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Article

Haplotype-resolved genome and mapping of freezing tolerance in the wild potato *Solanum commersonii*

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Abstract

Solanum commersonii ($2n = 2x = 24$, 1EBN, Endosperm Balance Number), native to the southern regions of Brazil, Uruguay, and northeastern Argentina, is the first wild potato germplasm collected by botanists and exhibits a remarkable array of traits related to disease resistance and stress tolerance. In this study, we present a high-quality haplotype-resolved genome of *S. commersonii*. The two identified haplotypes demonstrate chromosome sizes of 706.48 and 711.55 Mb, respectively, with corresponding chromosome anchoring rates of 94.2 and 96.9%. Additionally, the contig N50 lengths are documented at 50.87 and 45.16 Mb. The gene annotation outcomes indicate that the haplotypes encompasses a gene count of 39 799 and 40 078, respectively. The genome contiguity, completeness, and accuracy assessments collectively indicate that the current assembly has produced a high-quality genome of *S. commersonii*. Evolutionary analysis revealed significant positive selection acting on certain disease resistance genes, stress response genes, and environmentally adaptive genes during the evolutionary process of *S. commersonii*. These genes may be related to the formation of diverse and superior germplasm resources in the wild potato species *S. commersonii*. Furthermore, we utilized a hybrid population of *S. commersonii* and *S. verrucosum* to conduct the mapping of potato freezing tolerance genes. By combining BSA-seq analysis with traditional QTL mapping, we successfully mapped the potato freezing tolerance genes to a specific region on Chr07, spanning 1.25 Mb, with a phenotypic contribution rate of 18.81%. In short, current research provides a haplotype-resolved reference genome of the diploid wild potato species *S. commersonii* and establishes a foundation for further cloning and unraveling the mechanisms underlying cold tolerance in potatoes.

Introduction

Potato (*Solanum tuberosum* L.), which is a nutritionally and commercially important stable food, has already been grown in more than 100 countries worldwide, and more than 2 billion people use potatoes as their primary source of food or income [1–3]. However, cultivated potato varieties are sensitive to low temperatures, with common varieties being intolerant to frost and lacking cold acclimation ability [4, 5]. Low temperature is one of the most significant abiotic stress factors affecting the growth and development of potatoes, leading to an annual global economic loss of billions of dollars due to cold damage [6, 7]. Specifically, when the ambient temperature in the cultivation environment descends below 7°C, stalling occurs in plant growth, concomitant with arrested tuber enlargement. If the temperature falls below −3°C, irreversible death occurs in the aboveground parts of the potato plants [8]. Fortunately, potatoes possess a rich genetic resource base, ranging from diploid to octoploid distributions, with over 70% of wild potato resources in nature being diploid species [9, 10]. These diploid wild species encompass a wealth of excellent resistance germplasm, particularly in terms of freezing tolerance, rendering them crucial resources for the genetic improvement and functional research in potatoes [11, 12].

Plant survival in cold environments depends on two key factors: freezing tolerance in the non-acclimated state (NA) and cold acclimation capacity (CA), which refers to the increased resistance to cold temperatures through appropriate low-temperature training of materials. Previous research has demonstrated that these two factors are independently regulated by genetics and are not correlated with each other [13]. Li [14] utilized electrolyte leakage methods to assess the NA of 60 wild potato materials, among which materials such as *Solanum acaule*, *S. commersonii*, and *S. toralapanum* exhibited outstanding cold resistance. Vega and Bamberg [15] evaluated cold tolerance using natural frost methods in the field for 2635 samples from 101 species, categorizing the phenotypes of potato plants exposed to frost into seven grades (0–6) after frost. The frost experiment revealed that sensitive materials were killed by frost when the field temperature reached −2°C, while most materials were killed when it reached −5°C. Only a few highly cold-resistant wild species materials, such as *S. commersonii*, *S. acaule*, *S. albicans*, *S. paucissectum*, and *S. demissum*, could survive when the temperature dropped below −5°C. Currently, *S. acaule* and *S. commersonii* are considered the two major cold-resistant wild potato species [16]. Before cold acclimation, *S. acaule* exhibited cold resistance down to −6°C, which increased to

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of DNA from individuals exhibiting remarkably resistant or sensitive phenotypes, as determined by preliminary phenotypic evaluations, were combined to create the resistant and susceptible pools used for QTL-seq analysis. Resistant and sensitive pools consisted of 22 and 21 lines, respectively. The construction of paired-end libraries followed the Illumina library construction protocol. Sequencing of the DNA-seq libraries was performed at ~30× coverage relative to the CMM5_Hap1 reference genome. Raw data quality was assessed using FastQC software (https://github.com/s-andrews/FastQC). Data quality control and adapter sequence removal were executed using fastp (https://github.com/OpenGene/fastp) and Trimmomatic software [49]. Alignment of clean data to the CMM5_Hap1 and DM potato reference genome was accomplished using BWA software [60]. Variant detection and functional annotation of variation sites were conducted using GATK (https://github.com/broadinstitute/gatk) and ANNOVAR (https://github.com/WGLab/doc-ANNOVAR) software, respectively. Frost resistance regulatory regions were identified using the Δ(SNP-index) method. Candidate intervals were determined using DeepBSA software [66], with window size calculated based on the minimum Akaike information criterion for small sample sizes (AICc) [67]. The default threshold was set at three standard deviations above the genome-wide median. The tri-kernel-smooth fit method was used for all SNP sites in this study, and only the tri-kernel-smooth fit line that exceeded the threshold was considered a candidate region [66].

Genetic linkage map construction and quantitative trait locus mapping

We utilized MISA software [68] to develop a set of SSR markers proximal to the candidate interval located on Chr07. For the identification of indel markers, we selected those with a size difference exceeding 20 bp between the extreme pools in the BSA detection interval. Subsequently, we extracted sequence fragments of 150 bp upstream and downstream of the target site. To design primers and assess polymorphisms between the parental lines, TBtools [56] software was employed. Polymorphic markers identified between CMM5 and a resistant individual, and between Ver1 and a sensitive individual, were subsequently used for genotyping the backcross population (Supplementary Data Table S3). PCR amplification was performed using a C1000 Thermal Cycler (Bio-Rad Inc., USA). Each PCR reaction consisted of a 10-μl volume containing 1 μl DNA template (50 ng/μl), 5 μl 2 × Utaq PCR mix (Vazyme Co., Ltd., Nanjing, China), 3 μl ddH_2O, 0.5 μl 10 μM forward primers, and 0.5 μl 10 μM reverse primers, as described by Wang *et al.* [69]. Amplified products were resolved using 10% denaturing polyacrylamide gel electrophoresis (PAGE) and visualized through silver staining. All gel images were captured using a digital camera, and the resulting bands were analyzed to determine the genotypes of the offspring. For the construction of the genetic linkage map and mapping of the target locus, we employed QTL IciMapping software with default parameters [70].

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Author contributions

B.S. and X.C. conceived and designed the research. J.D. carried out most of the experiments, analyzed the results, and wrote the manuscript. J.W., W.T., Y.C., and H.W. collected and planted the experimental materials. J.L., Y.S., and Y.W. participated in freezing tolerance tests. Y.Z. and C.L. analyzed the high-throughput data. B.S. and J.D. revised the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets produced and analyzed in this study are accessible upon request from the corresponding author. The genome sequencing and assembly data have been deposited in the Genome Sequence Archive (GSA) database at BIG Data Center (https://ngdc.cncb.ac.cn/gsa/) under BioProject Number PRJCA023611. Additionally, the assembled genome and annotations have been submitted to FigShare [71] for public access.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary data

Supplementary data are available at *Horticulture Research* online.

References

1. Yousaf MF, Demirel U, Naeem M. *et al.* Association mapping reveals novel genomic regions controlling some root and stolon traits in tetraploid potato (*Solanum tuberosum* L.). *3 Biotech*. 2021;**11**:174–90
2. Yan L, Zhang Y, Cai G. *et al.* Genome assembly of primitive cultivated potato *Solanum stenotomum* provides insights into potato evolution. *G3 (Bethesda)*. 2021;**11**:11–9
3. Ma L, Zhang C, Zhang B. *et al.* A *nonS-locus F-box* gene breaks self-incompatibility in diploid potatoes. *Nat Commun*. 2021;**12**:4142
4. Tu W, Li J, Dong J. *et al.* Molecular marker-assisted selection for frost tolerance in a diallel population of potato. *Cells*. 2023;**12**:1226
5. Dong J, Li J, Deng G. *et al.* QTL analysis for low temperature tolerance of wild potato species *Solanum commersonii* in natural field trials. *Sci Hortic*. 2023;**310**:111689
6. Kou S, Chen L, Tu W. *et al.* The arginine decarboxylase gene *ADC1*, associated to the putrescine pathway, plays an important role in potato cold-acclimated freezing tolerance as revealed by transcriptome and metabolome analyses. *Plant J*. 2018;**96**:1283–98
7. Chen Y, Chen L, Sun X. *et al.* The mitogen-activated protein kinase kinase MKK2 positively regulates constitutive cold resistance in the potato. *Environ Exp Bot*. 2021;**194**:104702
8. Li H, Luo W, Ji R. *et al.* A comparative proteomic study of cold responses in potato leaves. *Heliyon*. 2021;**7**:e06002
9. Tang D, Jia Y, Zhang J. *et al.* Genome evolution and diversity of wild and cultivated potatoes. *Nature*. 2022;**606**:535–41
10. Zhang C, Yang Z, Tang D. *et al.* Genome design of hybrid potato. *Cell*. 2021;**184**:3873–3883.e12

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11. Bethke PC, Halterman DA, Jansky S. Are we getting better at using wild potato species in light of new tools? *Crop Sci*. 2017;**57**:1241–58
12. Machida-Hirano R. Diversity of potato genetic resources. *Breed Sci*. 2015;**65**:26–40
13. Stone JM, Palta JP, Bamberg JB. *et al.* Inheritance of freezing resistance in tuber-bearing *Solanum* species: evidence for independent genetic control of nonacclimated freezing tolerance and cold acclimation capacity. *Proc Natl Acad Sci USA*. 1993;**90**:7869–73
14. Li PH. Frost killing temperatures of 60 tuber-bearing *Solanum* species. *Am Potato J*. 1977;**54**:452–6
15. Vega SE, Bamberg JB. Screening the U.S. potato collection for frost hardiness. *Am Potato J*. 1995;**72**:13–21
16. Chen HH, Li PH. Biochemical changes in tuber-bearing *Solanum* species in relation to frost hardiness during cold acclimation. *Plant Physiol*. 1980;**66**:414–21
17. Liu T, Wang J, Chen L. *et al.* *ScAREB4* promotes potato constitutive and acclimated freezing tolerance associated with enhancing trehalose synthesis and oxidative stress tolerance. *Plant Cell Environ*. 2023;**46**:3839–57
18. Aversano R, Contaldi F, Ercolano MR. *et al.* The *Solanum commersonii* genome sequence provides insights into adaptation to stress conditions and genome evolution of wild potato relatives. *Plant Cell*. 2015;**27**:954–68
19. Li F, Bian CS, Xu JF. *et al.* Cloning and functional characterization of *SAD* genes in potato. *PLoS One*. 2015;**10**:e0122036
20. He F, Xu J, Jian Y. *et al.* Overexpression of galactinol synthase 1 from *Solanum commersonii* (*ScGolS1*) confers freezing tolerance in transgenic potato. *Hortic Plant J*. 2022;**9**:541–52
21. Esposito S, Aversano R, Bradeen J. *et al.* Coexpression gene network analysis of cold-tolerant *Solanum commersonii* reveals new insights in response to low temperatures. *Crop Sci*. 2021;**61**:3538–50
22. Carvallo MA, Pino MT, Jeknic Z. *et al.* A comparison of the low temperature transcriptomes and CBF regulons of three plant species that differ in freezing tolerance: *Solanum commersonii*, *Solanum tuberosum*, and *Arabidopsis thaliana*. *J Exp Bot*. 2011;**62**:3807–19
23. Xu X, Pan S, Cheng S. *et al.* Genome sequence and analysis of the tuber crop potato. *Nature*. 2011;**475**:189–95
24. Hosaka AJ, Sanetomo R, Hosaka K. A de novo genome assembly of *Solanum verrucosum* Schlechtendal, a Mexican diploid species geographically isolated from other diploid A-genome species of potato relatives. *G3 (Bethesda)*. 2022;**12**:jkac166
25. Marcais G, Kingsford C. A fast, lock-free approach for efficient parallel counting of occurrences of *k*-mers. *Bioinformatics*. 2011;**27**:764–70
26. Liu B, Shi Y, Yuan J. *et al.* Estimation of genomic characteristics by analyzing k-mer frequency in de novo genome projects. *Quant Biol*. 2020;**35**:62–7
27. Zhou Q, Tang D, Huang W. *et al.* Haplotype-resolved genome analyses of a heterozygous diploid potato. *Nat Genet*. 2020;**52**:1018–23
28. Yang X, Zhang L, Guo X. *et al.* The gap-free potato genome assembly reveals large tandem gene clusters of agronomical importance in highly repeated genomic regions. *Mol Plant*. 2022;**16**:314–7
29. Leisner CP, Hamilton JP, Crisovan E. *et al.* Genome sequence of M6, a diploid inbred clone of the high-glycoalkaloid-producing tuber-bearing potato species *Solanum chacoense*, reveals residual heterozygosity. *Plant J*. 2018;**94**:562–70
30. van Lieshout N, van der Burgt A, de Vries ME. *et al.* Solyntus, the new highly contiguous reference genome for potato (*Solanum tuberosum*). *G3 (Bethesda)*. 2020;**10**:3489–95
31. Liu B, Gu W, Yang Y. *et al.* Promoting potato as staple food can reduce the carbon–land–water impacts of crops in China. *Nat Food*. 2021;**2**:570–7
32. Tu W, Dong J, Zou Y. *et al.* Interspecific potato somatic hybrids between *Solanum malmeanum* and *S. tuberosum* provide valuable resources for freezing-tolerance breeding. *Plant Cell Tiss Org*. 2021;**147**:73–83
33. Liu T, Yu Y, Cai X. *et al.* Introgression of bacterial wilt resistance from *Solanum melongena* to *S. tuberosum* through asymmetric protoplast fusion. *Plant Cell Tiss Org*. 2016;**125**:433–43
34. Tiwari JK, Devi S, Ali N. *et al.* Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tiss Org*. 2017;**132**:225–38
35. Yermishin AP, Polyukhovich YV, Voronkova EV. *et al.* Production of hybrids between the 2EBN bridge species *Solanum verrucosum* and 1EBN diploid potato species. *Am J Potato Res*. 2014;**91**:610–7
36. Zhang H, Zhu J, Gong Z. *et al.* Abiotic stress responses in plants. *Nat Rev Genet*. 2021;**23**:104–19
37. Zhu Y, Zhu G, Xu R. *et al.* A natural promoter variation of *SlBBX31* confers enhanced cold tolerance during tomato domestication. *Plant Biotechnol J*. 2023;**21**:1033–43
38. Zeng R, Li Z, Shi Y. *et al.* Natural variation in a type-A response regulator confers maize chilling tolerance. *Nat Commun*. 2021;**12**:4713
39. Yang S. Cold responses in rice: from physiology to molecular biology. *J Plant Physiol*. 2022;**269**:153602
40. Song Y, Zhang X, Li M. *et al.* The direct targets of CBFs: in cold stress response and beyond. *J Integr Plant Biol*. 2021;**63**:1874–87
41. Li Z, Wang B, Luo W. *et al.* Natural variation of codon repeats in *COLD1* endows rice with chilling resilience. *Sci Adv*. 2023;**9**:eabq5506
42. Feng J, Li Z, Luo W. *et al.* COG2 negatively regulates chilling tolerance through cell wall components altered in rice. *Theor Appl Genet*. 2023;**136**:19
43. Ding Y, Yang S. Surviving and thriving: how plants perceive and respond to temperature stress. *Dev Cell*. 2022;**57**:947–58
44. Liu Z, Jia Y, Ding Y. *et al.* Plasma membrane CRPK1-mediated phosphorylation of 14-3-3 proteins induces their nuclear import to fine-tune CBF signaling during cold response. *Mol Cell*. 2017;**66**:117–128.e5
45. Stegner M, Schafernolte T, Neuner G. New insights in potato leaf freezing by infrared thermography. *Appl Sci (Basel)*. 2019;**9**:819
46. Chen Y, Bamberg J, Palta J. Expression of freezing tolerance in the interspecific F1 and somatic hybrids of potatoes. *Theor Appl Genet*. 1999;**98**:995–1004
47. Vega S, Rio A, Jung G. *et al.* Marker-assisted genetic analysis of non-acclimated freezing tolerance and cold acclimation capacity in a backcross Solanum population. *Am. J. Potato Res.* 2003;**80**:359–369
48. Saghai-Maroof MA, Soliman KM, Jorgensen RA. *et al.* Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci USA*. 1984;**81**:8014–8
49. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;**30**:2114–20
50. Cheng H, Concepcion GT, Feng X. *et al.* Haplotype-resolved de novo assembly using phased assembly graphs with hifiasm. *Nat Methods*. 2021;**18**:170–5
51. Zhou C, McCarthy SA, Durbin R. YaHS: yet another Hi-C scaffolding tool. *Bioinformatics*. 2023;**39**:btac808

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52. Durand NC, Robinson JT, Shamim MS. *et al.* Juicebox provides a visualization system for Hi-C contact maps with unlimited zoom. *Cell Syst*. 2016;**3**:99–101
53. Freire B, Ladra S, Parama JR. Memory-efficient assembly using Flye. *IEEE/ACM Trans Comput Biol Bioinform*. 2022;**19**:3564–77
54. Xu MY, Guo L, Gu S. *et al.* TGS-GapCloser: a fast and accurate gap closer for large genomes with low coverage of error-prone long reads. *Gigascience*. 2020;**9**:giaa094
55. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform*. 2013;**14**:178–92
56. Chen C, Wu Y, Li J. *et al.* TBtools-II: a "one for all, all for one" bioinformatics platform for biological big-data mining. *Mol Plant*. 2023;**16**:1733–42
57. Lin Y, Ye C, Li X. *et al.* quarTeT: a telomere-to-telomere toolkit for gap-free genome assembly and centromeric repeat identification. *Hortic Res*. 2023;**10**:uhad127
58. Rhie A, McCarthy S, Fedrigo O. *et al.* Towards complete and error-free genome assemblies of all vertebrate species. *Nature*. 2021;**592**:737–46
59. Simao FA, Waterhouse RM, Ioannidis P. *et al.* BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*. 2015;**31**:3210–2
60. Jung Y, Han D. BWA-MEME: BWA-MEM emulated with a machine learning approach. *Bioinformatics*. 2022;**38**:2404–13
61. Wang F, Xia Z, Zou M. *et al.* The autotetraploid potato genome provides insights into highly heterozygous species. *Plant Biotechnol J*. 2022;**20**:1996–2005
62. Huang H, Liu X, Arshad R. *et al.* Telomere-to-telomere haplotype-resolved reference genome reveals subgenome divergence and disease resistance in triploid Cavendish banana. *Hortic Res*. 2023;**10**:uhad153
63. Goll J, Rusch DB, Tanenbaum DM. *et al.* METAREP: JCVI metagenomics reports – an open source tool for high-performance comparative metagenomics. *Bioinformatics*. 2010;**26**:2631–2
64. Zhou Z-W, Yu ZG, Huang XM. *et al.* GenomeSyn: a bioinformatics tool for visualizing genome synteny and structural variations. *J Genet Genomics*. 2022;**49**:1174–6
65. Takagi H, Abe A, Yoshida K. *et al.* QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *Plant J*. 2013;**74**:174–83
66. Li Z, Chen X, Shi S. *et al.* DeepBSA: a deep-learning algorithm improves bulked segregant analysis for dissecting complex traits. *Mol Plant*. 2022;**15**:1418–27
67. Hurvich CM, Simonoff JS, Tsai C-L. Smoothing parameter selection in nonparametric regression using an improved Akaike information criterion. *J Roy Stat Soc Ser B (Stat Method)*. 1998;**60**:271–93
68. Beier S, Thiel T, Munch T. *et al.* MISA-web: a web server for microsatellite prediction. *Bioinformatics*. 2017;**33**:2583–5
69. Wang H, Cheng Z, Wang B. *et al.* Combining genome composition and differential gene expression analyses reveals that *SmPGH1* contributes to bacterial wilt resistance in somatic hybrids. *Plant Cell Rep*. 2020;**39**:1235–48
70. Meng L, Li H, Zhang L. *et al.* QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J*. 2015;**3**:269–83
71. Dong J. *Solanum commersonii* genome sequence and annotation files. *figshare* (2024)

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