds as demonstrated (Fig. 3a; 3b), and plants germinated well (Fig. 3c). Self-fertility in genotype BGB447 could be tested under three hypotheses. The first, if 2n pollen the S-Rnase do not activated in the style, and then making pollen grain able to germinate into the style and fertilizes (Mok et al. 1976; Camadro et al. 2004). Pre-zigotic barriers will be elucidate from the analysis of tube pollen growth under UV fluorescence microscopy. Second reason that BGB447 becomes self-compatible could be attributed to the presence of S-locus inhibitor gene (*Sli*). The *Sli* gene acts to overcome the self-incompatibility in diploid potatoes due to inhibition a function of S-alleles in pollen (Hosaka and Hanneman, 1998a). *Sli* gene has been mapped at chromosome 12 (Hosaka and Hanneman, 1998b) and many primers are available as those developed by Clot et al. (2020). According to Bryan et al. (2017) self-compatible species single plant is highly representative of a genebank accession. Also, the third hypothesis that BGB447 self-fertilizes could be attributed to the non-functioning of S-RNase (Dzidzienyo et al. 2016). Then, the self-compatibility of *S. malmeanum* genotype BGB447 needs to be further investigated according the 2n pollen production, screening for *Sli* gene and genotypes with non-functional S-RNase loci. Self-compatible wild potatoes plants, especially those with presence of *Sli* gene, is very desirable for development of diploid inbred lines of potatoes. This new modern potato breeding represents a new strategy to overcomes the main challenges encountered along the historical approach of tetraploid potato breeding (Birhman and Hosaka, 2000; Phumichai et al. 2005; Jansky et al. 2016; Alsahlany et al. 2021). Under treatment of natural conditions, whereas the flowers were not manipulated, BGB447 do not self-fertilizes but the plant can be pollinated with its own pollen with the aid of pollinators.

Fig. 7 Wild potatoes seedling four weeks old from open pollination with mixed pollen of *Solanum malmeanum* accessions.

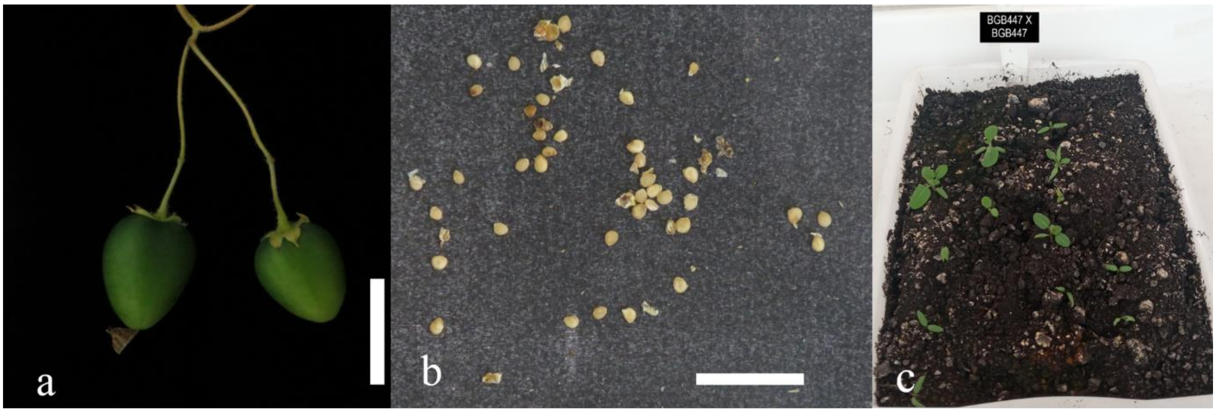

Fig. 8 Fruits (a), seeds (b), and seedlings (c) produced from self-compatible *Solanum malmeanum* accession BGB447 (*D.M.Costa 60*, from 26.18°S – 55.01°W). Bar = 10 mm.

At treatment open pollination a maximum fruit set was observed in accession BGB446 (89.3%) and the minimum in BGB015 (36.3%). BGB021, BGB081 and BGB084 did not produce any seed from all treatments, possibly due to its triploid nature inferred by flow cytometry analysis (Table 1), then these genotypes only propagate vegetatively. The diploid *S. malmeanum* accessions BGB015, BGB017, BGB446, BGB447 and BGB471 can be reproduced by seeds (sexually) and tubers (vegetatively), all are diploid inferred by flow cytometry analysis (Table 1).

S. malmeanum and *S. commersonii* can be easily intercrossed, and the seed germination followed by hybrid vigor evidence the possibility to hybridize in nature, if other barriers like geography and phenology are absent. According to the average of number of seeds produced per berry results (Table 5), *S. malmeanum* performed better when hybridized with *S. commersonii* comparing to *S. chacoense* and *S. tuberosum*. *Solanum malmeanum* produced an average of number of seeds per berry higher (116.3) when crossed as female parent with a male parent *S. commersonii*, but not differentiate statistically when *S. commersonii* was crossed as female parent with male parent *S. malmeanum* (114.7), followed by 8.8 seeds per berry when crossed as female parent with *S.*

chacoense, and 0.11 seed per berry when crossed as male parent in crosses with female *S. chacoense*. Crosses from *S. malmeanum* and *S. tuberosum* did not produce seeds.

Table 6 Average of number of seeds per berry produced from crossings involving *Solanum malmeanum*, *S. chacoense* and *S. commersonii*. Genotypic combination followed by average of cross efficiency.

Genotypic combination	**Average of number of seeds per berry**
S. malmeanum ♀ × ♂ *S. commersonii*	116.3 ^{a}
S. commersonii ♀ × ♂ *S. malmeanum*	114.7 ^{a}
S. malmeanum ♀ × ♂ *S. chacoense*	8.8 ^{b}
S. chacoense ♀ × ♂ *S. malmeanum*	0.11 ^{b}
S. malmeanum ♀ × ♂ *S. tuberosum*	0 ^{b}
S. tuberosum ♀ × ♂ *S. malmeanum*	0 ^{b}

*Means followed by the same letter in column are not significantly different from each other according to Tukey test ($p<0.05$). RStudio (v. 4.0.5, 2021).

Reciprocal crosses between *S. malmeanum* and *S. commersonii* formed berries which produced normal and plump seeds (Fig. 4a; 4b). When crosses involved *S. malmeanum* and *S. chacoense*, berries and seed production behave different. *Solanum malmeanum* accessions produced abnormal and malformed seeds when crossed with male *S. chacoense* (Fig. 5a). *Solanum chacoense* accession (BGB444) produced normal and plump seeds when crossed with the accessions of *S. malmeanum* (Fig. 5b). This differential performance on seed production and size observed from the intra- and interspecific crosses between *S. malmeanum*, *S. commersonii* and *S. chacoense* was reported by Summers and Grun (1981). Ehlendfeldt and Hanneman (1988) investigated the genetics of EBN using diploid hybrids exceptionally obtained from the 2x (1EBN) *S. commersonii*, 2x (1EBN) *S. malmeanum* (accession PI 320269 as *S. commersonii* subsp. *malmeanum*) and 2x (2EBN) *S. chacoense*. Under resulted sib-matting and backcrossing of F1 hybrids they assumed that three unlinked additive loci operate in a threshold-like system to control endosperm development, then, confirm a requisition of 2:1 maternal to paternal EBN gene dosage for normal seed development, and a slightly higher dosage of maternal dosage in endosperm produces small but viable seeds (Ehlenfeldt and Hanneman 1988). In this model, it is possible to observe that an excess of male dosage results in seed abortion, but a single excess of female dosage favor to produces small but viable seeds.

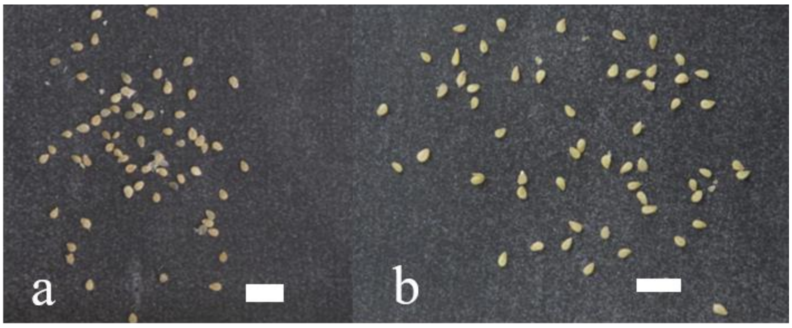

Fig. 4 Aspect of seeds produced from reciprocal crosses between *Solanum malmeanum* and *S. commersonii*: Normal and plump seeds from *S. malmeanum* ♀ × ♂ *S. commersonii* (a); Normal and plump seeds from *S. commersonii* ♀ × ♂ *S. malmeanum* (b). Bars = 5 mm.

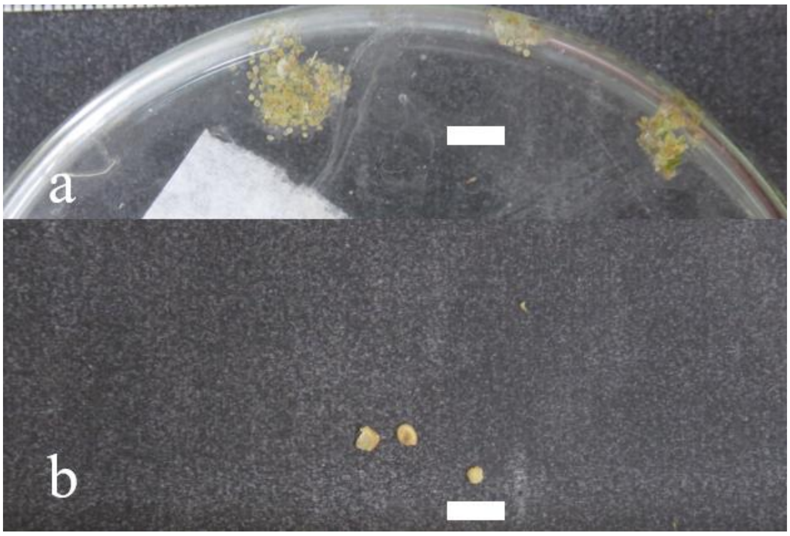

Fig. 5 Aspect of seeds produced from reciprocal crosses between *Solanum malmeanum* and *S. chacoense*: Abnormal seeds from *S. malmeanum* ♀ × ♂ *S. chacoense* (a); Normal and plump seeds from *S. chacoense* ♀ × ♂ S. *malmeanum* (b). Bars = 5 mm.

The differential performance on seed germination from intra and interspecific crosses is presented in Table 5. Seed germination from interspecific crosses was higher (40.9%) when crossed *S. malmeanum* as male with female (1EBN) *S. commersonii* (Fig. 6a) comparing to the crosses using *S. malmeanum* as female with male *S. commersonii* (32.8%) (Fig. 6b). Seed germination was 13.6% from *S. malmeanum* crossed as female with the male parent 2x (2EBN) *S. chacoense* (Fig. 6c), and no seed germinated from reciprocal crosses. Considering the EBN system, *S. malmeanum* performed better in crosses with its related tertiary gene pool 2x (1EBN) *S. commersonii*, however, the reduction of seed germination can evidence the acting of post-zygotic barrier of EBN, despite not complete, causes reduction of seed germination (Camadro et al. 2004). Under the assumption that inter-EBN occurs only with presence of 2n gametes, the species with 1EBN can hybridize as male parent with female parent 2EBN species, if 2n pollen in the male parental. From reciprocal way, 2EBN species can hybridize as male parent with 1EBN species if 2n eggs is present in the female parental. Observing the results on seed production and germination performance from crossings with *S. chacoense* (BGB444), is possible to infers that 2n pollen is absent in *S. malmeanum* accessions, but six *S. malmeanum* accessions (BGB017, BGB021, BGB080, bGB446, BGB447, BGB448) have 2n eggs. In the natural environmental, endosperm collapse acts as a strong barrier of hybridization, despite not complete, between *S. malmeanum* and *S. chacoense*. Barriers of hybridization can be either internal or external. External barriers can be classified in space-time, that is, when there is an asynchrony in the flowering period between two populations, not overlapping geography and distinct ecological preferences (Hawkes and Hjerting 1969). Internal barriers of hybridization, which are genetically determined, can be either pre-zygotic (e.g., occurs at stylar tissue, pollen-pistil incompatibility) or post-zygotic (e.g., occurs at embryo development level) (Johnston et al. 1980; Masuelli and Camadro 1997; Camadro et al. 2004).

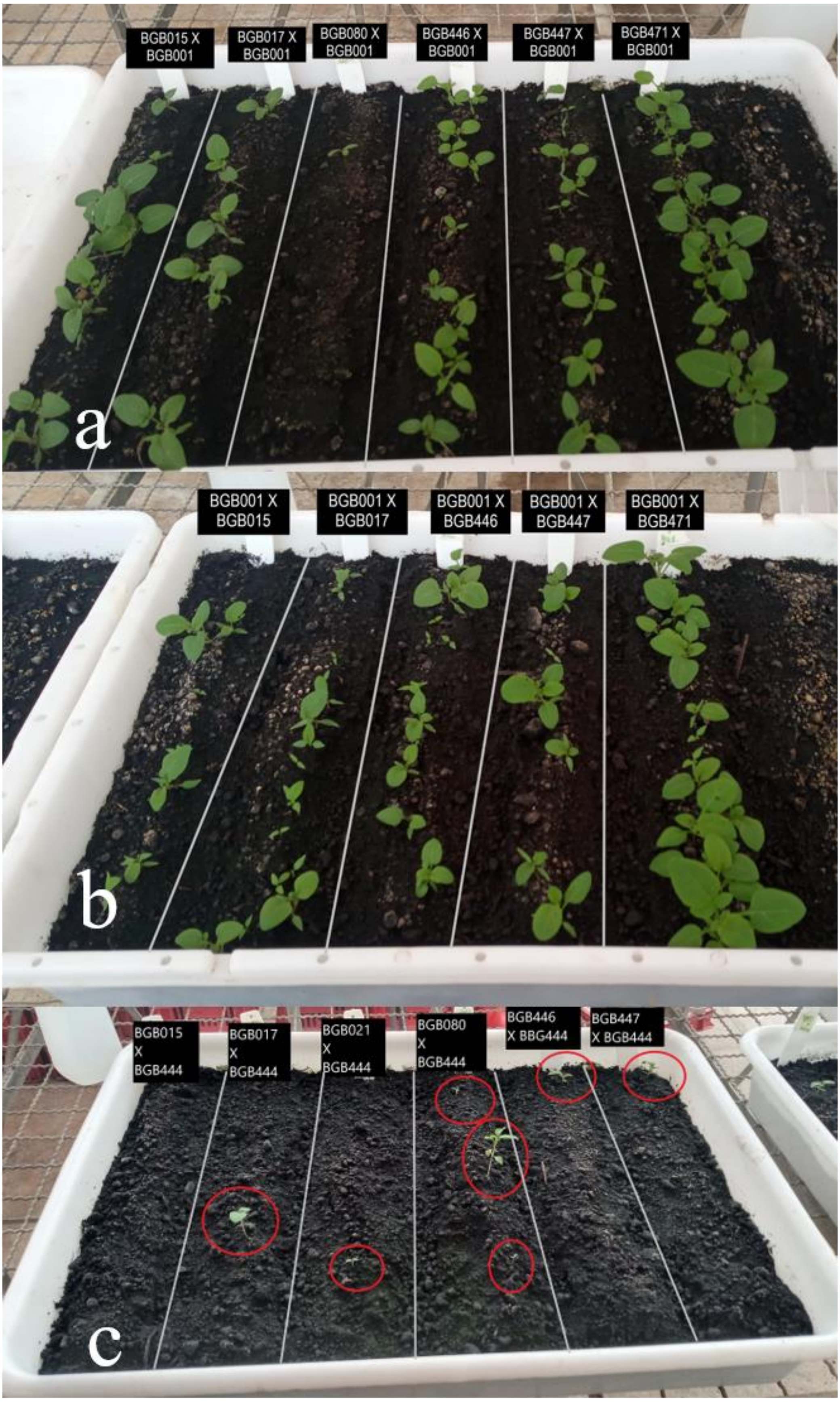

Fig. 6 Wild potatoes interspecific hybrid seedlings four-weeks-old from *Solanum commersonii* × *S. malmeanum* (a), *S. malmeanum* × *S. commersonii* (b), and *S. malmeanum* × *S. chacoense*.

Table 7 Seed germination from interspecific crossings between *Solanum malmeanum*, *S. chacoense*, and *S commersonii*. Genotypic combination followed by germination (%).

Genotypic combination	Germination (%)
S. commersonii ♀ × ♂ *S. malmeanum*	40.9 [a] *
S. malmeanum ♀ × ♂ *S. commersonii*	32.8 [ab]
S. malmeanum ♀ × ♂ *S. chacoense*	13.6 [bc]
S. chacoense ♀ × ♂ *S. malmeanum*	0 [c]

*Means followed by the same letter in column are not significantly different from each other according to Tukey test ($p<0.05$). RStudio (v. 4.0.5, 2021).

When 3x 1EBN *S. malmeanum* (genotypes BGB021 and BGB080) were crossed as female with 2x 1EBN *S. commersonii* (genotype BGB001) as male, no fruit with seeds formation was observed. But in other way, a few fruits with seeds were produces from crossing 3x *S. malmeanum* (genotypes BGB021 and BGB080) as female with 2x 2EBN *S. chacoense* (genotype BGB444). The differential behavior of two 3x accessions of *S. malmeanum* (BGB021 and BGB080) from intra and interspecific crosses should be further investigated due to non-formation of berries in crosses with related 1EBN *S. commersonii* any from the open pollination using bulked pollen of *S. malmeanum* accessions.

According to many authors, wild potato not only hybridize with those of its own ploidy level but also with others of higher ploidy levels, providing a means for genetic exchange between members of different ploidy levels (den Nijs and Peloquin 1977b; Peloquin et al. 1999; Carputo et al. 2000), however, a balanced EBN is required for successful crosses, according to the EBN theory (Johnston et al. 1980; Ehlenfeldt and Ortiz 1995; Hanneman 1999; Jansky 2006), exemplified in Figure 7 Because the cross-ability and reproductive behavior could be reflected on the taxonomical determination as early discussed by Hawkes and Jackson (1992). Other possibility on seed production is if n gametes are present in sporophytic *S. chacoense* that could unit with the n gametes of gametophyte of S. *malmeanum*, then progeny will produce triploid plant. Could BGB021 and BGB080 present other mechanism which act due to block the berries set in triploid accessions of *S. malmeanum* when submitted to the crosses?

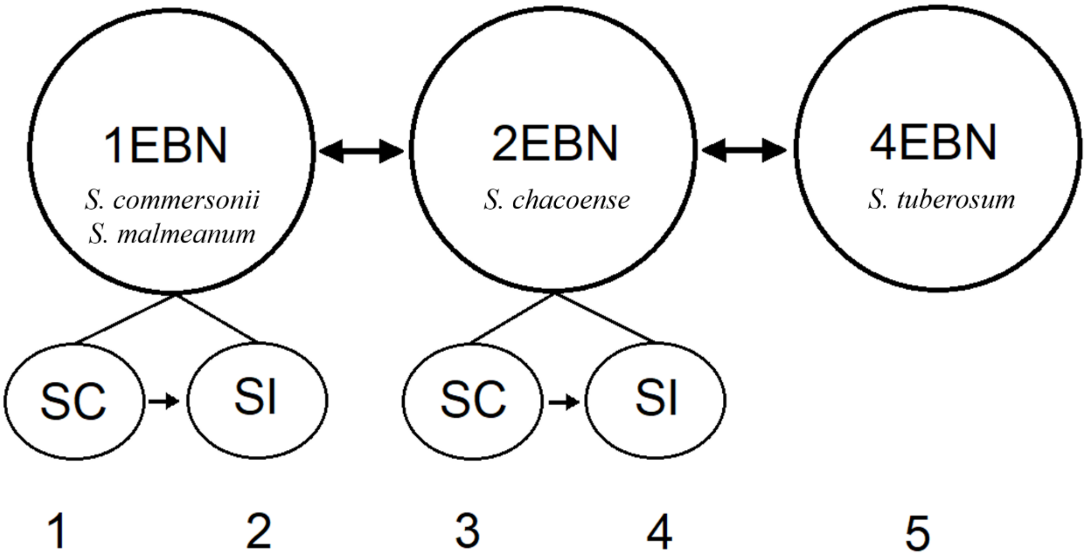

Fig. 7 Cross-ability groups (1 – 5) of wild potato species (*Solanum* sect. *Petota*, Solanaceae) based on EBN and self-compatible/ self-incompatible systems. Connected lines allow freely crosses, arrowhead allows freely crosses if 2n gametes are present (based on Spooner et al. 2014).

It is widely known that 2n gametes in the 2x wild potato species are a potential mechanism to overcome barriers of hybridization between species from different EBN values. 2n gametes in *S. malmeanum* could capture around 80% of the genetic diversity of the species of 2EBN groups, and the 2n pollen present in *S. malmeanum* genotypes could contribute with 40% of its own allelic diversity as well to introgression of some interesting trait into the bridge species of upper groups of 2 EBN (Mendiburu and Peloquin, 1977; Peloquin et al. 1989; Hanneman 1999; Carputo et al. 2000).

Conclusions

Solanum malmeanum is mostly an allogamous species, some self-compatible genotypes are present pointing to a promising development of inbred lines for diploid breeding. The mechanism that allows accession BGB447 self-fertilize needs to be explored.

Solanum malmeanum diploid accessions can freely cross with *S. commersonii* and produce normal, plump and viable seeds. Barriers of hybridization between *S. malmeanum* and *S. chacoense* are not complete, evidencing the likely presence of 2n gametes in *S. malmeanum*., berries set abnormal and malformed seeds, however, some of these seeds can germinate. *S. malmeanum* is cross-incompatible with *S. tuberosum* cultivars. However, the partial successful results when crossed with *S. chacoense* points to alternative pathways for introgression with bridge species allowing further advances to unlock the reproductive isolation of this promising wild relative to broaden the potato crop genetic basis by the mean of introgression of desirable traits.

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Concluding remarks

Solanum malmeanum is a wild relative into tertiary gene pool of the potato crop (*S. tuberosum*) with high potential for its uses in potato breeding. The review of Chapter 1 showed that this species received conflicting taxonomic concepts, which made difficult to define the taxon via morphological traits, as well it was formely considered as a subspecies or a form of *S. commersonii*. Also, because these two taxa are partially distributed sympatrically and a wide morphological variability can be encountered reflecting diverse natural environments where they grow. Phylogeography studies aligned with morphological and reproductive approaches could better elucidate the circumscription of *S. malmeanum* under species level. Despite these constrains and changes to define the taxon, *S. malmeanum* has been proved to contains several traits that are remarkably interesting for potato breeding, including those genes to confer resistance to combat the main pathogens that reduces production of the crop, as well to tolerate abiotic stresses like frost and heat, including potential genes to increase quality traits for industrial purpose as higher dry matter content, low reducing sugar levels, and starch content in tubers.

In Chapter 2, the main characteristics about breeding system of *S. malmeanum* accessions were assessed according to its mode of reproduction and the cross-ability with its related species 2x 1EBN *S. commersonii*, 2x 2EBN *S. chacoense*, and the potato crop 4x 4EBN *S. tuberosum*. *Solanum malmeanum* is a diploid wild potato with presence of triploid specimens according to inference analysis by flow cytometry. This species sometimes produces parthenocarpic fruit, but does not produce apomictic seeds, is mainly allogamous with the rare presence of self-compatible genotypes. Some triploid genotypes reproduces only vegetatively. Diploid *S. malmeanum* accessions, buttriploids, easily hybridizes with the related 2x 1EBN *S. commersonii*. The success of hybridization between *S. malmeanum* accessions and its related 2x 2EBN *S. chacoense* are lower due to reducedseed germination. *Solanum malmeanum* is cross-incompatible with 4x 4EBN *S. tuberosum* cultivars. Under these results it is possible to infer the acting of the post-zygotic barriers of hybridization that resides on endosperm development. Thus,cies assigned as the same EBN groups such as *S. malmeanum* and *S. commersonii* of 1EBN can freely crosses, when other barriers are absent, but crossing species of different EBN groups as demonstrated via reciprocal crosses between 1 point difference EBN groups like 1EBN S*. malmeanum* and 2EBN

S. chacoense, seed germination are reduced. Finally, we confirmed again the cross-incompatibility between the most distal EBN species groups as 1EBN *S. malmeanum* and 4EBN *S. tuberosum*.

Solanum malmeanum, despite historically neglected, proved to be a promising wild species for potato breeding to face new demands. However, because its cross-incompatibility with the potato crop, it is difficult to introgress its genetic diversity, especially those for agronomical traits, into the potato primary genepool. The post-zygotic barrier of EBN is strong but not complete. Several strategies are available to overcome this barrier. On the other hand, some self-compatible accesions of *S. malmeanum* herein identififed are very desirable for the development of inbred lines for diploid potato breeding.

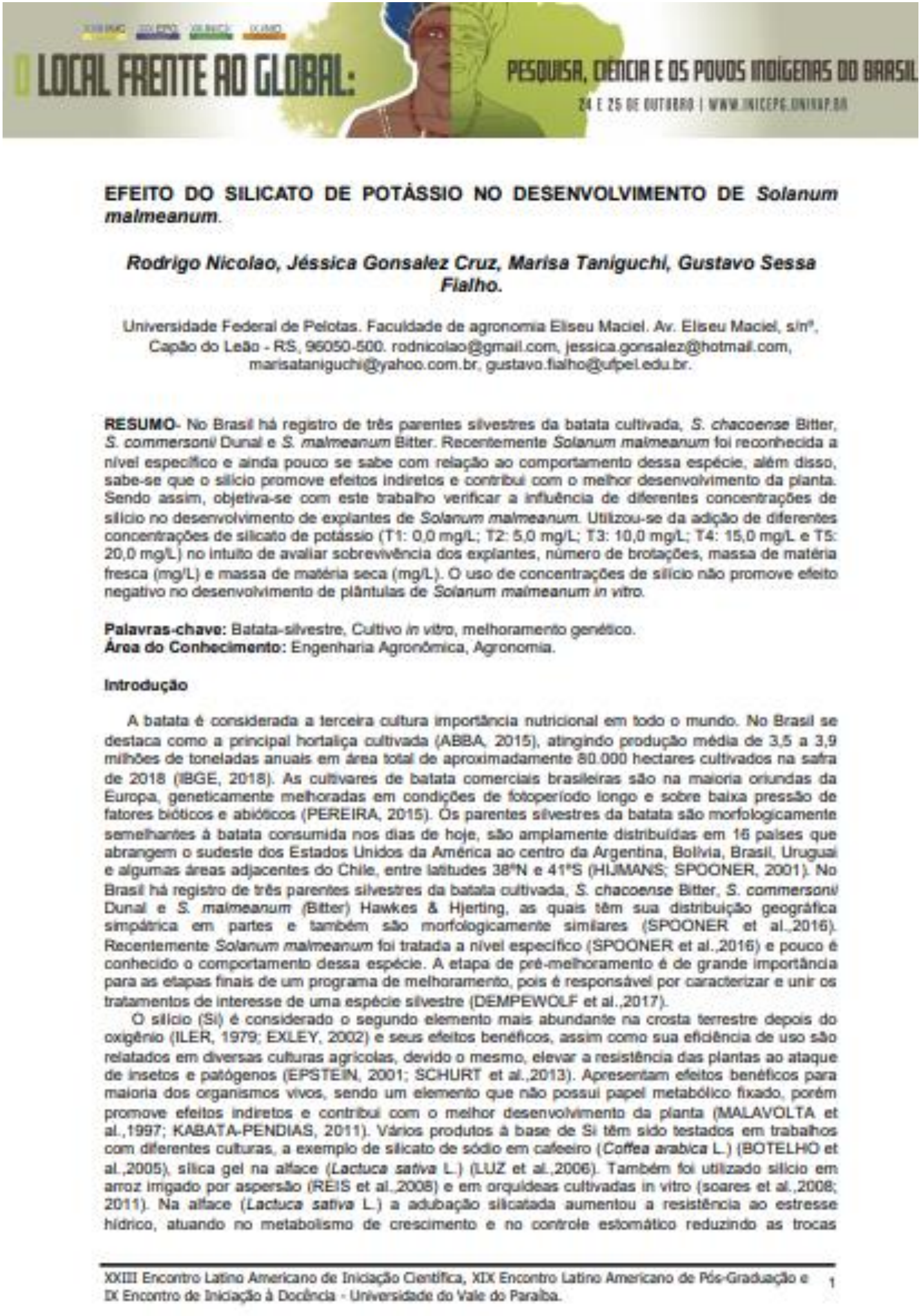
Cytogenetic data available are paving the way to overcome reproductive barriers and although deeper knowledge is needed concerning breeding systems, several attempts based on different techniques for hybridization with different levels of successful outcomes have been made demonstrating that introgression is a reachable goal. Potato breeding is facing a revolution with the proposal of innovative ways to face constraints imposed by the breeding at tetraploid level and theoretical and applied models are evolving faster than ever to broaden the genetic basis of the crop with the aid of wild species. Critical information towards the use of *S. malmeanum* for potato breeding is lacking. There is a lack of data concerning the functioning of self-compatibility (SC) and self-incompatibility (SI) at genetic level, and a comprehensive elucidation related to the meiotic mechanisms that lead for 2n gametes. Furthermore, the un-reduced pollen grain size must be verified. The reproductive system behavior must be systematically assessed for a comprehensive understanding of the pattern of its variability, as well for its adequate conservation, genetic studies, and to unlock ways to transmit its genetic diversity to the cultivated potato.

Future efforts on genetic sequencing, cytogenetics characterization, and phenotypic screening for desirable traits, considered in an integrative way, will enhance the fully unlocking of this promising potato wild relative for potato breeding!

Appendices

Appendix A – Articles and abstracts published in proceedings.

A.1 NICOLAO, R.; CRUZ, J. G.; TANIGUCHI, M.; FIALHO, G.S. **Efeito do silicato de potássio no desenvolvimento de *Solanum malmeanum***, 2019, Paraíba. XXIII INIC / XIX EPG / XIII INIC Jr / IX INID, 2019. Full text available in: http://www.inicepg.univap.br/cd/INIC_2019/anais/indice_geral.html



EFEITO DO SILICATO DE POTÁSSIO NO DESENVOLVIMENTO DE *Solanum malmeanum*.

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RESUMO- No Brasil há registro de três parentes silvestres da batata cultivada, *S. chacoense* Bitter, *S. commersonii* Dunal e *S. malmeanum* Bitter. Recentemente *Solanum malmeanum* foi reconhecida a nível específico e ainda pouco se sabe com relação ao comportamento dessa espécie, além disso, sabe-se que o silício promove efeitos indiretos e contribui com o melhor desenvolvimento da planta. Sendo assim, objetiva-se com este trabalho verificar a influência de diferentes concentrações de silício no desenvolvimento de explantes de *Solanum malmeanum*. Utilizou-se da adição de diferentes concentrações de silicato de potássio (T1: 0,0 mg/L; T2: 5,0 mg/L; T3: 10,0 mg/L; T4: 15,0 mg/L e T5: 20,0 mg/L) no intuito de avaliar sobrevivência dos explantes, número de brotações, massa de matéria fresca (mg/L) e massa de matéria seca (mg/L). O uso de concentrações de silício não promove efeito negativo no desenvolvimento de plântulas de *Solanum malmeanum in vitro*.

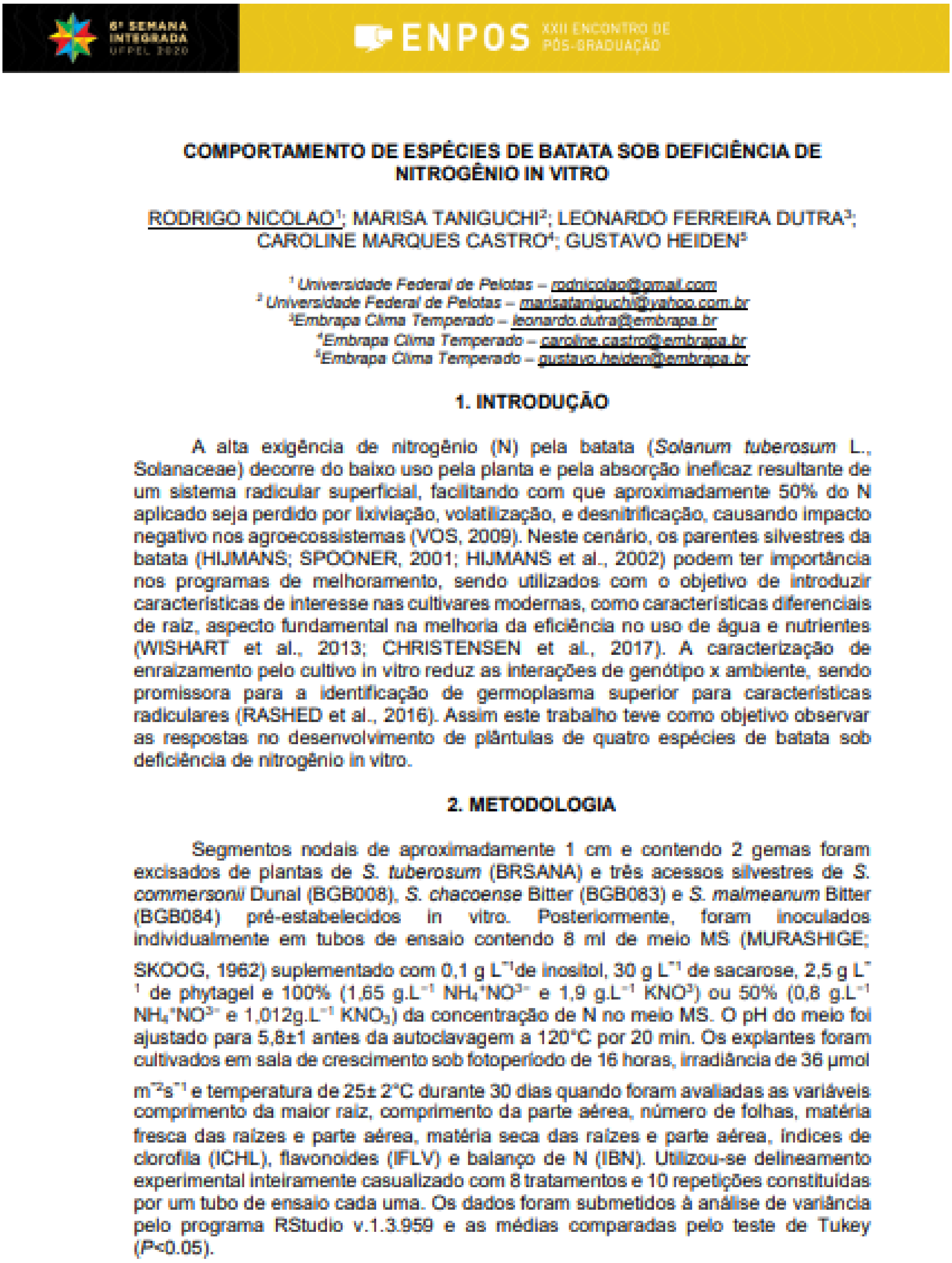
Palavras-chave: Batata-silvestre, Cultivo *in vitro*, melhoramento genético.
Área do Conhecimento: Engenharia Agronômica, Agronomia.

Introdução

A batata é considerada a terceira cultura importância nutricional em todo o mundo. No Brasil se destaca como a principal hortaliça cultivada (ABBA, 2015), atingindo produção média de 3,5 a 3,9 milhões de toneladas anuais em área total de aproximadamente 80.000 hectares cultivados na safra de 2018 (IBGE, 2018). As cultivares de batata comerciais brasileiras são na maioria oriundas da Europa, geneticamente melhoradas em condições de fotoperíodo longo e sobre baixa pressão de fatores bióticos e abióticos (PEREIRA, 2015). Os parentes silvestres da batata são morfologicamente semelhantes à batata consumida nos dias de hoje, são amplamente distribuídas em 16 países que abrangem o sudeste dos Estados Unidos da América ao centro da Argentina, Bolívia, Brasil, Uruguai e algumas áreas adjacentes do Chile, entre latitudes 38ºN e 41ºS (HIJMANS; SPOONER, 2001). No Brasil há registro de três parentes silvestres da batata cultivada, *S. chacoense* Bitter, *S. commersonii* Dunal e *S. malmeanum* (Bitter) Hawkes & Hjerting, as quais têm sua distribuição geográfica simpátrica em partes e também são morfologicamente similares (SPOONER et al.,2016). Recentemente *Solanum malmeanum* foi tratada a nível específico (SPOONER et al.,2016) e pouco é conhecido o comportamento dessa espécie. A etapa de pré-melhoramento é de grande importância para as etapas finais de um programa de melhoramento, pois é responsável por caracterizar e unir os tratamentos de interesse de uma espécie silvestre (DEMPEWOLF et al.,2017).

O silício (Si) é considerado o segundo elemento mais abundante na crosta terrestre depois do oxigênio (ILER, 1979; EXLEY, 2002) e seus efeitos benéficos, assim como sua eficiência de uso são relatados em diversas culturas agrícolas, devido o mesmo, elevar a resistência das plantas ao ataque de insetos e patógenos (EPSTEIN, 2001; SCHURT et al.,2013). Apresentam efeitos benéficos para maioria dos organismos vivos, sendo um elemento que não possui papel metabólico fixado, porém promove efeitos indiretos e contribui com o melhor desenvolvimento da planta (MALAVOLTA et al.,1997; KABATA-PENDIAS, 2011). Vários produtos à base de Si têm sido testados em trabalhos com diferentes culturas, a exemplo de silicato de sódio em cafeeiro (*Coffea arabica* L.) (BOTELHO et al.,2005), sílica gel na alface (*Lactuca sativa* L.) (LUZ et al.,2006). Também foi utilizado silício em arroz irrigado por aspersão (REIS et al.,2008) e em orquídeas cultivadas in vitro (soares et al.,2008; 2011). Na alface (*Lactuca sativa* L.) a adubação silicatada aumentou a resistência ao estresse hídrico, atuando no metabolismo de crescimento e no controle estomático reduzindo as trocas

A.2 NICOLAO, R.; TANIGUCHI, M.; DUTRA, L.F.; CASTRO, CM; HEIDEN, G. **Comportamento de espécies de batata sob deficiência de nitrogênio in vitro**, 2020, Pelotas. Anais 2020, 2020. Full text available in: https://wp.ufpel.edu.br/enpos/anais/anais-2019



6ª SEMANA INTEGRADA UFPEL 2020
ENPOS XXII ENCONTRO DE PÓS-GRADUAÇÃO

COMPORTAMENTO DE ESPÉCIES DE BATATA SOB DEFICIÊNCIA DE NITROGÊNIO IN VITRO

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1. INTRODUÇÃO

A alta exigência de nitrogênio (N) pela batata (*Solanum tuberosum* L., Solanaceae) decorre do baixo uso pela planta e pela absorção ineficaz resultante de um sistema radicular superficial, facilitando com que aproximadamente 50% do N aplicado seja perdido por lixiviação, volatilização, e desnitrificação, causando impacto negativo nos agroecossistemas (VOS, 2009). Neste cenário, os parentes silvestres da batata (HIJMANS; SPOONER, 2001; HIJMANS et al., 2002) podem ter importância nos programas de melhoramento, sendo utilizados com o objetivo de introduzir características de interesse nas cultivares modernas, como características diferenciais de raiz, aspecto fundamental na melhoria da eficiência no uso de água e nutrientes (WISHART et al., 2013; CHRISTENSEN et al., 2017). A caracterização de enraizamento pelo cultivo in vitro reduz as interações de genótipo x ambiente, sendo promissora para a identificação de germoplasma superior para características radiculares (RASHED et al., 2016). Assim este trabalho teve como objetivo observar as respostas no desenvolvimento de plântulas de quatro espécies de batata sob deficiência de nitrogênio in vitro.

2. METODOLOGIA

Segmentos nodais de aproximadamente 1 cm e contendo 2 gemas foram excisados de plantas de *S. tuberosum* (BRSANA) e três acessos silvestres de *S. commersonii* Dunal (BGB008), *S. chacoense* Bitter (BGB083) e *S. malmeanum* Bitter (BGB084) pré-estabelecidos in vitro. Posteriormente, foram inoculados individualmente em tubos de ensaio contendo 8 ml de meio MS (MURASHIGE; SKOOG, 1962) suplementado com 0,1 g L^{-1}de inositol, 30 g L^{-1} de sacarose, 2,5 g L^{-1} de phytagel e 100% (1,65 $g.L^{-1}$ $NH_4^+NO^{3-}$ e 1,9 $g.L^{-1}$ KNO^3) ou 50% (0,8 $g.L^{-1}$ $NH_4^+NO^{3-}$ e 1,012$g.L^{-1}$ KNO_3) da concentração de N no meio MS. O pH do meio foi ajustado para 5,8±1 antes da autoclavagem a 120°C por 20 min. Os explantes foram cultivados em sala de crescimento sob fotoperíodo de 16 horas, irradiância de 36 μmol $m^{-2}s^{-1}$ e temperatura de 25± 2°C durante 30 dias quando foram avaliadas as variáveis comprimento da maior raiz, comprimento da parte aérea, número de folhas, matéria fresca das raízes e parte aérea, matéria seca das raízes e parte aérea, índices de clorofila (ICHL), flavonoides (IFLV) e balanço de N (IBN). Utilizou-se delineamento experimental inteiramente casualizado com 8 tratamentos e 10 repetições constituídas por um tubo de ensaio cada uma. Os dados foram submetidos à análise de variância pelo programa RStudio v.1.3.959 e as médias comparadas pelo teste de Tukey (*P*<0.05).

A.3 NICOLAO, R.; CASTRO, CM; HEIDEN, G. **Tuber dry matter content in *Solanum malmeanum* accessions from Embrapa Clima Temperado Potato Genebank**, 2020. SOL International online meeting 2020. Abstract Book, 2020. p. 144-144. Full text available in: https://solgenomics.net/sol2020

Abstract

Crop Wild Relatives (CWR's) are considered a unique reserve of useful traits for human needs, which are not present in the genetic basis of current crops, however, are increasingly attracting the attention of breeders due to their great genetic diversity. *Solanum malmeanum* Bitter (Solanaceae) is a wild relative' species of cultivated potatoes (*S. tuberosum* L) from Cone Sur of South America. The objective of this study was to characterize the tuber dry matter of eleven accessions of *Solanum malmeanum* (BGB015, BGB017, BGB021, BGB080, BGB081, BGB084, BGB443, BGB446, BGB447, BGB448, BGB471) and two clones of *S. tuberosum* (BRSANA and C1750_15_95) conserved at Active Gene Bank of Potato from Embrapa Clima Temperado – Brazil, cultivated from in vitro plants at greenhouse on Spring 2019. Dry matter content was quantified by the oven drying the sample in an oven to constant weight at a temperature of 60ºC. Tuber dry matter content of samples was calculated by the formula; %DM = (dry weight/fresh weight) X 100, and the means of triplicate samples was calculated and separated in cluster using Scott-Knott multiple comparison ($p<0.05$). All accessions were separated in three groups (A, B and C) and CV = 14.99%. Into group A, BGB021 (51.74%), BGB017 (50.48%) and BGB015 (45.45%). Into group B, BGB471 (42.46%), BGB446 (40.45%), BGB443 (40.33%), BGB080 (38%), BGB084 (37.9%), BGB447 (37.46%), BGB448 (36.91%) and BGB081 (33.93%). Into group C, the two clones of *S. tuberosum*, C1750_15_95 (23.20%) and BRSANA (21.36%). *S. malmeanum* accessions contains higher tuber dry matter content than recommended levels, and higher than commercial clones of *S. tuberosum*.

Tuber dry matter content in *Solanum malmeanum* accessions from Embrapa Clima Temperado Potato Genebank

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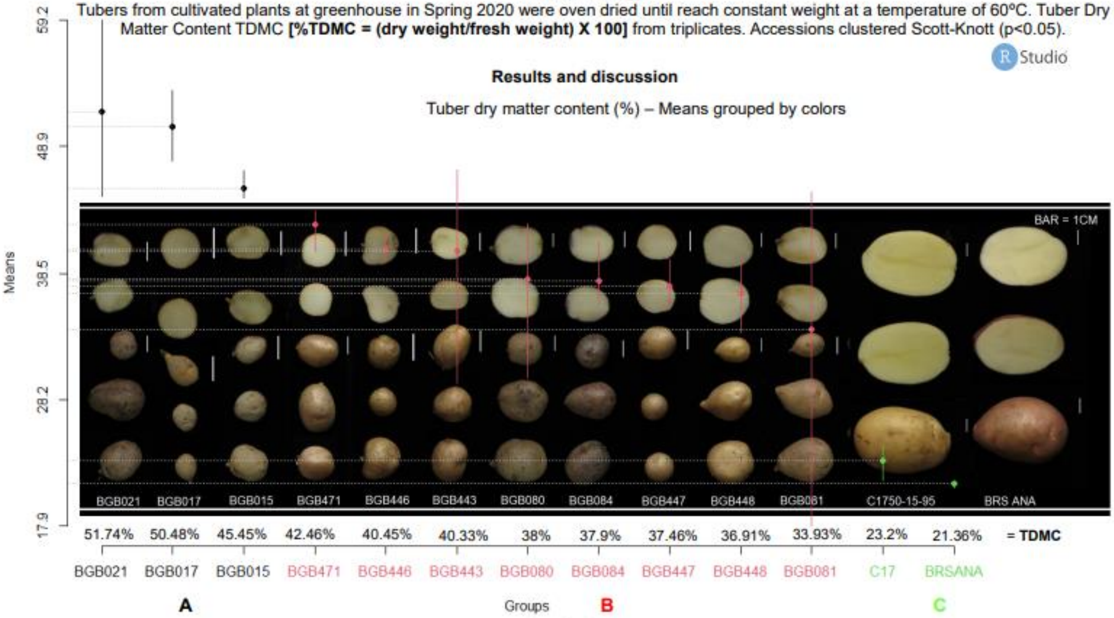
Methodology

Tubers from cultivated plants at greenhouse in Spring 2020 were oven dried until reach constant weight at a temperature of 60ºC. Tuber Dry Matter Content TDMC **[%TDMC = (dry weight/fresh weight) X 100]** from triplicates. Accessions clustered Scott-Knott ($p<0.05$).

Results and discussion


Tuber dry matter content (%) – Means grouped by colors

Conclusion

S. malmeanum accessions contains much higher TDMC than minimum recommended levels (>20%) and are promising sources for this trait for potato breeding.

Acknowledgments

CAPES/PROAP , CNPq (429368/2016-0) and FAPERGS (19/2551-0001703-0) for research funding, and CNPq for the master's scholarship (830425/1999-3).

Embrapa

CNPq

FAPERGS

A.4 NICOLAO, R.; CASTRO, CM; HEIDEN, G. **Biologia reprodutiva de *Solanum malmeanum* aplicada a conservação e uso do germoplasma no melhoramento genético da batata**, 2020. Anais do VI Congresso Brasileiro de Recursos Genéticos, 2020. Full text available in: http://recursosgeneticos.org/publicacao/anais-vi-congresso-brasileiro-de-recursos-geneticos

Anais
VI Congresso Brasileiro de Recursos Genéticos
09 a 12 de novembro de 2020
ISBN: 978-65-88187-01-2

Realização:
Apoio:
Patrocínio:

Biologia reprodutiva de *Solanum malmeanum* aplicada a conservação e uso do germoplasma no melhoramento genético da batata

O conhecimento da biologia reprodutiva de *Solanum malmeanum* B. (Solanaceae) é importante para compreender a variabilidade genética e morfológica, a dinâmica das populações naturais in situ, e para a conservação ex situ e uso do germoplasma em programas de melhoramento genético. Esta espécie é um parente silvestre da batata cultivada nativo do Brasil, Argentina, Paraguai e Uruguai. Possui resistência a alguns estresses bióticos e abióticos, além de características agronômicas qualitativas diferenciais nos tubérculos como o alto conteúdo de matéria seca e baixa concentração de açúcares redutores. Está classificada no pool gênico terciário (1EBN) e é considerada reprodutivamente isolada de outras espécies de batatas pelos diferentes grupos de cruzamento 2EBN e 4EBN. Este estudo visa caracterizar a biologia reprodutiva dos acessos BGB081 (triploide) e BGB471 (diploide) de *S. malmeanum*, do Banco Ativo de Germoplasma (BAG) de Batata da Embrapa Clima Temperado. O experimento foi conduzido em casa de vegetação (31°40’53.16”S, 52°26’23.60”W) entre agosto e dezembro de 2019. Cinco tratamentos foram aplicados em no mínimo 30 flores: T1-Controle (flores não emasculadas e nem polinizadas manualmente); T2-Apomixia (flores emasculadas e não polinizadas); T3-Autogamia (emasculação e autopolinização); T4-Compatibilidade com *S. tuberosum* L. (4x) (emasculação e polinização com pólen da linhagem C1750-15-95); T5- Alogamia (emasculação e polinização manual com mistura de pólens dos acessos de S. malmeanum do BAG). A emasculação foi feita em pré-antese, as polinizações ocorreram na manhã seguinte e todas as flores foram ensacadas para evitar contaminação. O T3 não foi realizado no BGB081, uma vez que este não produziu pólen. Nos tratamentos 1, 2, 3 e 4 não houve formação de frutos e sementes em nenhum acesso. No tratamento 5, o BGB081 não formou frutos e, das 30 flores polinizadas do BGB471, 15 frutificaram produzindo 210 sementes no total e em média 14 sementes/fruto. Portanto, o BGB081 (3x) não produz pólen, não é apomítico, não foi avaliado quanto a auto-incompatibilidade por não produzir pólen, não é compatível com *S. tuberosum* e não frutifica ao receber pólen dos demais genótipos, tendo reprodução apenas vegetativa. O BGB471 (2x) produz pólen, não é apomítico, é auto-incompatível, não é compatível com *S. tuberosum*, e é alógamo, pois frutifica e produz sementes ao receber pólen de outros genótipos, tendo reprodução sexuada e vegetativa.

Palavras-chave: batata-silvestre, parentes silvestres, pré-melhoramento

A.5 NICOLAO, R.; KLASEN, G.L.; CAMPOS, A.; SOUZA, S.M.; CASTRO, CM; HEIDEN, G. **Conteúdo de DNA (2C) e ploidia de *Solanum malmeanum* (Solanaceae)**, 2020. Anais do VI Congresso Brasileiro de Recursos Genéticos, 2020. Full text available in: http://recursosgeneticos.org/publicacao/anais-vi-congresso-brasileiro-de-recursos-geneticos

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ISBN: 978-65-88187-01-2

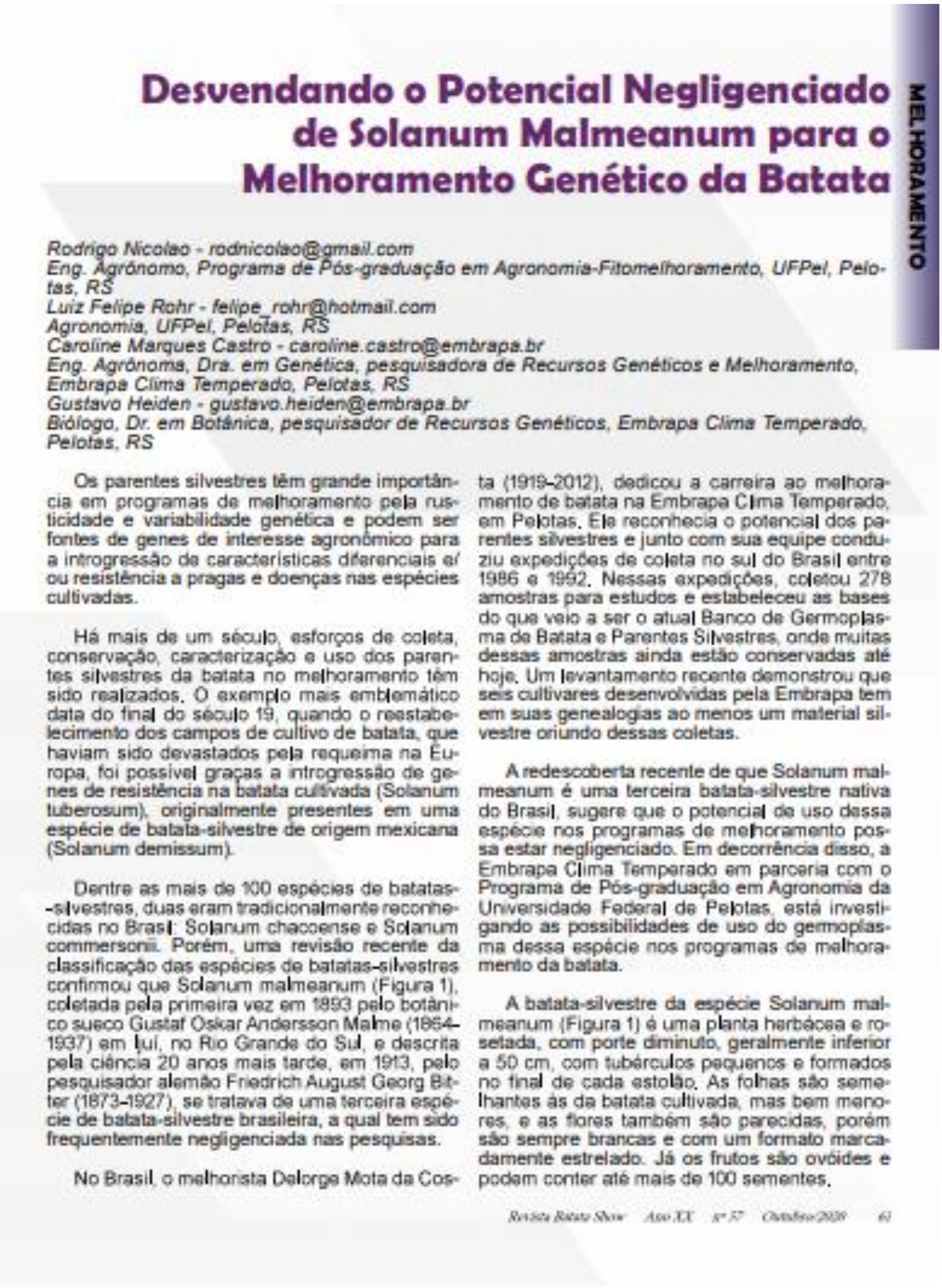
Conteúdo de DNA (2C) e ploidia de *Solanum malmeanum* (Solanaceae)

Solanum malmeanum Bitter (Solanaceae) é um parente silvestre da batata (*S. tuberosum* L.) nativo do Brasil, Argentina, Paraguai e Uruguai. O conteúdo do DNA nuclear é uma informação importante em programas de melhoramento genético. O valor C refere-se à estabilidade da quantidade de DNA nuclear de um organismo em um complemento cromossômico monoploide, independentemente da ploidia. Uma vez que o conteúdo de DNA (2C) está relacionado ao nível de ploidia, a citometria de fluxo é uma alternativa eficiente a outros métodos como a contagem de cromossomos. O estudo visou estimar o conteúdo de DNA (2C) e inferir a ploidia de oito acessos de *S. malmeanum* (BGB015, BGB017, BGB021, BGB080, BGB081, BGB443, BGB446, BGB471), do Banco Ativo de Germoplasma (BAG) de Batata da Embrapa Clima Temperado. Foram coletadas folhas jovens (30 mg por acesso), que foram fragmentadas em 700 µL de tampão de extração WPB. A solução foi filtrada em uma tela de nylon (50 µm) e adicionada de 25 µL de tampão de extração com 1 mg/mL de iodeto de propídeo e 5 µL de RNase. A suspensão obtida permaneceu por 1 hora no escuro a temperatura ambiente. Para cada amostra 10000 núcleos foram analisados usando escala logarítmica, em citômetro de fluxo CytoFLEX LX (Beckman Coulter). Folhas de Pisum sativum L. foram utilizadas como padrão interno (9,09 pg/2C). Para cada acesso foram realizadas 3 leituras. O conteúdo 2C foi calculado usando o pico médio de 3 repetições/amostra e do padrão interno. Somente medidas com coeficientes de variação menores que 5% foram consideradas. Os coeficientes de variação encontrados para os picos G1 variaram de 3,63% a 5%. Foram inferidos dois níveis de ploidia em *S. malmeanum*: diploide (2x) e triploide (3x). O conteúdo de DNA (2C) observado variou entre 1,89 a 3,03 pg. A variação no conteúdo 2C de DNA nuclear foi de 1,89 a 2,18 pg nos diploides e 2,82 a 3,03 nos triploides. BGB017 apresentou o menor conteúdo de DNA entre os diploides (1,89 pg), seguido por BGB015 (1,95 pg), BGB471 (2,05 pg), BGB443 (2,05 pg), e o maior valor foi observado no BGB446 (2,18 pg). Entre os triploides, BGB021 e BGB081 apresentaram os menores conteúdos de DNA (ambos com 2,82 pg), já o maior conteúdo foi observado no BGB080 (3,03 pg). Portanto, sugerimos que os acessos BGB015, BGB017, BGB443, BGB446, BGB471 são diploides, enquanto BGB021, BGB080 e BGB081 são triploides. Estudos citogenéticos futuros possibilitarão confirmar a ploidia inferida.

Palavras-chave: batata-silvestre, caracterização, germoplasma

Appendix B – Article in Magazine.

B.1 NICOLAO, R.; ROHR, LF; CASTRO, CM; HEIDEN, G. Desvendando o potencial negligenciado de Solanum malmeanum para o melhoramento genético da batata. Por que a cadeia da batata prospera, estagna ou implode?, Revista Batata Show, p. 61 - 63, 02 out. 2020. Text available in: http://www.abbabatatabrasileira.com.br/site/revista/edicao-57



MELHORAMENTO

Desvendando o Potencial Negligenciado de Solanum Malmeanum para o Melhoramento Genético da Batata

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Os parentes silvestres têm grande importância em programas de melhoramento pela rusticidade e variabilidade genética e podem ser fontes de genes de interesse agronômico para a introgressão de características diferenciais e/ou resistência a pragas e doenças nas espécies cultivadas.

Há mais de um século, esforços de coleta, conservação, caracterização e uso dos parentes silvestres da batata no melhoramento têm sido realizados. O exemplo mais emblemático data do final do século 19, quando o reestabelecimento dos campos de cultivo de batata, que haviam sido devastados pela requeima na Europa, foi possível graças a introgressão de genes de resistência na batata cultivada (Solanum tuberosum), originalmente presentes em uma espécie de batata-silvestre de origem mexicana (Solanum demissum).

Dentre as mais de 100 espécies de batatas--silvestres, duas eram tradicionalmente reconhecidas no Brasil: Solanum chacoense e Solanum commersonii. Porém, uma revisão recente da classificação das espécies de batatas-silvestres confirmou que Solanum malmeanum (Figura 1), coletada pela primeira vez em 1893 pelo botânico sueco Gustaf Oskar Andersson Malme (1864–1937) em Ijuí, no Rio Grande do Sul, e descrita pela ciência 20 anos mais tarde, em 1913, pelo pesquisador alemão Friedrich August Georg Bitter (1873–1927), se tratava de uma terceira espécie de batata-silvestre brasileira, a qual tem sido frequentemente negligenciada nas pesquisas.

No Brasil, o melhorista Delorge Mota da Costa (1919–2012), dedicou a carreira ao melhoramento de batata na Embrapa Clima Temperado, em Pelotas. Ele reconhecia o potencial dos parentes silvestres e junto com sua equipe conduziu expedições de coleta no sul do Brasil entre 1986 e 1992. Nessas expedições, coletou 278 amostras para estudos e estabeleceu as bases do que veio a ser o atual Banco de Germoplasma de Batata e Parentes Silvestres, onde muitas dessas amostras ainda estão conservadas até hoje. Um levantamento recente demonstrou que seis cultivares desenvolvidas pela Embrapa tem em suas genealogias ao menos um material silvestre oriundo dessas coletas.

A redescoberta recente de que Solanum malmeanum é uma terceira batata-silvestre nativa do Brasil, sugere que o potencial de uso dessa espécie nos programas de melhoramento possa estar negligenciado. Em decorrência disso, a Embrapa Clima Temperado em parceria com o Programa de Pós-graduação em Agronomia da Universidade Federal de Pelotas, está investigando as possibilidades de uso do germoplasma dessa espécie nos programas de melhoramento da batata.

A batata-silvestre da espécie Solanum malmeanum (Figura 1) é uma planta herbácea e rosetada, com porte diminuto, geralmente inferior a 50 cm, com tubérculos pequenos e formados no final de cada estolão. As folhas são semelhantes às da batata cultivada, mas bem menores, e as flores também são parecidas, porém são sempre brancas e com um formato marcadamente estrelado. Já os frutos são ovóides e podem conter até mais de 100 sementes.

Na natureza, essa espécie tem preferência por ambientes de campos nativos ou bordas de matas, mas às vezes também é encontrada em áreas cultivadas, roçadas ou pastejadas. De modo geral, ela ocorre no sul do Brasil, Argentina, Paraguai e Uruguai (Figura 2).

Figura 1. Batata-silvestre da espécie Solanum malmeanum sob cultivo para pesquisa na Embrapa Clima Temperado: (A) Aspecto da planta; (B) Sistema subterrâneo com raízes, estolões e tubérculos; (C) Flores; (D) Frutos; (E) Tubérculos.

Com base nos estudos em andamento, estamos revisando as coleções de batatas-silvestres brasileiras e redescobrindo antigos registros na literatura, em herbários e no banco de germoplasma que eram equivocadamente atribuídos a outras espécies de batata-silvestre (Solanum chacoense e Solanum commersonii) e que na realidade são Solanum malmeanum. Até o momento, já localizamos 97 registros de ocorrência (pontos no mapa da Figura 2) e 11 acessos de germoplasma da Embrapa que correspondem a essa espécie. Uma revisão de literatura, também em andamento, está revelando que acessos já estudados de Solanum malmeanum possuem diferentes níveis de resistência à murcha bacteriana e à requeima, à insetos como afídeos e o besouro-da-batata, à nematoides e a alguns vírus. Alguns acessos avaliados também possuem alta capacidade de aclimatação ao frio, uma característica muitas vezes correlacionada também com a resistência a outros estresses abióticos como seca e calor. Além disso, alguns acessos desta espécie possuem conteúdo de matéria seca nos tubérculos superiores a 20% e baixas concentrações de açúcares redutores (causadores do escurecimento na batata frita pela formação de acrilamida, é tóxico para o consumo humano). Todas essas, são características importantes e promissoras para o desenvolvimento de novas cultivares de batata mais resistentes e com aptidão ao processamento industrial.

Figura 2. Mapa de ocorrência de Solanum malmeanum na Argentina, Brasil, Paraguai e Uruguai.

Contudo, devido à barreiras genéticas, como o fato de a batata cultivada ser tetraploide e Solanum malmeanum ser diploide ou triploide, não é possível realizar um cruzamento direto entre as duas espécies para se obter uma progênie híbrida, devido ao desbalanço no número endospérmico entre os parentais, impossibilitando a fertilização e desenvolvimento de sementes botânicas facilmente. Com o objetivo de superar essas barreiras, as etapas atuais da pesquisa visam caracterizar a biologia reprodutiva (Figura 3) de Solanum malmeanum, possibilitando o desenvolvimento e aplicação de formas de contornar essas dificuldades e possibilitar a introgressão de características de interesse do parente silvestre na batata cultivada.

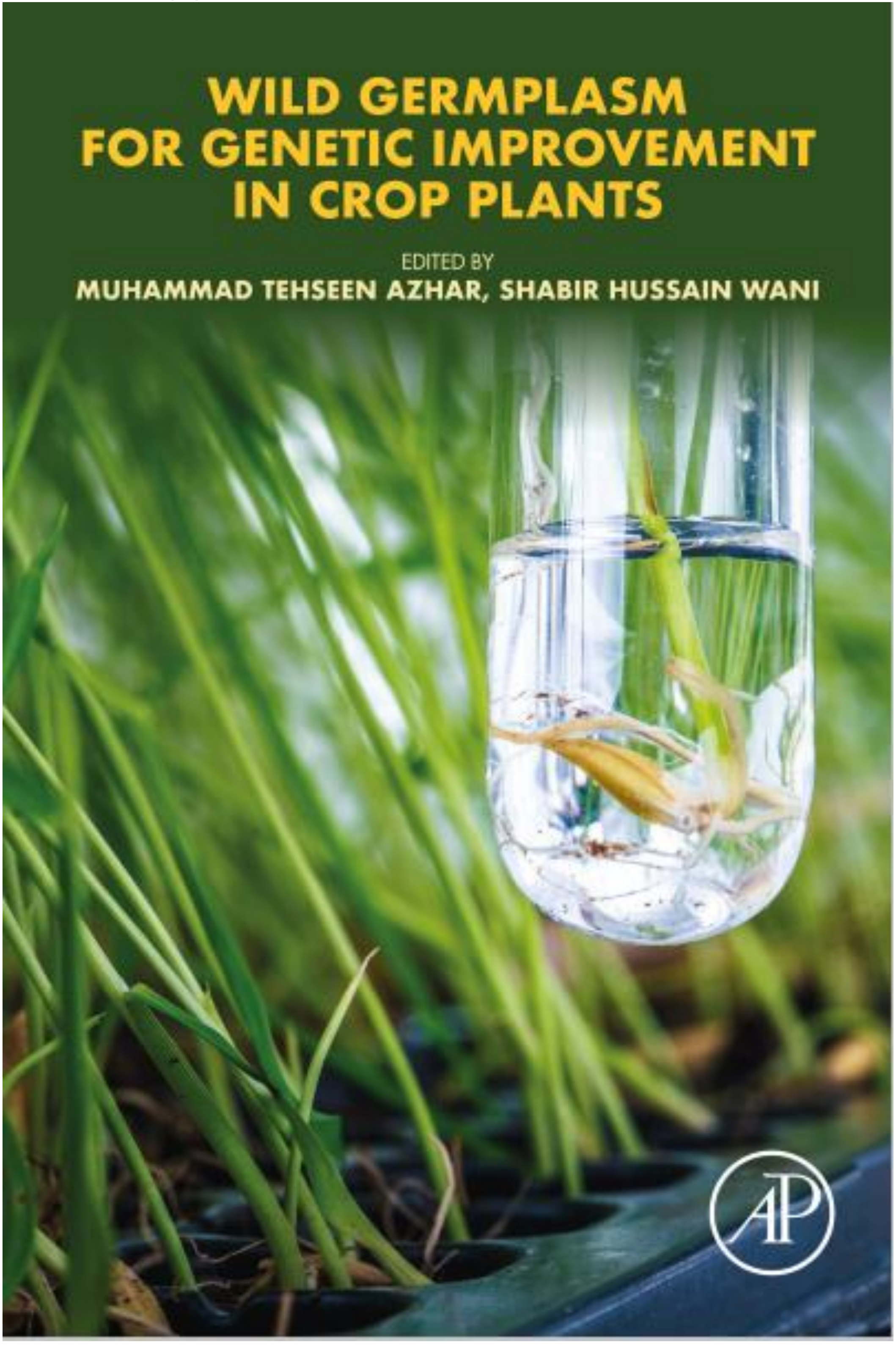
Figura 3. Ensaios para a caracterização reprodutiva e avaliação da cruzabilidade de Solanum malmeanum em casa-de-vegetação do Programa de Melhoramento da Batata na Embrapa Clima Temperado em Pelotas, RS.

Além da compreensão da biologia reprodutiva e da cruzabilidade de *Solanum malmeanum*, a introgressão de características da espécie silvestre pode ser facilitada também por meio de outras técnicas que manipulam o número cromossômico como poliploidização ou fusão de protoplastos, pela identificação e uso de gametas não-reduzidos ou cruzamento indireto por meio de espécies-ponte. Outras possibilidades são a incorporação da espécie no cada vez vais promissor melhoramento diploide ou o desenvolvimento e popularização de técnicas biotecnológicas como cisgenia e edição gênica (CRISPR). Contudo, as etapas atuais de resgate e revisão do conhecimento acumulado sobre a espécie e as ações de pré-melhoramento são os primeiros passos fundamentais para desvendar o potencial negligenciado deste parente silvestre brasileiro para o melhoramento genético da batata, assegurando a sustentabilidade e o futuro desse cultivo no longo prazo por meio da conservação, caracterização, avaliação e uso das fontes de variabilidade genética disponíveis.

Agradecimentos: Embrapa (Coleta de germoplasma de parentes silvestres de batata; Banco Ativo de Germoplasma de Batata e Parentes Silvestres; Prospecção de germoplasma silvestre de batata como fonte de genes de características especiais; Melhoramento genético de batata para ecossistemas tropicais e subtropicais do Brasil - 5º Ciclo); CAPES/PROAP; CNPQ (processo 429368/2016-0); e FAPERGS (processo 42860.540.28778.19072019).

Appendix C – Book Chapter.

C.1 BASHIR, I.; NICOLAO, R.; HEIDEN, G. Wild Potatoes: A Genetic Reservoir for Potato Breeding. In: Azhar, M.T. & Wani, S. (Org.). **Wild Germplasm for Genetic Improvement in Crop Plants**. 1 ed. Massachusetts: Elsevier, 2021, v. 1, p. 215-240. Available in: https://www.elsevier.com/books/wild-germplasm-for-genetic-improvement-in-crop-plants/azhar/978-0-12-822137-2



Wild Germplasm for Genetic Improvement in Crop Plants

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12. Wild Potatoes: A Genetic Reservoir for Potato Breeding

Ikram Bashir, Rodrigo Nicolao and Gustavo Heiden

13. Wild Relatives of Sweetpotato; Distribution and Prospectus for Crop Improvement

Muhammad Qadir Ahmad, Mohamed Hamed Arisha and Qiang Li

Chapter 12

Wild Potatoes: A Genetic Reservoir for Potato Breeding

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12.1 Introduction

Wild potatoes are a large genetic reservoir for potato breeding with current use and potential to provide genes for novel traits, and resistances for abiotic and biotic stresses absent in commercial cultivars. The first steps to use wild relatives in modern potato breeding started almost two centuries ago. Wild species from South America had been introduced in Europe in 1824, especially *S. commersonii* (Fig. 12.1) and *S. maglia*. Before 1920 many efforts were made to cross *S. demissum* with *S. tuberosum* and by 1932 introgression of resistance genes succeed first for late blight, later for virus (1941) and potato cyst nematode [1]. Since then, many resistance genes have been introduced into modern potato cultivars.

Potatoes (*Solanum* sect. *Petota*) taxonomy is a matter of debate. Accepted wild species ranges from 107 [2] to 180 [3], and domesticated from 4 (*S. ajanhuiri*, *S. curtilobum*, *S. juzepczukii*, *S. tuberosum* group *Andigenum*) [2] to 7, being 4 of them hybrids (*S.* × *ajanhuir*, *S.* × *chaucha*, *S.* × *curtilobum*, *S.* × *juzepczukii*), 2 of them with 4 subspecies (*S. stenotomum* subsp. *goniocalyx*, *S. stenotomum* subsp. *stenotomum*, *S. tuberosum* subsp. *andigenum*, *S. tuberosum* subsp. *tuberosum*) and 1 with no subspecies (*S. phureja*) [3].

Wild potatoes occur from southwestern United States of America (38°N) to central Argentina and Chile (41°S) and the Juan Fernández Archipelago. Most species are from South America with the diversity peaking at 21°S and a secondary center of diversity lies around 20°N in the central Mexican highlands [4]. They occur along a wide range of habitats from deserts to rainforests and from sea level to elevations around 4,700 m above sea level in the tropical Andean mountains. Potato wild relatives grow in sunny to partially sunny areas and in preserved or anthropogenic disturbed environments, including tussocks and grazed areas and edges or glades in temperate and subtropical forests [5].

Wild Germplasm for Genetic Improvement in Crop Plants. http://dx.doi.org/10.1016/B978-0-12-822137-2.00012-6

Appendix D - Science communication.

D.1 **Batata silvestre garante variabilidade para melhoramento genético - Parte 1 |** Programa Terra Sul. 2020. Documentary available in: https://www.youtube.com/watch?v=9VWJ2lVM5II&list=PLrvZulKr0UxBoUlBfstsjiJfP_mbCsb3c&index=5.

D.2 **Batata silvestre garante variabilidade para melhoramento genético - Parte 2 |** Programa Terra Sul. 2020. Documentary available in: https://www.youtube.com/watch?v=dWChO7-1Oaw&list=PLrvZulKr0UxBoUlBfstsjiJfP_mbCsb3c&index=6

D.3 **Comportamento de espécies de batata sob deficiência de nitrogênio in vitro**. XXII Encontro de Pós-Graduação, VI SIIEPE - UFPel. 2020. Video available in: https://www.youtube.com/watch?v=wC5E7lqbYx0

D.4 **Popularizando os Recursos Genéticos – Batata Silvestre**. Concurso Cultural de Vídeos: “Popularizando os Recursos Genéticos: o que são e onde estão no seu dia a dia”. Sociedade Brasileira de Recursos Genéticos. 2020. Video available in: https://www.youtube.com/watch?v=tHG7QseFHgo