The discovery of self-compatibility in the 1EBN Solanum malmeanum (Solanaceae) reveals novel source of genetic variability for diploid potato breeding

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1 **The discovery of self-compatibility in the 1EBN *Solanum malmeanum* (Solanaceae)**
2 **reveals novel source of genetic variability for diploid potato breeding**

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7 **F1 hybrid potato breeding at the diploid level requires the development of new inbred lines from self-**
8 **compatible germplasm. This study was carried out to search for new sources of self-compatible**
9 **diploid wild potatoes focusing on the 1EBN species *Solanum malmeanum*. Five diploid accessions**
10 **from Embrapa potato genebank were screened to evaluate their reproductive behavior and check if**
11 **the production of viable seeds when self-pollinated results from successful fertilizations or apomixis.**
12 **Measurements of the pollen were taken, and pollen viability was evaluated. Five treatments based on**
13 **controlled crosses assessed gametes compatibility; response to self-pollination; occurrence of**
14 **parthenocarpy and/or apomixis; and tested the accessions for autogamy and allogamy. Pollen**
15 **viability vary from 96% to 100%, with viable pollen size varying from 20 μm to 22 μm. Cross-**
16 **pollinations confirmed that all accessions produced viable gametes and are allogamous, since pollen**
17 **tube growth confirmed fertilization in treatments where seeds were set. No accession produced**
18 **parthenocarpic fruit nor apomictic seeds. Four self-pollinated accessions were self-incompatible with**
19 **no fruit and seeds set. Self-incompatibility was due to a pre-zygotic barrier confirmed by inhibited**
20 **pollen tube growth at 1/3 style length in BRA 00183778-0 and 2/3 in BRA 00167071-0, BRA 00167093-**
21 **4, and BRA 00183754-1. One self-pollinated accession (BRA 00183755-8) produced 3 fruits totalizing**
22 **51 seeds. The self-compatibility in this accession was confirmed based on the image record of pollen**
23 **tubes reaching the ovules, thus rejecting apomixis, which also did not occur in control (non-**
24 **manipulated bagged flowers) and emasculated bagged flowers. The 51 endogamic seeds obtained**
25 **were sown, and 17 seedlings were obtained, from which 16 tuberized. Still, further investigations are**
26 **needed to elucidate the mechanism responsible for the self-compatibility, such as *S*-locus inhibitor**
27 **(*Sli*), malfunction of *S*-RNase or HT genes.**

28 Potato wild relatives (*Solanum* sect. *Petota* Dumort., Solanaceae) are more diverse than the domesticated
29 potato *Solanum tuberosum* L [1,2,] and represent an important source of genetic diversity for potato breeding[3].
30 More than 100 potato wild species are present into the gene pool of the potato crop and are widely
31 distributed in natural landscapes of the American continent[4], from which around 70% of the wild species
32 and landraces are diploid[5]. Diploid potatoes (2n=2x=24) are known to be self-incompatible (SI) due to the
33 genetically controlled gametophytic incompatibility system that enable the pistil to identify and reject its
34 self-pollen[6, 7]. This mechanism limits the use of the valuable genetic resources of diploid potato germplasm
35 in potato breeding. Despite this constraint, self-compatibility has been found in some wild and cultivated
36 potato species (i.e., *S. kurtzianum* Bitter & Wittm., *S. phureja* Juz. & Bukasov, *S. polyadenium* Greenm, *S.*
37 *raphanifolium* Cárdenas & Hawkes, *S. stenotomum* Juz. & Bukasov, and *S. verrucosum* Schltdl.)[8].
38 Notwithstanding, the identification of a dominant self-incompatibility inhibitor (*Sli*) gene in the diploid
39 wild species *S. chacoense* Bitter[9, 10, 11] opened the doors to exploit the genetic resources implicit into the
40 wild potatoes[12, 13, 14, 15, 16].

41 The discovery of diploid self-compatible potatoes leads to proposals for the re-domestication of
42 the potato crop from an autotetraploid and vegetatively propagated tuber crop to a F1-hybrid ideotype bred
43 at diploid level and propagated by true-potato-seeds (TPS)[17]. This approach is considered a very promising
44 way to accelerate potato breeding to deal with climate change and ensure food security due to the feasibility
45 to unlock the potential genetic diversity present into the potato wild relatives[18, 19] because self-compatibility
46 allows the generation of inbred lines of diploid potatoes. Diploid inbred lines favor potato breeders to
47 exclude unfavorable alleles by inbreeding processing and fix and increase genetic gains of the interesting
48 traits through crossing contrasting genotypes and cycles of selection and backcrossing[20, 21].

49 *Solanum malmeanum* Bitter is a tertiary (2x, 1EBN) wild potato belonging to *Solanum* ser.
50 *Commersoniana* Buk.[22, 23]. This species occurs in Argentina, Brazil, Paraguay and Uruguay, from the sea
51 level up to 700 m of elevation[24, 25]. This potato wild relative is ex situ conserved in several genebanks
52 around the world[26] and encloses several interesting traits to contribute for the development of new potato
53 cultivars. Some accessions are resistant against bacterial (*Ralstonia solanacearum* (Smith) Yabuuchi) and
54 verticillium wilt (*Verticillium dahlia* Kleb.); ring rot (*Corynebacterium michiganensis* subsp. *Sepedonicus*
55 Spieck. & Kotth.); late (*Phytophthora infestans* Mont.) and early blight (*Alternaria solani* Elis & Martin);
56 fusarium dry rot (*Fusarium sambucinum* Fuckel); hapla (*Meloidogyne hapla* Chitwood) and cyst nematode
57 (*Globodera rostochiensis* Wollenweber); colorado potato beetle (*Leptinotarsa decemlineata* Say); potato

58 leaf hopper (*Empoasca fabae* Harris); green peach aphid (*Myzus persicae* Sulzer); potato aphid
59 (*Macrosiphum euphorbiae* Thomas); and potato leafroll virus (PLRV) (*Polerovirus* sp.)[27, 28, 29, 30, 31, 32, 33, 34].
60 In addition, it is a genetic reservoir for quality traits for industrial purposes such as high dry matter and
61 protein contents, and low content of reducing sugar and glycoalkaloids in tubers[35, 36, 37]. Additionally, some
62 authors emphasized its good performance under low temperatures and frost[38, 39] as well as its sister species
63 *Solanum commersonii* Dunal.

64 Based on this background, we screened the five available diploid accessions of the potato wild
65 relative *S. malmeanum* to assess their self-compatibility/self-incompatibility behavior looking for novel
66 sources of germplasm to increase the genetic basis readily available for diploid potato breeding efforts.

67 **Results**

68 **Pollen viability.** Pollen viability was 96% in BRA 00183754-1 and 100% in BRA 00167071-0,
69 BRA 00167093-4, BRA 00183755-8, and BRA 00183778-0 (Table 6; Supplementary Fig. S1 and Fig. S2).

70 **Measurement of viable pollen size.** The size of viable pollen of diploid *S. malmeanum* accessions
71 vary from 20 to 22 µm. BRA 00167071-0, BRA 00183755-8, and BRA 00183778-0 have viable pollen
72 grains with diameter of 20 µm, and the accessions BRA 00167093-4 and BRA 00183754-1 have viable
73 pollen size with 22 µm diameter. The average of pollen size of all *S. malmeanum* accessions is 20.8 µm.
74 Unreduced pollen grains are absent in all accessions (Supplementary Table S1).

75 **Control (spontaneous self-pollination; parthenocarpy):** From 30 flowers treated there were no
76 development of fruit in all accessions (BRA 00167071-0, BRA 00167093-4, BRA 00183754-1, BRA
77 00183755-8, BRA 00183778-0). Percent fruit set is presented in Table 1.

78 **Apomixis and parthenocarpy**: From 30 flowers treated there were no development of apomictic
79 seeds in any accession (BRA 00167071-0, BRA 00167093-4, BRA 00183754-1, BRA 00183755-8, BRA
80 00183778-0) Percent fruit set, average of seeds produced per fruit and germination rate (%) of produced
81 seeds are presented in Table 2.

82 **Autogamy (geitonogamy; self-compatibility-SC/self-incompatibility-SI)**: Four accessions
83 (BRA 00167071-0, BRA 00167093-4, BRA 00183754-1, BRA 00183778-0) were self-incompatible (SI)
84 and accession BRA 00183755-8 had pollen tube elongation reaching the ovary and behaved as self-
85 compatible (SC) as follows:

86 BRA 00167071-0 self-pollen germinated but is inhibited at 2/3 of style length (Supplementary Fig.
87 S3), thus it did not produce fruit from 14 self-pollinations and is self-incompatible;

88 BRA 00167093-4 self-pollen germinated but is inhibited at 2/3 of style length (Supplementary Fig.
89 S4), thus it did not produce fruit from 14 self-pollinations and is self-incompatible;

90 BRA 00183754-1 self-pollen germinated but is inhibited at 2/3 of style length (Supplementary Fig.
91 S5), thus it did not produce fruit from 22 self-pollinations and is self-incompatible;

92 BRA 00183755-8 self-pollen germinated and reached the ovary (Figure 1), thus it produced three
93 fruits with 51 seeds in total (average of 17 seeds per fruit) from 14 pollinations (Figure 2) and is self-
94 compatible. Germination of endogamous seeds was 37.1% and from 17 plants germinated and fully grown,
95 16 tuberized (Figure 3);

96 BRA 00183778-0 self-pollen germinated but is inhibited at 1/3 of style length, (Supplementary
97 Fig. S6) thus it did not produce fruit from 30 self-pollinations and is self-incompatible.

98 Percent fruit set, average of seeds produced per fruit and germination rate (%) of produced seeds
99 of autogamy treatment are presented in Table 3.

100 **Allogamy (female fertility/female sterility; induced parthenocarpy)**: five accessions (BRA
101 00167071-0, BRA 00167093-4, BRA 00183754-1, BRA 00183755-8, BRA 00183778-0) are allogamous
102 and female fertile as follows:

103   BRA 00167071-0 produced four fruits from 11 pollinations, 103 seeds in total, average 25.7 seeds
104 per fruit and had 75% seed germination;

105   BRA 00167093-4 produced 13 fruits from 16 pollinations, 1,542 seeds in total, average 118.6
106 seeds per fruit and had 50% seed germination;

107   BRA 00183754-1 produced 25 fruits from 28 pollination, 1,743 seeds in total, average 69.7 seeds
108 per fruit and had 61.1% seed germination;

109   BRA 00183755-8 produced seven fruits from 15 pollinations, 425 seeds in total, average 60.7
110 seeds per fruit and had 100% seed germination;

111   BRA 00183778-0 produced 20 fruits from 30 pollinations, 1,625 seeds in total, average 81.2 seeds
112 per fruit and had 100% seed germination.

113   Percent fruit set, average of seeds produced per fruit and germination rate (%) of produced seeds
114 of allogamy treatment are presented in Table 4. The results of all treatments of crossings are summarized
115 in Table 5.

Accession Genesys code	Fruit Set %	Seeds/Fruit	Germination Rate
BRA 00167071-0	0	N/A	N/A
BRA 00167093-4	0	N/A	N/A
BRA 00183754-1	0	N/A	N/A
BRA 00183755-8	0	N/A	N/A
BRA 00183778-0	0	N/A	N/A

116 **Table 1.** Control treatment. Percent fruit set, average number of seeds per fruit and germination
117 rate for the diploid wild potato *S. malmeanum* accessions from Embrapa Potato Genebank, Pelotas, RS,
118 Brazil. N/A=seed count and germination rate could not be collected for plant accessions that did not produce
119 fruit.
120

Accession Genesys code	Fruit Set %	Seeds/Fruit	Germination Rate
BRA 00167071-0	0	N/A	N/A
BRA 00167093-4	0	N/A	N/A
BRA 00183754-1	0	N/A	N/A
BRA 00183755-8	0	N/A	N/A
BRA 00183778-0	0	N/A	N/A

121 **Table 2.** Apomixis treatment. Percent fruit set, average number of seeds per fruit and germination
122 rate for the diploid wild potato *S. malmeanum* accessions from Embrapa Potato Genebank, Pelotas, RS,
123 Brazil. N/A=seed count and germination rate could not be collected for plant accessions that did not produce
124 fruit.
125

Accession Genesys code	Fruit Set %	Seeds/Fruit	Germination Rate
BRA 00167071-0	0	N/A	N/A
BRA 00167093-4	0	N/A	N/A
BRA 00183754-1	0	N/A	N/A
BRA 00183755-8	21	17	37.1
BRA 00183778-0	0	N/A	N/A

126 **Table 3.** Autogamy treatment. Percent fruit set, average number of seeds per fruit and germination
127 rate for the diploid wild potato *S. malmeanum* accessions from Embrapa Potato Genebank, Pelotas, RS,
128 Brazil. N/A=seed count and germination rate could not be collected for plant accessions that did not produce
129 fruit.

Accession Genesys code	Fruit Set %	Seeds/Fruit	Germination Rate
BRA 00167071-0	36	25.75	75
BRA 00167093-4	81	118.62	50
BRA 00183754-1	89	69.72	61.1
BRA 00183755-8	50	60.71	50
BRA 00183778-0	66	81.25	100

130 **Table 4.** Allogamy treatment. Percent fruit set, average number of seeds per fruit and germination
131 rate for the diploid wild potato *S. malmeanum* accessions from Embrapa Potato Genebank, Pelotas, RS,
132 Brazil. N/A=seed count and germination rate could not be collected for plant accessions that did not produce
133 fruit.

Accession Genesys code	Pollen viability	Unreduced pollen	Control (self-pollination)	Parthenocarpy	Apomixis	Autogamy* (manual self-pollination)	Allogamy (manual bulk pollination, female fertility)
BRA 00167071-0	100%	Absent	Absent	Absent	Absent	$SI_{2/3}$	Fertile
BRA 00167093-4	100%	Absent	Absent	Absent	Absent	$SI_{2/3}$	Fertile
BRA 00183754-1	96%	Absent	Absent	Absent	Absent	$SI_{2/3}$	Fertile
BRA 00183755-8	100%	Absent	Absent	Absent	Absent	SC	Fertile
BRA 00183778-0	100%	Absent	Absent	Absent	Absent	$SI_{2/3}$	Fertile

Table 5. Summary of presence or absence of the breeding system behavior of five diploid wild potato *Solanum malmeanum* accessions from Embrapa Potato Genebank, Pelotas, RS, Brazil. * Self-Compatible (SC); Self-Incompatible ($SI_{1/3}$ region of the style, $SI_{2/3}$ region of the style, $SI_{3/3}$ region of the style)
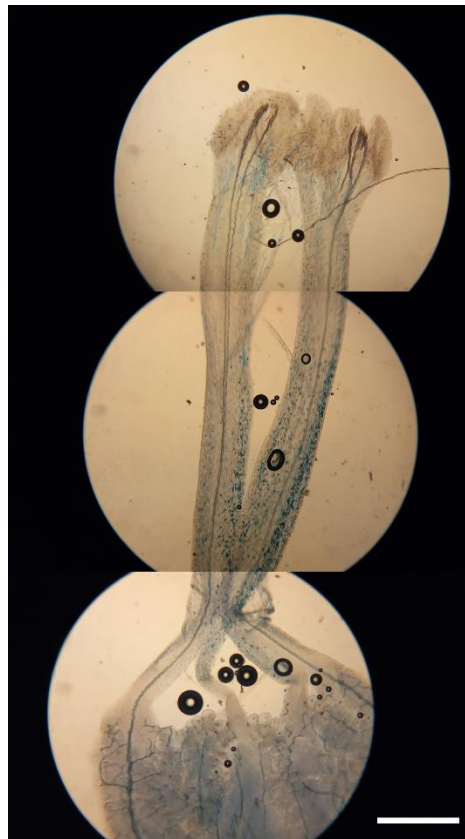
Figure 1. Pollen tube growth reaction of self-pollinated diploid *Solanum malmeanum* accessions. a) Pollen tube elongation in the style of self-compatible diploid wild potato *Solanum malmeanum* accession BRA 00183755-8 (Genesys code) from Embrapa Potato Genebank, Pelotas, RS, Brazil, using LACMOID staining technique and observation using an optical microscope. Detailed of a) Pollen tubes elongated and reaching the ovary (100× magnification). Bar = 0.5 mm.

Figure 2. Fruits (a), and seeds (b) obtained from self-fertilized diploid wild potato *Solanum malmeanum* accession BRA 00183755-8 (Genesys code) from Embrapa Potato Genebank, Pelotas, RS, Brazil. Bars = 1 cm.

Figure 3. Four of the 17 first-generation endogamous plants from self-pollinated diploid wild potato *Solanum malmeanum* accession BRA 00183755-8 (Genesys code) from Embrapa Potato genebank growing under greenhouse conditions at Pelotas, RS, Brazil.

Discussion

Diploid wild potato *Solanum malmeanum* accessions produces viable pollen grains, which vary in diameter size from 20 to 22 µm. According to the categories for describing pollen viability[41], all accessions evaluated here are into the category 7, whereas the pollen viability ranges from 80 to 100%, considered as high. Tarn and Hawkes[42] reported that some diploid clones of *S. malmeanum* (published as *S. commersonii* subsp. *malmeanum*) presented lower pollen stainability, varying from 37% to 56%, with 23,2 µm in size.

Wild potato diploid *Solanum malmeanum* accessions produces viable pollen, are female fertile, do not set apomictic fruits nor set parthenocarpic fruits. Most accessions are self-incompatible, but sometimes self-compatible genotypes may occur, and this condition could be more widely distributed among the potato crop genepool than previously thought, considering the set of evaluated accessions in the current study. Additionally, more investigations are needed to explain the molecular mechanisms that allows the diploid accession BRA 00183755-8 self-fertilizes.

All diploid *S. malmeanum* accessions evaluated were not able to self-fertilize in natural conditions (control) and did not set fruit in the treatment whereas the flowers were emasculated in pre-anthesis. These results evidence the non-preference for autogamy nor the occurrence of apomixis, respectively. Otherwise, *S. malmeanum* accessions are confirmed as fertile when hand-pollinated with bulked pollen, evidencing its preference for allogamy. The manual self-pollination showed that from the five diploid accessions evaluated, only BRA 00183755-8 was able to self-fertilize. Most diploid wild potatoes are known to be

171 self-incompatible (SI)[8, 43, 44, 45, 46], as observed in BRA 00167071-0, BRA 00167093-4, BRA 00183754-1,
172 and BRA 00183778-0. Self-fertilization in diploid potato is limited by the gametophytic self-
173 incompatibility (GSI) system that is controlled by a single multiallelic *S-locus* on chromosome 1 that
174 contains tightly linked genes encoding *S-RNase* (female determinant) and multiple pollen-specific *S*-locus
175 *F*-box (SLF/SFB) (male determinant) genes[47, 48, 49]. *S*-RNase is the main determinant for self-
176 incompatibility in flowering plants[7]. Each *S*-allele encodes an *S*-RNase and multiple *SLF/SFB* with different
177 specificities, which together can recognize all *S*-RNases except the *S*-RNase present on the same allele[48].
178 If the pollen *S*-haplotypes coincide either of the *S*-haplotype in the diploid pistil, the ribonuclease activity
179 of *S*-RNases exerts cytotoxicity and inhibit pollen tube growth in the style[50, 51]. The self-compatibility
180 resulted of the ubiquitination of *S*-RNases is mediated by the pollen determinant SLF/SFB. The pollen-
181 expressed *S-locus F-box* (SLF/SFB) proteins recognize all *S*-RNases and direct them to the proteasomal
182 degradation manner, preventing the cytotoxicity reaction, in this way it allows pollen tube growth towards
183 the ovaries where fertilization occurs[49, 52, 53, 54]. Gametophytic self-incompatibility is found in many
184 important groups of cultivated plants such as Poaceae, Plantaginaceae, Rosaceae and Solanaceae[47, 53, 55].
185 For example, most tomato wild relatives (*Solanum* sect. *Lycopersicon* (Mill.) Wettst.) are self-incompatible,
186 but the cultivated tomato is self-compatible due to the loss of function of *S*-RNase and other self-
187 incompatibility-related genes (HT genes)[56]. In the self-compatible edited diploid wild potato whereas
188 researchers silenced the *S*-RNase, the number of seeds per fruits ranged from 67 to 288 seeds per fruit[57].

189   The self-compatibility behavior found in BRA 00183755-8, a diploid *S. malmeanum* accession,
190 could be caused by diverse factors. One is the putative presence of a *S-locus inhibitor (Sli)* gene which
191 break the self-incompatibility reaction. The self-compatibility was previously identified in the potato wild
192 relatives *S. chacoense*[58] and *Sli* gene was mapped at chromosome 12[9, 10]. Clot and collaborators[15] found that
193 *Sli* allele (SC haplotype) is widespread in the cultivated gene pool of potato, in which has been early
194 originated from the introduction of the tetraploid cultivar Rough Purple Chili introduced into the USA in
195 1851[59]. From the potato wild relative *S. chacoense*, Eggers and collaborators[11] reported the mapping of *Sli*
196 gene to an interval from 628 kb to 12.6 kb on chromosome 12, and the 533 bp in the promotor of the *Sli*
197 leads to a mal-function of *S*-RNases. Kaiser and collaborators[60] pointed out that *Sli* markers can predict SC
198 in potato germplasm, but due to some discrepancies observed in the phenotype response, the authors
199 highlighted that is necessary to identify other regions responsible for controlling SC as well the
200 environmental forces acting in the expression of genes enrolled in the SC reaction. However, many *Sli*
201 KASP™ markers are available[60]. The diploid potato clone 'M6' is a successful example of fertile and vigor
202 diploid potato inbred lines create by seven generation of self-pollination of the wild diploid species *S.*
203 *chacoense* relying on the *Sli* gene[14]. Ma and collaborators[61] identified a *non-S-locus F-box (NSF)* gene in
204 RH-89-039-16 (RH), line derived diploid *S. tuberosum*. The *NSF* gene interact with the multiple-allelic
205 variants of *S*-RNases, operating as the same way of a general *S-locus inhibitor* that allows self-fertilization,
206 however, the authors attested that *NSF* is the *Sli* gene and confers SC in M6[61]. Another hypothesis for SC
207 behavior in BRA 00183755-8 could be the presence of *SLF/SFB* gene, HT genes or mal-function of *S*-
208 RNases, as explained above[56, 60, 61].

209   Two possible approaches could overcome SI in diploid potatoes. The first is the transfer of *Sli*
210 through conventional breeding[14, 60, 61, 62]. It is widely demonstrated that the *Sli* gene can be efficiently used
211 to introduce SC from the diploid potato lines to other SI lines diploid potatoes[14, 15, 60, 61, 63]. The second way
212 to overcomes SI could be the manipulation of *S*-locus. Some researchers showed the state-of-the art to re-
213 invent self-compatible diploid potato by knocking out the *S*-RNase alleles using the CRISPR-Cas9
214 system[57, 65] and searching for mutants of *S*-RNase[64]. The knockout of *S*-RNases by genetic engineering
215 permits to overcomes self-incompatibility in diploid potato without introducing any exogenous DNA[57, 65].

216   The identification of self-compatibility in a diploid *S. malmeanum* accession for the first time
217 allows to generate future novel sources of self-compatible diploid germplasm, with efficient production of
218 self-fertilized seeds and the development of recombinant inbred lines (RILs), introgression lines (ILs),
219 multi-parent advanced generation intercrosses populations (MAGICs), and nested association mapping
220 populations (NAMs)[12, 13, 14, 16, 66]. Diploid inbred lines are fundamental materials to address some limitations
221 for potato breeding and functional genetics[12, 57, 63, 66], and novel sources of self-compatible diploid plants is
222 desirable.

223   Diploid potato germplasm facilitates a high throughput phenotyping and efficient QTL detection
224 because of reduced genome complexity and homozygosity for example[67, 68, 69, 70]. The genome design of
225 hybrid diploid potato was elucidated[21] and some protocols for 'speed breeding' are proposed to create a
226 precise controlled environmental of chamber growth combined with the most sophisticated technologies
227 (i.e., photoperiod and temperature control, with genomic-assisted and artificial intelligence supports), and
228 can be implanted in any plant breeding program[71, 72, 73, 74, 75]. Hybrid diploid breeding enables potato genetic
229 resources to be exploited with less time and more precision compared to conventional potato allotetraploid

230 breeding, still, the potential process of breeding and the generation of new F1-hybrid potato cultivars at the
231 diploid level for developing one climate-smart potato crop is promising.

232 To move forward with the first generation of self-compatible *S. malmeanum* BRA 00183755-8 offspring,
233 the next steps involve evaluating the fertility of the S1 population. This assessment will facilitate the
234 advancement of the hybrid breeding process, with numerous strategies available in literature, including
235 bioinformatics, genomics, genetic engineering, and environmental control. Furthermore, a plan is required
236 to incorporate this 1EBN species into diploid potato breeding, pre-breeding, or hybrid breeding (2EBN),
237 such as by employing chromosome doubling or 2n gametes.

238 We consider this study relevant for valuation of germplasm conservation and utilization.
239 Considering the advances achieved so far and the technologies available, *S. malmeanum* can be considered
240 one valuable genetic resource which can contribute to potato breeding programs at diploid level.

241 **Methods**

242 **Plant material.** Five diploid *Solanum malmeanum* accessions conserved at Embrapa Potato Genebank
243 were available for this study (Table 6, Figure 4).

244 **Study site.** The experiment was conducted under greenhouse conditions at Embrapa Temperate
245 Agriculture, a research unit of the Brazilian Agriculture Research Corporation (EMBRAPA) at Pelotas, RS,
246 Brazil. The average temperature is 20 ±5 ℃ day/ 15 ±5 ℃ night (Supplementary Fig. S7) with 10 to 14h of
247 photoperiod. The experiment was implanted in July 2020. Five tubers of uniform size from each accession
248 were selected and individually planted in five pots per treatment with volume of 10 L filled with
249 TurfaFértil® substrate. Supplemental fertilization with 05-30-10 (NPK) dose of 3 grams per 5L pot (3
250 ton/ha) was made at the time of planting. Pollinations were made from August to September. Fruits were
251 harvested in October 2020 (~5 weeks after pollination). Seeds were extracted, dried at 25 ℃ chamber with
252 air circulation for 24 hours. The dried seeds were stored in paper envelopes in a recipient with silica gel.
253 Germination test was carried out in January 2021. For germination essay, seeds were submerged into 1500
254 ppm gibberellic acid for 24 hours.

255

Figure 4. Diploid wild potato *Solanum malmeanum* (Solanaceae) accessions from Embrapa Potato Genebank (Pelotas, RS, Brazil) assessed to characterize the breeding system. Bar = 10 cm.

Table 6 Diploid wild potato accessions of *Solanum malmeanum* (Solanaceae) available at Embrapa Clima Temperado Potato Genebank (Pelotas, RS, Brazil) evaluated to characterize the breeding system.

Accession Institute code – AleloVegetal *	International code – Genesys	Origin	Lat	Long
BGB015	BRA 00167071-0	Augusto Pestana, RS, Brazil	-28.517	-53.992
BGB017	BRA 00167093-4	Santo Ângelo, RS, Brazil	-28.299	-54.263
BGB446	BRA 00183754-1	Pelotas, RS, Brazil	-31.772	-52.342
BGB447	BRA 00183755-8	Porto Lucena, RS, Brazil	-27.856	-55.016
BGB471	BRA 00183778-0	Canoinhas, SC, Brazil	-26.187	-50.368

* https://alelobag.cenargen.embrapa.br/.

261

262 **Breeding system treatments.** To determine the reproductive mode of *S. malmeanum*, five treatments were
263 applied following Dafni[40] (1992), with some modifications: Control (spontaneous self-pollination;
264 spontaneous parthenocarpy), Apomixis (emasculation, no pollination), Parthenocarpy (emasculation, no
265 pollination), Autogamy (manual self-pollination: self-compatibility-SC/self-incompatibility-SI; induced
266 parthenocarpy), and Allogamy (manual cross-pollination: female fertility/female sterility; induced
267 parthenocarpy).

268 **Samples preparation for treatments and crosses.** The emasculation was performed in flower buds in the
269 pre-anthesis stage, one day prior to pollination, by extracting gently the anthers one by one with the aid of
270 a forceps sterilized in 70º ethylic alcohol. Pollen was collected separately from all accessions from mature
271 flowers in anthesis stage, processed and stored in short period for using in pollinations. For pollen
272 processing, the anthers from flowers in anthesis were collected in an identified plastic container and later
273 packed in waxed paper envelopes identified with the respective accession code and stored in a BOD (Bio-
274 Oxygen Demand) incubators at 25°C for 24 hours. After 24 hours, the envelopes were removed from the
275 BOD and the pollen extracted from the anthers was transferred to test tubes of 1.5 mL identified with the
276 respective accession code, and later stored in freezer (0°C) for medium term conservation. The pollinations
277 were done in pistils from buds emasculated one day before anthesis using fresh pollen according to each
278 treatment in the morning, period of best stigma receptivity. The process was carried out by lightly touching
279 the stigma of the pistil with the pollen. After the procedure, the inflorescences were bagged to avoid pollen
280 contamination from other sources. When possible, it was prioritized to treat at least 30 flowers per
281 accession.

282 **Pollen viability evaluation by acetocarmine staining.** Pollen viability test was performed for plants
283 grown in the greenhouse following the methodology described by the International Center of Potato (CIP)[41],
284 and calculating according to equation (1). Pollen grains were collected from three flowers of the five plants
285 of the same accession and bulked. Acetocarmine stain (20 µl) was mixed with pollen and placed on a clean
286 glass slide with coverslip. Viable and non-viable pollen grains were observed and counted under a PZO
287 optical microscope at 200x, and images were captured using a digital camera. Field of view representative
288 of the entire slide was used for analysis. Round and turgid pollen grains that stained in red were considered
289 viable (Supplementary Fig. S1).

290 $\quad$ (1) $\ \%viability = \frac{number\ of\ stained\ pollen\ grains}{total\ pollen\ grains} \times 100$

291

292 **Measurement of viable pollen size and identification of viable 2n pollen grains.** Pollen grains were
293 collected from three flowers of the five plants of the same accession and bulked, and then were observed
294 microscopically after acetocarmine staining, following the protocol of International Center of Potato
295 (CIP)[41], measuring the diameter of 50 grains of pollen with 200x of magnification. Based on CIP, unreduced
296 viable pollen (2n) can be detected by the bimodal distribution of their diameter and viable 2n pollen will
297 have a diameter 1.2 times larger than the viable reduced pollen (n).

298 **Pollen tube growth observation.** Pistils were collected between 48-72 hours after pollinations in
299 treatments of autogamy and allogamy. The samples were fixed in 3:1 (100% ethanol: glacial acetic acid).
300 Pistils were then washed three times in distilled water, softened for 24 h in sodium hydroxide 0.8 N. Finally,
301 pistils were washed again in distilled water and cleaned in hypochlorite 20%. Pollen tube growth was
302 observed using a 0.5% lacmoid (Sigma L-7512). The observations were made with an optical microscope
303 (6,3 x). Pollen tubes reaches the ovary or growth inhibition by stigma occurs at 1/3 of style length, 2/3 of
304 style length or 3/3 of style length.

305 Fruit set percentage (FSP) was calculated according to the following equation (2).

306 $\quad$ (2) $\ FSP = \frac{Number\ of\ total\ fruits\ per\ plant}{Number\ of\ total\ flowers\ pollinated} \times 100$

307

308 Total number of seeds (TNS) was calculated indirectly using the following equation (3).

309 $\quad$ (3) $\ TNS = \frac{Total\ seed\ weight}{Weight\ of\ 100\ seeds} \times 100$

310 Seed set (SS) was calculated according to the following equation (4).

311 $\quad$ (4) $\ SS = \frac{Total\ number\ of\ seeds}{Total\ number\ of\ fruits}$

312 Percentage of germinated seeds (%) was calculated according to the following equation (5).

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

314 **References**

315 1. Hardigan, M.A., Bamberg, J., Buell, C.R., & Douches, D.S. Taxonomy and genetic differentiation among
316 wild and cultivated germplasm of *Solanum* sect. *Petota*. The Plant Genome. 8(1):plantgenome2014-06.
317 https://doi.org/10.3835/plantgenome2014.06.0025 (2015).

318 2. Hardigan, M.A., Laimbeer, F.P.E., Newron, L., Crisovan, E., Hamilton, J.P., Vaillancourt, B., Wiegert-
319 Rininger, K., Wood, J.C., Douches, D.S., Farré, E.M., Veilleux, R.E., & Nuell, C.R. Genome diversity of
320 tuber-bearing *Solanum* uncovers complex evolutionary history and targets of domestication in the cultivated
321 potato. Proceedings of the National Academy of Sciences of the United States of America. 114(46):9999-
322 10008. https://doi.org/10.1073/pnas.1714380114 (2017).

323 3. Bradshaw, J.E., Bryan, G.J., & Ramsay, G. Genetic resources (including wild and cultivated *Solanum*
324 species) and progress in their utilization in potato breeding. Potato Research. 49:49-65.
325 https://doi.org/10.1007/s11540-006-9002-5 (2006).

326 4. Hijmans, R.J., & Spooner, D.M. Geographic distribution of wild potato species. American Journal of
327 Botany. 88:2101-2112. https://doi.org/10.2307/3558435 (2001).

328 5. Spooner, D.M., Ghislain, M., Simon, R., Jansky, S.H., & Gavrilenko, T. Systematics, diversity, genetics,
329 and evolution of wild and cultivated potatoes. Botanical Review. 80:283-383.
330 https://doi.org/10.1007/s12229-014-9146-y (2014).

331 6. De Jong, H., & Rowe, P.R. Inbreeding in cultivated diploid potatoes. Potato Research. 14(2):74-83.
332 https://doi.org/10.1007/BF02355931 (1971).

333 7. Takayama, S., & Isogai, A. Self-incompatibility in plants. Annual Review of Plant Biology. 56: 467.
334 https://doi.org/10.1146/annurev.arplant.56.032604.144249 (2005).

335 8. Cipar, M.S., Peloquin, S.J., & Hougas, R.W. Variability in the expression of self-incompatibility in tuber-
336 bearing diploid *Solanum* species. American Potato Journal. 41(6):155-162.
337 https://doi.org/10.1007/BF02855317 (1964).

338 9. Hosaka, K., & Hanneman, R.E. Genetics of self-compatibility in a self-incompatible wild diploid potato
339 species *Solanum chacoense*. 1. Detection of an S locus inhibitor (*Sli*) gene. Euphytica. 99(3):191-197.
340 https://doi.org/10.1023/A:1018353613431 (1998a).

341 10. Hosaka, K., & Hanneman, R.E. Genetics of self-compatibility in a self-incompatible wild diploid potato
342 species *Solanum chacoense*. 2. Localization of an S locus inhibitor (*Sli*) gene on the potato genome using
343 DNA markers. Euphytica. 103(2):265-271. https://doi.org/10.1023/A:1018380725160 (1998b).

344 11. Eggers, E.J., van der Burgt, A., van Heusden, S.A., de Vries, M.E., Visser, R.G., Bachem, C.W., &
345 Lindhout, P. Neofunctionalisation of the *Sli* gene leads to self-compatibility and facilitates precision
346 breeding in potato. Nature Communications. 12(1):1-9. https://doi.org/10.1038/s41467-021-24267-6
347 (2021).

348 12. Phumichai, C., Mori, M., Kobayashi, A., Kamijima, O., & Hosaka, K. Toward the development of
349 highly homozygous diploid potato lines using the self-compatibility controlling *Sli* gene. Genome.
350 48(6):977-984. https://doi.org/10.1139/g05-066 (2005).

351 13. Lindhout, P., Meijer, D., Schotte, T., Hutten, R.C.B., Visser, R.G.F., & Van Eck, H.J. Towards F1
352 hybrid seed potato breeding. Potato Research. 54:301-12. https://doi.org/10.1007/s11540-011-9196-z
353 (2011).

354 14. Jansky, S.H., Chung, Y.S., & Kittipadukal, P.I.Y.A. M6: a diploid potato inbred line for use in breeding
355 and genetics research. Journal of Plant Registrations. 8(2):195-199.
356 https://doi.org/10.3198/jpr2013.05.0024crg (2014).

357 15. Clot, C.R., Polzer, C., Prodhomme, C., Schuit, C., Engelen, C.J., Hutten, R.C., & van Eck, H.J. The
358 origin and widespread occurrence of *Sli*-based self-compatibility in potato. Theoretical and Applied
359 Genetics. 133(9):2713-2728. https://doi.org/10.1007/s00122-020-03627-8 (2020).

16. Alsahlany, M., Enciso-Rodriguez, F., Lopez-Cruz, M., Coombs, J., & Douches, D.S. Developing self-compatible diploid potato germplasm through recurrent selection. Euphytica. 217(3):1-16. https://doi.org/10.1007/s10681-021-02785-0 (2021).

17. Jansky, S.H., Charkowski, A.O., Douches, D.S., Gusmini, G., Richael, C., Bethke, P.C., Spooner, D.M., Novy, R.G., de Jong, H., de Jong, W.S., Bamberg, J.B., Thompson, A.L., Bizimungu, B., Holm, D.G., Brown, C.R., Haynes, K.G., Sathuvalli, V.R., Veilleux, R.E., Miller, J.C., Bradeen, J.M., & Jiang, J. Reinventing potato as a diploid inbred line-based crop. Crop Science. 56:1412-1422. https://doi.org/10.2135/cropsci2015.12.0740 (2016).

18. Jansky, S.H., & Spooner, D.M. The evolution of potato breeding. Plant Breeding Reviews. 41:169-214. https://doi.org/10.1002/9781119414735.ch4 (2018).

19. Ter Steeg, E., Struik, P.C., Visser, R.G., & Lindhout, P. Crucial factors for the feasibility of commercial hybrid breeding in food crops. Nature Plants. 8(5):463-473. https://doi.org/10.1038/s41477-022-01142-w (2022).

20. Bachem, C.W., van Eck, H.J., & de Vries, M.E. Understanding genetic load in potato for hybrid diploid breeding. Molecular Plant. 12(7):896-898. https://doi.org/10.1016/j.molp.2019.05.015 (2019).

21. Zhang, C., Yang, Z., Tang, D., Zhu, Y., Wang, P., Li, D., Zhu, G., Xiong, X., Shang, Y., Li, C., & Huang, S. Genome design of hybrid potato. Cell. 184(15):3873-3883. https://doi.org/10.1016/j.cell.2021.06.006 (2021).

22. Bitter, G. *Solanum* (Tuberarium) Malmeanum Bitter, nov. spec. In: Repertorium specierum novarum regni vegetabilis. 12:447-448 (1913).

23. Spooner, D.M., Alvarez, N., Peralta, I.E., & Clausen, A.M. Taxonomy of wild potatoes and their relatives in southern South America (*Solanum* sects. *Petota* and *Etuberosum*). Systematic Botany Monographs. 1-240 (2016).

24. Hawkes, J.G., & Hjerting, J.P. The potatoes of Argentina, Brazil, Paraguay and Uruguay. (ed. Oxford University Press, London) A biosystematic study (1969)

25. Nicolao, R. *Solanum malmeanum*, a promising wild relative for potato breeding. Dissertação (Mestrado em Agronomia – Fitomelhoramento) – Universidade Federal de Pelotas (UFPel). Pelotas, RS, p.123. < https://tinyurl.com/2p8w84hv> (2021)./

26. Nicolao, R.; Gaiero, P., Castro, C.M., & Heiden, G. *Solanum malmeanum*, a promising wild relative for potato breeding. Front. Plant Sci. 13:1046702. doi: 10.3389/fpls.2022.1046702 (2023).

27. Radcliffe, E.B., & Laufr, F.I. Resistance to green peach aphid and potato aphid in introductions of wild tuber-bearing *Solanum* species. Journal of Economic Entomology. 64(5):1260-1266. https://doi.org/10.1093/jee/64.5.1260 (1971).

28. Flanders, K.L., & Radcliffe, E.B. Host plant resistance in *Solanum* germplasm. An Appraisal of Resistance to Colorado Potato Beetle, Potato Leafhopper and Potato Flea Beetle. St. Paul, Minnesota. (1992).

29. Laferriere, LT., Helgeson, J.P., & Allen, C. Fertile *Solanum tuberosum*+ *S. commersonii* somatic hybrids as sources of resistance to bacterial wilt caused by *Ralstonia solanacearum*. Theoretical and Applied Genetics. 98(8):1272-1278. https://doi.org/10.1007/s001220051193 (1999).

30. Micheletto, S., Andreoni, M., & Huart, M.A. Vertical resistance to late blight in wild potato species from Argentina. Euphytica. 110(2):133-138. https://doi.org/10.1023/A:1003793227338 (1999).

31. Micheletto, S., Boland, R. & Huarte, M. Argentinian wild diploid Solanum species as sources of quantitative late blight resistance. Theoretical and Applied Genetics. 101(5);.902-906. https://doi.org/10.1007/s001220051560 (2000).

32. Siri, M.I., Galván, G.A., Quirici, L., Silvera, E., Villanueva, P., Ferreira, F., Franco-Fraguas, L., & Pianzzola, M.J. Molecular marker diversity and bacterial wilt resistance in wild *Solanum commersonii* accessions from Uruguay. Euphytica. 165(2):371-382. https://doi.org/10.1007/s10681-008-9800-8 (2009).

407 33. Bashir I., R. Nicolao, & G. Heiden. Wild Potatoes: A Genetic Reservoir for Potato Breeding. In: Azhar,
408 MT & Wani, S (Org.). Wild Germplasm for Genetic Improvement in Crop Plants. 1ed., 215-240.
409 Cambridge, Massachusetts: Elsevier. Doi.org/10.1016/B978-0-12-822137-2.00012-6 (2021).

410 34. Genesys. Global Portal on Plant Genetic Resources. https://www.genesys-pgr.org. Accessed March 31,
411 2023. (2023).

412 35. Rocha, B.H.G., Augustin, E., Silva, J.B.D., & Pereira, A.D.S. Associação entre isoenzimas e matéria
413 seca em batata silvestre. Pesquisa Agropecuária Brasileira. 35(12):2415-2421.
414 https://doi.org/10.1590/S0100-204X2000001200012 (2000).

415 36. Chala, C.D.A., Pereira, A.D.S., Campos, A.D., Viegas, J. & Salamoni, A.T. Genetic variability for
416 reducing sugar content in wild potatoes that ocurr in southern Brazil. Ciência Rural. 31:43-47.
417 https://doi.org/10.1590/S0103-84782001000100007 (2001).

418 37. Jansen, G., Flamme, W., Schüler, K., & Vandrey, M. Tuber and starch quality of wild and cultivated
419 potato species and cultivars. Potato Research. 44:137-146. https://doi.org/10.1007/BF02410100 (2001).

420 38. Ross, R.W., & Rowe, P.R. Frost resistance among the *Solanum* species in the IR-1 Potato Collection.
421 American Potato Journal. 42(7):177-185. https://doi.org/10.1007/BF02860948 (1965).

422 39. Tu, W., Dong, J., Zou, Y., Zhao, Q., Wang, H., Ying, J., Wu, J., Du, J., Cai, X., & Song, B. Interspecific
423 potato somatic hybrids between *Solanum malmeanum* and *S. tuberosum* provide valuable resources for
424 freezing-tolerance breeding. Plant Cell, Tissue and Organ Culture (PCTOC), v. 147, n. 1, p. 73-83 (2021).

425 40. Dafni, A. Pollination Ecology. New York Press 1992. 250p.

426 41. Ordoñez, B., Orrillo, M., & Bonierbale, M. Manual on potato reproductive and cytological biology.
427 Lima (Peru). International Potato Center (CIP). ISBN 978-92-9060-480-8. 65 pp (2017).

428 42. Tarn, T.R., & Hawkes, J.G. Cytogenetic studies and the occurrence of triploidy in the wild potato
429 species *Solanum commersonii* Dun. Euphytica, 35, 293-302. https://doi.org/10.1007/BF00028567 (1986).

430 43. Carson, G.P., & Howard, H.W. Self-incompatibility in certain diploid potato species. Nature.
431 150(3801):290-290. https://doi.org/10.1038/150290a0 (1942).

432 44. Swaminathan, M.S., & Howard, H.W. The cytology and genetics of the potato (*Solanum tuberosum*)
433 and related species. Bibliographia Genetica. 16: 1-192 (1953).

434 45. Hawkes, J.G. Significance of wild species and primitive forms for potato breeding. Euphytica. 7(3):257-
435 270. https://doi.org/10.1007/BF00025267 (1958).

436 46. Hawkes, J.G. The potato: evolution, biodiversity and genetic resources (ed.Belhaven Press) (1990).

437 47. de Nettancourt, D. Incompatibility and incongruity in wild and cultivated plants (2nd ed. Springer-
438 Verlag, Berlin, Germany) (2001).

439 48. Kubo, K.I., Entani, T., Takara, A., Wang, N., Fields, A.M., & Hua, Z. Collaborative non-self recognition
440 system in *S*-RNase—based self-incompatibility. Science. 330:796–799.
441 https://doi.org/10.1126/science.1195243 (2010).

442 49. Kubo, K.I., Paape, T., Hatakeyama, M., Entani, T., Takara, A., Kajihara, K., Tsukahara, M., Shimizu-
443 Inatsugi, R., Shimizu, K.K., & Takayama, S. Gene duplication and genetic exchange drive the evolution of
444 *S*-RNase-based self-incompatibility in Petunia. Nature Plants. 1(1):1-9.
445 https://doi.org/10.1038/nplants.2014.5 (2015).

446 50. Hua, Z.H., Fields, A., & Kao, T.H. Biochemical models for *S*-RNase-based self-incompatibility.
447 Molecular Plant. 1(4):575-585. https://doi.org/10.1093/mp/ssn032 (2008).

448 51. Dzidzienyo, D.K., Bryan, G.J., Wilde, G., & Robbins, T.P. Allelic diversity of *S*-RNase alleles in
449 diploid potato species. Theoretical and Applied Genetics. 129, 1985-2001. https://doi.org/10.1007/s00122-
450 016-2754-7 (2016).

451 52. Sijacic, P., X. Wang, A.L. Skirpan, Y. Wang, P.E. Dowd, A.G. McCubbin, S. Huang, & T.H. Kao.
452 Identification of the pollen determinant of *S*-RNase-mediated self-incompatibility. Nature. 429:302-305.
453 https://doi.org/10.1038/nature02523 (2004).

454 53. McClure, B., Cruz-Garcia, F., & Romero, C. Compatibility and incompatibility in *S*-RNase-based
455 systems. Annals of Botany. 108:647-658. https://doi.org/10.1093/aob/mcr179 (2011).

456 54. Entani, T., Kubo, K., Isogai, S., Fukao, Y., Shirakawa, M., Isogai, A., & Takayama, S. Ubiquitin
457 proteasome-mediated degradation of *S*-RNase in a solanaceous cross-compatibility reaction. Plant Journal.
458 78:1014-1021. doi:10.1111/tpj.12528 (2014).

459 55. de Nettancourt, D. Incompatibility in angiosperms. Sexual Plant Reproduction. 10(4):185-199.
460 https://doi.org/10.1007/s004970050087 (1997).

461 56. Kondo, K., Yamamoto, M., Matton, D. P., Sato, T., Hirai, M., Norioka, S., Hattori, T., & Kowyama, Y.
462 Cultivated tomato has defects in both *S*-RNase and HT genes required for stylar function of self-
463 incompatibility. The Plant Journal. 29(5):627-636. https://doi.org/10.1046/j.0960-7412.2001.01245.x
464 (2002).

465 57. Ye, M., Peng, Z., Tang, D., Yang, Z., Li, D., Xu, Y., Zhang, C., & Huang, S. Generation of self-
466 compatible diploid potato by knockout of *S*-RNase. Nature Plants. 4(9):651-654.
467 https://doi.org/10.1038/s41477-018-0218-6 (2018).

468 58. Hanneman, Jr.R.E. Self-fertility in *Solanum chacoense*. American Potato Journal. 62:428-429 (1985).

469 59. Goodrich, C.E. The origination and test culture of seedling potatoes. Transactions of New York State
470 Agricultural Society. 23:89-134 (1985).

471 60. Kaiser, N.R., Jansky, S., Coombs, J.J., Collins, P., Alsahlany, M., & Douches, D.S. Assessing the
472 contribution of *Sli* to self-compatibility in North American diploid potato germplasm using KASP™
473 markers. American Journal of Potato Research. 98(2):104-113. https://doi.org/10.1007/s12230-021-09821-
474 8 (2021).

475 61. Ma, L., Zhang, C., Zhang, B., Tang, F., Li, F., Liao, Q., Tang, D., Peng, Z., Jia, Y., Gao, M., Guo, H.,
476 Zhang, J., Luo, X., Yang, H., Gao, D., Lucas, W.J., Li, C., Huang, S., & Shang, Y. A *nonS-locus F-box*
477 gene breaks self-incompatibility in diploid potatoes. Nature Communications. 12(1):1-8.
478 https://doi.org/10.1038/s41467-021-24266-7 (2021).

479 62. O'Brien, M., Kapfer, C., Major, G., Laurin, M., Bertrand, C., Kondo, K., Kowyama, Y., & Matton, D.P.
480 Molecular analysis of the stylar-expressed *Solanum chacoense* small asparagine-rich protein family related
481 to the HT modifier of gametophytic self-incompatibility in Nicotiana. The Plant Journal. 32(6):985-996
482 (2002).

483 63. Birhman, R.K., & Hosaka, K. Production of inbred progenies of diploid potatoes using an S-locus
484 inhibitor (*Sli*) gene, and their characterization. Genome. 43:495-502. https://doi.org/10.1139/g00-012
485 (2000).

486 64. Zhang, C., Wang, P., Tang, D., Yang, Z., Lu, F., Qi, J., Tawari, N.R., Shang, Y., Li, C., & Huang, S.
487 The genetic basis of inbreeding depression in potato. Nature Genetics. 51(3):374-378.
488 https://doi.org/10.1038/s41588-018-0319-1 (2019).

489 65. Enciso-Rodriguez, F., Manrique-Carpintero, N.C., Nadakuduti, S.S., Buell, C.R., Zarka, D., &
490 Douches, D. Overcoming self-incompatibility in diploid potato using CRISPR-Cas9. Frontiers in Plant
491 Science. 10:376. https://doi.org/10.3389/fpls.2019.00376 (2019).

492 66. Phumichai, C., & Hosaka, K. Cryptic improvement for fertility by continuous selfing of diploid potatoes
493 using *Sli* gene. Euphytica. 149(1):251-258. https://doi.org/10.1007/s10681-005-9072-5 (2006).

494 67. Fournet, S., Eoche-Bosy, D., Kerlan, M.C., Grenier, E., & Montarry, J. Phenotypic and genomic
495 modifications associated with *Globodera pallida* adaptation to potato resistances. Potato Research.
496 61(1):65-71. https://doi.org/10.1007/s11540-018-9358-3 (2018).

497   68. Hara-Skrzypiec, A., Śliwka, J., Jakuczun, H., & Zimnoch-Guzowska, E. QTL for tuber morphology
498   traits in diploid potato. Journal of Applied Genetics. 59(2):123-132. https://doi.org/10.1007/s13353-018-
499   0433-x (2018).

500   69. Getahun, B.B., Visser, R.G., & van der Linden, C.G. Identification of QTLs associated with nitrogen
501   use efficiency and related traits in a diploid potato population. American Journal of Potato Research.
502   97(2):185-201. https://doi.org/10.1007/s12230-020-09766-4 (2020).

503   70. Parra-Galindo, M.A., Soto-Sedano, J.C., Mosquera-Vásquez, T., & Roda, F. Pathway-based analysis of
504   anthocyanin   diversity   in   diploid   potato.   PloS   One.   16(4):e0250861.
505   https://doi.org/10.1371/journal.pone.0250861 (2021).

506   71. Ghosh, S., Watson, A., Gonzalez-Navarro, O.E., Ramirez-Gonzalez, R.H., Yanes, L., Mendoza-Suárez,
507   M., Simmonds, J., Wells, R., Rayner, T., Green, P., Hafeez, A., Hayta, S., Melton, R.E., Steed, A., Sarkar,
508   A., Carter, J., Perkins, L., Lord, J., Taster, M., Osbourn, A., Moscou, M.J., Nicholson, P., Hardwood, W.,
509   Martin, C., Domoney, C., Uauy, C., Hazard, B., Wulff, B.B.H., & Hickey, L.T. Speed breeding in growth
510   chambers and glasshouses for crop breeding and model plant research. Nature Protocols. 13(12):2944-2963.
511   https://doi.org/10.1038/s41596-018-0072-z (2018).

512   72. Watson, A., Ghosh, S., Williams, M.J., Cuddy, W.S., Simmonds, J., Rey, M.D., Hatta, M.A.M.,
513   Hinchliffe, A., Steed, A., Reynolds, D., Adamski, N.A., Breakspear, A., Korolev, A., Rayner, T., Dixon,
514   L.E., Riaz, A., Martin, W., Ryan, M., Edwards, D., Batley, J., Raman, H., Carter, J., Rogers, C., Domoney,
515   C., Moore, G., Harwood, W., Nicholson, P., Dieters, M.J., DeLacy, I.H., Zhou, J, Uauy, C., Boden, S.A.,
516   Park, R.F., Wulff, B.B.H., & Hickey, L.T. Speed breeding is a powerful tool to accelerate crop research
517   and breeding. Nature Plants. 4:23-29. https://doi.org/10.1038/s41477-017-0083-8 (2018).

518   73. Watson, A., Hickey, L.T., Christopher, J., Rutkoski, J., Poland, J., & Hayes, B.J. Multivariate genomic
519   selection and potential of rapid indirect selection with speed breeding in spring wheat. Crop Science.
520   59(5):1945-1959. https://doi.org/10.2135/cropsci2018.12.0757 (2019).

521   74. Pandey, S., Singh, A., Parida, S.K., & Prasad, M. Combining speed breeding with traditional and
522   genomics-assisted   breeding   for   crop   improvement.   Plant   Breeding.   141(3):301-303.
523   https://doi.org/10.1111/pbr.13012 (2022).

524   75. Rai, K.K. Integrating speed breeding with artificial intelligence for developing climate-smart crops.
525   Molecular Biology Reports. 1-18. https://doi.org/10.1007/s11033-022-07769-4 (2022).

526

Author contributions statement

528   RN, CMC, and GH discussed the original idea. RN and GH delineated the hypothesis and methodology.
529   RN conducted the experiments and analyzed the data. RN wrote the draft manuscript, CC reviewed the text,
530   and GH reviewed the text and provided advisory.

Additional information

532   The authors attest no conflict of interests.

Data availability

540   The datasets generated during and/or analyzed during the current study are available in the presented study.

541

Supplementary Files

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