Localization of Potato browning resistance genes based on BSA-seq Technology (#95338)

Second revision

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Localization of Potato browning resistance genes based on BSA-seq Technology

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Browning during processing is a common issue with potatoes, and the usual solution involves adding chemicals during production. However, as consumer demand for green and healthy diets increases, there is a need to develop potato varieties that are resistant to browning. This study initially identified 275 potato resources with resistance to browning and then narrowed it down to 8 resistant potato resources, with 4 of them being highly resistant. A hybrid population was developed by crossing the highly resistant potato resource CIP395109.29 with the easily browning resource Kexin 23. Subsequently, Bulked Segregant Analysis (BSA) was conducted on the population, leading to the identification of 21 potato genes associated with anti-browning properties through sequencing data analysis and organization. The findings of this study lay a solid groundwork for future research on breeding potatoes with anti-browning traits, offer molecular markers for identifying anti-browning varieties, and serve as a valuable reference for further investigations into potato browning mechanisms.

Localization of potato browning resistance genes based on BSA-seq technology

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Abstract

Browning is a common problem that occurs during potato processing; it is typically resolved by adding chemicals during the production process. However, there is a need to develop potato varieties that are resistant to browning due to a growing consumer interest in healthier diets. This study initially identified 275 potato varieties that are resistant to browning; these were narrowed down to eight varieties, with four of them being highly resistant. A hybrid population was developed by crossing the highly resistant CIP395109.29 with the easily browned Kexin 23. Bulked segregant analysis (BSA) was conducted, which identified 21 potato genes associated with anti-browning properties through sequencing data analysis and organization. The findings of this study lay a solid groundwork for future research on breeding potatoes with anti-browning traits, offer molecular markers for identifying anti-browning varieties, and serve as a valuable reference for further investigations into potato browning mechanisms.

Introduction

Potatoes are the third largest food crop in the world and the fourth largest in China (Wang et al. 2023; Yuhan et al. 2023). As the largest non-cereal grain crop, it is significant in agriculture (Li et al. 2019), industry (Kaur & Singh 2016), and other fields due to its exceptional nutritional and economic value. In recent years, as potato applications have expanded, the demand for high-quality potatoes has also increased. However, the occurrence of potato browning has become a prevalent issue during storage and processing, negatively affecting the quality, taste, and nutritional value of potatoes (Ali et al. 2016). Potato browning primarily occurs due to the enzymatic reaction between phenolic substances within potatoes and polyphenol oxidase (PPO) (Vaughn et al. 1988; Yu et al. 2022), resulting in the production of phenolic compounds. Additionally, non-enzymatic browning reactions associated with the Maillard reaction also contribute to potato browning (Lee & Park 2005). These processes lead to the strengthening of pigments, browning, and the formation of unsightly spots. Browning frequently occurs in potato processing industries, significantly impacting food production, food safety, and environmental pollution, resulting in avoidable losses. Therefore, preventive measures must be taken (McEvily et al. 1992; Vámos-Vigyázó 1981).

Eliminating or delaying potato browning is a significant concern in product processing and home applications. Common methods to prevent browning include physical and chemical approaches. Physical methods encompass low-temperature preservation, low-frequency ultrasound (Xu et al. 2022), vacuum and modified atmosphere preservation (Rocculi et al. 2009). Chemical methods mainly involve a preservative food coating (Shun-Shun 2010) or the use of anti-browning agents (Nascimento et al. 2020; Ru et al. 2020). In the processing industry, the most prevalent method for preventing potato browning is the use of anti-browning agents. These agents effectively and simply slow down the occurrence of potato browning. Sulfite is an efficient browning inhibitor, and potato producers control browning by applying sulfite. But with widespread application, adverse factors have also been exposed, which may have adverse effects on physical health. Therefore, the US Food and Drug Administration has restricted the use of sulfites. As a result, various substitutes are being sold on the market, such as ascorbic acid or a combination of isoascorbic acid with citric acid and cysteine. Lapea et al. studied the anti-browning treatment of sliced potatoes using two antioxidant solutions, and demonstrated the effectiveness of vitamin C as a substitute for sulfite in preserving fresh cut potatoes and delaying their browning (Nicolau-Lapea et al. 2021). However, these products are oxidized irreversibly and therefore do not meet the shelf life requirements in pre-peeled potatoes without special packaging or cover solutions (Sapers & Miller 1993). The limitations of some of the anti-browning agents and the pressure from regulatory agents point to the need for developing alternative technologies for the prevention of enzymatic browning that will be effective and safe. Therefore, solving the potato browning problem from a genetic perspective is the most fundamental approach.

Bulked segregant analysis (BSA) was performed by selecting parents with relative traits to construct a separate population. We chose a specific number of individuals with extreme phenotypes to form a mixed pool and used the differences in DNA molecular markers between the two mixed pools to achieve quantitative trait locus (QTL). BSA, when combined with highthroughput genome resequencing technology, offers an efficient and accurate way to identify trait determining genes, while also reducing research costs. This technology has found widespread application in various animal and plant breeding research, with promising prospects for future applications. For example, Qiu et al. successfully used BSA-seq technology to locate the carpel number of watermelon and identified a 44.7 Mb chromosome fragment related to the carpel number trait on chromosome 7. The candidate gene Cla97C07G143260 was screened (Qiu et al. 2022). Chen et al. conducted research on the slow melting flush (SMF), and BSA results identified two adjacent main QTLs on chromosome 4, resulting in a total of 29 genes that may be related to SMF (Chen 2021). Yang et al. obtained the candidate gene CsaV3_ 6G050410 for controlling trichomes through BSA-seq technology (Yang et al. 2021). Li et al. used BSA-seq technology to map potato starch traits, located them on chromosome 2, and obtained six candidate genes (Li et al. 2021). Sharma et al. studied the dormancy and germination of potato tubers(Sharma et al. 2021). At present, there is relatively little research on the application of BSA seq technology in potatoes, especially in potato browning. Therefore, our research is particularly significant. This study aimed to evaluate the degree of browning in 275 potato samples, create hybrid combinations, and use BSA-seq technology to identify candidate genes associated with potato browning. The findings of this study will provide a theoretical basis for future screening of anti-browning materials in potatoes.

Materials and Methods

Experimental materials and identification

Identification of potato resources browning

This study was conducted at the Keshan branch of the Heilongjiang Academy of Agricultural Sciences from 2018 to 2019 and relied on platforms such as the National Potato Improvement Center, the National Potato Germplasm *in vitro* Seedling Bank (Keshan), and the Key Laboratory of Potato Biology and Genetic Breeding of the Ministry of Agriculture. A total of 275 potato varieties were used (*Table S1*). We then identified how each variety browns through indicators such as browning index, browning intensity, and cooking browning intensity.

Construction of offspring population and identification of browning

Based on the results of our browning identification research, the anti-browning material CIP395109.29 was selected as the female parent, and the low resistance browning material KX 23 was selected as the male parent. A hybrid F1 population containing 362 families was constructed. From 2021 to 2022, planting and sowing were conducted on the experimental site of Keshan Branch of Heilongjiang Academy of Agricultural Sciences. After identifying the degree of browning in the offspring population, 30 light browning and 30 heavy browning potato resources were selected for mixed pool construction.

Experimental methods

Browning index

The methods developed by Wang et al (2007) were used to determine the browning index (Wang et al. 2007). We selected potato tubers of uniform size, with no pests or diseases, and no green skin. Three samples of each potato were selected with two tubers per repeat. The tubers were cut evenly and then sectioned into 0.5 cm thick potato chips. The samples were photographed every 30 minutes, 2 hours, 5 hours, and 7 hours after cutting. Classification of browning levels based on the browning area on the cutting surface (*Table S1*):

Note: The browning index is calculated according to browning grading standards,. The higher the browning index, the heavier the degree of browning.

$$Browning\ Index = \Sigma\left[\frac{\text{level} \times\ tubers\ \text{number of this index}}{top\ \text{level} \times all\ of\ tubers\ \text{number}}\right] \times 100\%$$

Browning strength

We referred to Li et al. (2010) to determine the intensity of browning (Li et al. 2010). After peeling the potato, 3 mm of subcutaneous flesh was removed and a sample was taken from the surface of the tuber for skin samples. Heart samples were obtained approximately 3 cm from the center of the potato tuber. We weighed 1 g of the sample, chopped it, added deionized water at a ratio of 1:4 (m/m), homogenized it with a high-speed tissue grinder for 5 minutes, and placed it in a 30 ℃ water bath for 20 minutes. We then took a portion of the homogenization at 4 ℃, centrifuged it at 12,000 r/min for 5 minutes, and removed the supernatant to measure the absorbance value at 420 nm. The remaining homogenate was kept at 4 ℃ for 24 hours and centrifuged at 12,000 r/min for 5 minutes; the resulting supernatant was taken to measure the absorbance value at 420 nm. Using distilled water as the blank control, each treatment was repeated three times and parallel samples were obtained. The results are calculated as the average value, and A420 was the browning intensity (BD). Here, materials with a core browning strength less than 0.25 after 20 minutes of browning at 30 ℃ and a change value of no more than 0.15 after being placed at 4 ℃ for 24 hours were selected as anti-browning materials due to the lack of a unified standard for browning strength.

Steam-cooked browning

We referred to Bradshaw (2013) to determine cooked browning (Bradshaw 2013). Three potato tubers with no mechanical damage, no pests or diseases, no green skin, and moderate size were selected as samples for testing. The potatoes were peeled and washed and were kept under the water until cooking to avoid enzymatic discoloration. The samples were placed in an electric rice cooker with just enough water to reach the tubers. The tubers were cooked for 25 minutes, checked for doneness and the undercooked pieces were cooked for another 5 minutes. The steamed tubers were left under natural conditions for 24 hours and their discoloration was evaluated. A time delay may deepen the discoloration and improve ability to evaluate any differences. According to Dutch breeders, the degree of browning after cooking can be categorized into six levels: 9, 8, 7, 6, 5, and 4. The 9 represents no discoloration, and 4 represents dark gray or black. The color of the steamed potato tubers was used in conjunction with this standard to determine the grade.

Mixed pool construction and DNA extraction

To construct the extreme material pools, 30 families exhibiting extreme anti-browning and 30 families with extreme low anti-browning were selected from the F1 generation segregation population, along with their male and female parents. DNA was extracted from these samples using the CTAB method and stored at -20 ℃.

Library construction and sequencing

The reference genome was selected from the *S. tuberosum* group phureja DM 1-3 V 6.1 (http://spuddb.uga.edu/). Each offspring in the mixed pool has a sequencing depth of ≥ 1 x, and each parent has a sequencing depth of ≥ 20 x. The detection of SNP and InDel was mainly achieved using the GATK software toolkit. The specific process was determined by the best practices found on the GATK official website (https://www.broadinstitute.org/gatk/guide/best-practices?bpm=DNAseq#variant-discovery-ovw). The data was then filtered and analyzed by the Baike Company. Resequencing was used to obtain two extreme trait pools of potato, one with high browning (Z1) and the other with low browning (Q1), as well as genotype data of the two parental parents. The extreme pool was derived from the F1 family population, and a BSA analysis strategy was used to locate trait-related loci. The Euclidean Distance (ED) method was employed for localization analysis.

$$ED = \sqrt{(A_{mut} - A_{wt})^2 + (C_{mut} - C_{wt})^2 + (G_{mut} - G_{wt})^2 + (T_{mut} - T_{wt})^2}$$

where A_{mut} is the frequency of A base in the mutation pool, A_{wt} is the frequency of A base in the wild-type pool; C_{mut} is the frequency of C bases in the mutation pool, and C_{wt} is the frequency of C bases in the wild-type pool; G_{mut} is the frequency of G bases in the mutation pool, and G_{wt} is the frequency of G bases in the wild-type pool; T_{mut} is the frequency of T bases in the mutation pool, and T_{wt} is the frequency of T bases in the wild-type pool.

The larger the ED value, the greater the difference between the two mixing pools.

Results

Evaluation of browning in 275 potato resources

A comprehensive evaluation was conducted on 275 potato samples, assessing their browning index, browning intensity, and cooking browning index. The results revealed a wide variation in the anti-browning abilities of the potatoes. From this evaluation, eight anti-browning samples were selected, including four high anti-browning materials, namely Yunshu 501, CIP395109.29, CIP393615.6, and Yunshu 401 (Table 1).

Formation of extreme bulks and sequencing data quality control

Following the identification of the varieties used to determine potato browning, Kexin 23, was identified as a susceptible browning resource and was chosen as the male parent. Sexual hybridization was conducted between Kexin 23 and the four potato varieties that were highly resistant to browning. During the evaluation, it was observed that Yunshu 401 did not exhibit typical fruiting behavior, while CIP395109.29 displayed a high fruit setting rate. Consequently, we selected CIP395109.29 as the female parent for sexual hybridization in the population construction process. A total of 4,922 actual seeds were obtained. In 2020, 700 seeds were randomly selected and treated with 1.5 mg/ml gibberellin, resulting in a total of 421 actual seedlings. From 2021 to 2022, the degree of browning of the F1 population was identified. Thirty individuals with milder browning and 30 individuals with heavier browning were selected from the offspring population to construct a mixed pool, along with the two parents (*Table S3*). The sample concentration was ≥ 30 ng/ μl, the total sample quantity was ≥ 2 μg (not less than 15 μl), and the sample purity OD260/280 was 1.6-2.5.

The results indicated that the GC content ranged between 34.58% and 36.29%, the genome coverage depth was greater than 95% (with at least three base covers), and Q30 was higher than 93%. These parameters confirm that the sequencing sample data was sufficient, the sequencing data quality is qualified, and it aligns with the potato reference genome (http://spuddb.uga.edu/dm_v6_1_download.shtml). The comparison efficiency is high, indicating its suitability for subsequent mutation detection and gene localization of traits (Table 2)

The distribution of browning sites on chromosomes

Following the filtering of SNPs and InDels, a total of 14,339,422 original SNPs and InDel sites were obtained. Subsequent screening resulted in 7,910,511 high-quality and reliable SNPs and InDel sites (Table 3 and Fig. 1). The ED values of each point were calculated and multiplied, and the 5th power of the original ED value was taken as the correlation value to eliminate background noise. The LOESS method was employed to fit the ED values, and the correlation threshold was set at the median + 5 sd of the fitted values of all sites. This analysis revealed a single interval associated with the region at 24.08-27.10Mb on chromosome 8, where genes related to controlling potato browning were identified (Fig. 2).

Functional annotation of associated interval genes

Utilizing the localization region, gene location, and reference genome information, potato browning-related genes were mapped onto chromosome 8, resulting in the annotation of a total of 21 candidate genes. Functional annotation of these candidate genes was carried out using seven functional databases, including NR, TrEMBL, KEGG, GO, KOG, SwissProt, and PFAM. The functions and annotations of each gene are detailed in the Table S1. For detailed results, please refer to Date S1

Discussion

Screening for potato resistance to browning: a vital step in browning resistance breeding

Traditionally, inhibiting potato browning has heavily relied on the use of anti-browning chemical reagents during processing, which has proven in the past to be the most effective method. However, with the growing emphasis on health concerns, there is a rising demand for green, healthy, and environmentally-friendly processed products (Hon-Ming et al. 2013). Consequently, enhancing the natural ability of potatoes to resist browning has become increasingly important. We used 275 potato samples for anti-browning identification, resulting in the identification of eight anti-browning varieties, including four highly effective anti-browning potatoes. A total of 421 offspring resources were obtained through the population configuration, with the highly resistant browning resource CIP395109.29 serving as the female parent and the easily browning resource Kexin 23 as the male parent. After two years of browning degree identification, 30 anti-browning and 30 susceptible browning resources were selected. Given that potatoes are not native to our country, resources are relatively limited. Typically, chemical agents are the primary method used to prevent browning during processing. Sulfite, a commonly used additive, is widely employed in the potato processing industry. However, sulfite is an allergen with potential adverse effects on human health. Additionally, studies have suggested that vitamin C can be utilized as an anti-browning substance in products. While these methods can effectively alleviate or mitigate the browning issue during potato processing, they may negatively impact processing quality and increase processing costs, ultimately hindering the development of the potato processing industry. Improving variety defects through breeding methods has consistently been the most widely used and effective approach in agriculture. Therefore, enhancing the natural resistance of potatoes to browning is the most direct, green, and environmentally friendly solution to address processing browning concerns.

Screening of potato browning resistance related genes

Through the tireless efforts of scientific researchers, genes related to potato traits have been continuously discovered and emerged. Of particular interest are genes related to brown traits, as demonstrated by Bachem et al (Bachem et al. 1994). Who showed that the expression of antisense RNA from a potato PPO cDNA can decrease PPO activity and inhibit enzymatic browning in European potato varieties selected for blackspot resistance. Similarly, Coetzer et al. demonstrated that sense and antisense RNA from a heterologous tomato PPO gene can control the level of PPO activity and enzymatic browning in the major commercial potato cultivar in the United States, the Russet Burbank potato (Coetzer et al. 2001). Enzymatic browning mediated by PPO is particularly evident in potato tubers (Corsini et al. 1992; Matheis 1987), where the enzyme is localized within amyloplasts of the tuber cells (Mayer & Harel 1979). Two PPO genes previously isolated from potato by Hunt et al. were found to be expressed in leaves, flowers, roots, and petioles, but no expression was detected in tubers (Hunt et al. 1993). In a recent study, BSA technology was utilized to locate browning-related genes on chromosome 8, identifying a total of 21 candidate genes that may be involved in the browning process. Therefore, further in-depth research into these genes holds significant importance.

Research has revealed that 08G009100 and 08G009110 are potentially associated with phosphoinositol phosphatase SAC9, while 08G009160 is speculated to be a gene related to the F-box protein family. Additionally, 08G009210 may be linked to methylthioribose-1-phosphate isomerase, and 08G009230, 08G009370, and 08G009420 may translate proteins containing pentapeptide repeat sequences that are potentially related to the RPF2, TPR, and PPR families, respectively. Meanwhile, 08G009280 and 08G009290 are speculated to be associated with chlorophyll (ide) b reductase NOL, and 08G009310 and 08G009320 may have the potential 3-hydroxybutyryl CoA hydrolase 3 function. The function of 08G009400 may be related to the WAT1 protein, while 08G009450 may be related to ADP ribosylation factor GTPase activating protein AGD2 like isoform X1. From this, it is clear that potato browning is a complex physiological process that requires multiple genes to cooperate and regulate in order to have an anti-browning effect. Further screening and investigation are required to determine the primary functional roles of these 21 candidate genes.

Conclusion

We have successfully identified the browning characteristics of 275 potato samples, leading to the discovery of high-quality anti-browning varieties. This breakthrough serves as a solid foundation for future advancements in breeding potatoes that do not brown. Notably, we have pioneered the use of BSA technology to pinpoint the anti-browning related genes on chromosome 8 within the 24.08-27.10Mb range, resulting in the identification of 21 candidate genes. This groundbreaking revelation lays the groundwork for further in-depth exploration of potato browning. Building upon these pivotal findings, our subsequent research aims to uncover even more compelling insights.

In summary, the experimental research results will hopefully serve as useful feedback information to improve browning resistance in potatoes. These results may catalyze advancements in the potato industry and improve food security measures.

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Table 1(on next page)

Table 1 Screening and identification of anti browning potato resources

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Resource Name	Browning Index	Browning Intensity		Cooking browning Index
		30℃ 20min	4℃ 24h	
Yunshu 501	0	0.117	0.245	9
CIP395109.29	0	0.185	0.255	7
CIP393615.6	0	0.151	0.180	5
Yunshu 401	12.50	0.112	0.133	8
NH High starch	25.00	0.089	0.121	8
CIP397100.9	29.17	0.134	0.159	9
K200950-3	33.33	0.154	0.210	9
S.goniocalyx	37.50	0.121	0.158	8

Table 2(on next page)

Table 2 Quality inspection of mixing pool

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Sample	Total reads	Comparison rate between sequencing data of each sample and genome(%)	Genome coverage depth （>3）	Alkali base mass value greater than 30
fuben	127075230	87.68	95	94.14
muben	146439062	78.71	95	94.04
Q1	375407052	88.64	98	93.64
Z1	376789854	88.91	98	93.42

Table 3(on next page)

Table 3 The distribution of high-quality credible loci on chromosomes

Table 3 The distribution of high-quality credible loci on chromosomes

chr	SNP_number	InDel_number	Variants_number
chr01	828616	134189	962805
chr02	439850	88117	527967
chr03	568264	93970	662234
chr04	747313	110071	857384
chr05	576186	91108	667294
chr06	575172	93196	668368
chr07	631002	95277	726279
chr08	435291	79120	514411
chr09	648282	89206	737488
chr10	403903	53818	457721
chr11	552832	82504	635336
chr12	426189	67035	493224
total	6832900	1077611	7910511

Table 4(on next page)

Table S1 275 Potato Resources

Table S1 275 Potato Resources

Number	Name	Number	Name	Number	Name
1	-9	93	Hu H97-9	185	Pepo416
2	194	94	Hu H99-1	186	Pepo418
3	295	95	Hu H99-9	187	Pepo426
4	04P48-3	96	Hu83210	188	Qibaiwan
5	05HE5-43	97	Hu9058-2	189	Qingshu 9
6	06H26	98	IH01-5	190	Quarta
7	08CA0979	99	IMRALA-1	191	s.goniocalyx
8	08CA9728-04	100	J10828	192	$S_{5-2-7-44-1-10-5-2-1-1-6-1-(11)}$
9	08HE042-2	101	Jin90-7-23	193	Saikai35
10	2013Y45	102	Jizhang	194	Sante
11	292-20	103	Jizhang 11	195	sebago
12	83Y64	104	Jizhang 14	196	SH11R-6
13	Amsel	105	Jizhang 8	197	Shancheng
14	Andover	106	Jizhang12	198	shepody
15	Anemone	107	K1	199	Solist
16	Aquila	108	K16-6	200	spunta
17	Astilla	109	K2	201	Superior
18	Atlantic	110	K200001-24	202	Taihe
19	Atzimba	111	K200373-13	203	Vester
20	Aula	112	K200632-12	204	Villas
21	Ba 90-2-6	113	K200809-90	205	Vitara
22	Bashu 7	114	K200846-10	206	Weishu 1
23	BE200158-3	115	K200852-13	207	WYJ1
24	BE200170-10	116	K200852-205	208	WYJ2
25	BE20079-24	117	K200852-24	209	WYJ3
26	BE20413-22	118	K200856-6	210	WYJ4
27	Bintje	119	K200858-22	211	WYJ5
28	Bo-B2	120	K200867-15	212	WYJ7
29	Bo-BR	121	K200904-37	213	WYJ8
30	Bo-C	122	K200908-16	214	WYJ9
31	Bo-S	123	K200920-11	215	WYJ11
32	Boyou 2	124	K200920-28	216	WYJ12
33	Cal White	125	K200920-35	217	WYJ13
34	Carola	126	K200927-13	218	WYJ14
35	Chenggong	127	K200931-1	219	WYJ15
36	Chuanyi	128	K200935-43	220	Xindaping
37	Chunshu 1	129	K200935-51	221	Xingjia 2
38	CIP388611.22	130	K200938-48	222	Xisen 3
39	CIP388615.22	131	K200950-3	223	Xisen 4

40	CIP388676.1	132	K200961-5	224	Yan 0225-432
41	CIP388972.22	133	K200968-12	225	Yan 2005-1
42	CIP390663.8	134	k200969-11	226	Yanshu 3
43	CIP391002.6	135	K200969-2	227	Yanshu 4
44	CIP391011.17	136	K200969-4	228	Yiselie
45	CIP391180.6	137	K200979-17	229	Youjin
46	CIP392633.54	138	K200979-25	230	Yunshu 103
47	CIP392797.22	139	K200979-3	231	Yunshu 201
48	CIP393077.159	140	K3	232	Yunshu 202
49	CIP393077.54	141	K4	233	Yunshu 205
50	CIP393371.157	142	K9201-10	234	Yunshu 303
51	CIP393615.6	143	K9412-13	235	Yunshu 401
52	CIP393617.1	144	Kangheijingbing	236	Yunshu 501
53	CIP395037.107	145	Kangyibai	237	Yunshu 505
54	CIP395109.29	146	katadin	238	Yunshu827
55	CIP395112.9	147	Kende	239	Zaodabai
56	CIP395434.1	148	Kennebec	240	Zhengshu 5
57	CIP396004.263	149	Kexin 1	241	Zhong A9215-84
58	CIP396033.102	150	Kexin 2	242	Zhong C9305-6
59	CIP396311.1	151	Kexin 4	243	Zhongshu 1
60	CIP397073.16	152	Kexin 6	244	Zhongshu 4
61	CIP397077.16	153	Kexin 12	245	Zhongshu 5
62	CIP397100.9	154	Kexin 13	246	Zhongshu 6
63	CIP399004.19	155	Kexin 14	247	Zhongshu 12
64	CIP703831	156	Kexin 17	248	Zhongshu 17
65	Скарб	157	Kexin 18	249	Zhukefu
66	Colmo	158	Kexin 19	250	Архидея
67	Dadi	159	Kexin 20	251	Атлант
68	Delta	160	Kexin 21	252	Вилъня
69	Denali	161	Kexin 22	253	вир244(Suniax-S.Stolonig)
70	Dianella	162	Kexin 23	254	вишневый
71	Diniela	163	Kexin 25	255	выток
72	Dongnong 00-33048	164	Kexin 26	256	гибрид 59/m-56
73	Dongnong 0733-125	165	Khongor	257	гибрид 728-6
74	DY4-5-10	166	Kunta	258	гибрид 90.1/11
75	E-13	167	KURODA	259	гибрид MB-168
76	E-3	168	Long201207-4	260	гибрид59/m-69
77	E-421	169	Long201208-13	261	гибрид80-1
78	E-5	170	Longshu 3	262	гибридMB-168

79	Eramosa	171	Longshu 6	263	Дельфин
80	Eyin	172	Longshu 7	264	Журавинка
81	F00070	173	Longxinxuan 1	265	зарево
82	F70021-1	174	Lt-5	266	Здабыток
83	Favorita	175	Maiken 1	267	Лилея
84	Feixing	176	Maiken 4	268	Маг
85	FL1533	177	Maiken 5	269	Максимум
86	FL1625	178	Minshu 1	270	Московский
87	Fujian	179	Nanjue	271	никунский
88	GADRE	180	Ne 16	272	орбита
89	GaRant(тараит)	181	Nehe gaodianfen	273	Ранный
90	Hu 5	182	Norland	274	Тарант
91	Hu 8212-3	183	NS51-5	275	Уладар
92	Hu 9707-116	184	NS78-11		

Table 5(on next page)

Table S2 Browning area of Leve

Table S2 Browning area of Level

level	Browning area
0	area <25%
1	25%⩽area <50%
3	50%⩽area <75%
4	area⩾75%

Table 6(on next page)

Table S3 Construction of potato populations with light and dark browning

Table S3 Construction of potato populations with light and dark browning

Number	Browning light（Q）						Number	Browning dark（Z）					
	2021			2022				2021			2022		
	Browning Index %	Browning Intensity	Cooking browning Index	Browning Index %	Browning Intensity	Cooking browning Index		Browning Index %	Browning Intensity	Cooking browning Index	Browning Index %	Browning Intensity	Cooking browning Index
T37	16.67	0.045	8.00	8.33	0.080	7.00	T5	100.00	0.334	6.75	91.67	0.104	6.33
T41	33.33	0.109	7.50	25.00	0.000	7.00	T6	100.00	0.303	5.25	91.67	0.154	6.67
T44	0.00	0.035	8.00	25.00	0.039	6.00	T18	100.00	0.248	5.00	91.67	0.038	6.33
T46	16.67	0.110	7.75	0.00	0.053	7.00	T30	100.00	0.380	5.00	100.00	0.251	7.00
T60	41.67	0.036	7.00	5.56	0.041	7.00	T40	100.00	0.212	7.25	75.00	0.247	6.33
T83	44.44	0.119	7.75	33.33	0.065	8.00	T53	100.00	0.241	7.50	100.00	0.240	7.00
T120	0.00	0.104	8.00	8.33	0.091	8.00	T72	100.00	0.312	7.00	50.00	0.206	7.33
T150	66.67	0.033	8.00	8.33	0.022	8.00	T74	16.67	0.187	6.25	12.50	0.079	6.50
T166	0.00	0.071	7.50	41.67	0.050	7.33	T76	100.00	0.328	6.50	75.00	-0.011	6.50
T175	62.50	0.041	7.00	25.00	0.070	7.00	T86	100.00	0.231	7.00	75.00	0.146	7.00
T183	33.33	0.139	7.00	16.67	0.144	7.00	T142	91.67	0.396	7.00	100.00	0.488	6.00
T199	0.00	0.079	8.00	16.67	0.011	6.83	T146	100.00	0.167	6.75	100.00	0.059	7.67
T201	8.33	0.187	7.00	16.67	0.045	6.00	T149	100.00	0.238	7.00	75.00	0.194	5.67
T230	8.33	0.076	8.00	66.67	0.023	7.67	T156	100.00	0.566	5.25	100.00	0.160	6.50
T250	32.50	0.083	9.00	100.00	0.251	8.00	T169	100.00	0.547	6.00	100.00	0.147	6.00
T256	16.67	0.128	7.00	16.67	0.051	8.00	T190	91.67	0.368	8.00	100.00	0.376	7.33
T286	25.00	0.081	7.00	8.33	0.086	7.00	T232	100.00	0.071	5.50	66.67	0.174	7.00
T299	0.00	0.086	8.50	0.00	0.055	7.17	T238	100.00	0.352	7.00	83.33	0.077	7.00
T303	12.50	0.055	8.50	0.00	0.109	8.00	T253	100.00	□	5.75	100.00	0.102	7.00
T304	25.00	0.088	6.75	33.33	0.060	4.67	T257	100.00	0.219	7.25	100.00	0.320	7.00
T331	33.33	0.165	7.25	58.33	0.031	7.00	T258	66.67	0.402	4.00	100.00	0.260	6.67
T338	0.00	0.048	7.50	0.00	0.033	7.67	T261	100.00	0.229	9.00	100.00	0.103	6.67
T354	8.33	0.100	7.25	0.00	0.016	8.00	T270	100.00	0.436	7.00	91.67	0.220	6.00

T371	8.33	0.119	8.00	16.67	0.051	8.00	T274	100.00	0.212	6.00	77.78	0.101	6.00
T377	58.33	0.078	8.00	25.00	0.035	8.00	T302	100.00	0.478	6.25	86.11	0.184	6.67
T384	25.00	0.015	7.00	37.50	0.072	6.50	T321	100.00	0.498	7.00	100.00	0.178	6.00
T396	0.00	0.087	8.00	8.33	0.065	7.83	T328	100.00	0.212	7.25	100.00	0.218	6.67
T404	0.00	0.151	8.00	0.00	0.018	8.00	T337	100.00	0.354	6.75	83.33	0.102	6.67
T405	0.00	0.154	8.25	50.00	0.074	7.00	T353	100.00	0.159	6.75	100.00	0.235	7.33
T414	8.33	0.172	4.75	25.00	0.109	7.00	T359	100.00	0.220	6.00	83.33	0.187	6.00

Table 7(on next page)

Table S4 Function and annotation of candidate genes

Table 7 Function and annotation of candidate genes

	gene_ID	start	end	nr_annotation
1	Soltu.DM.08G009080.1	24148809	24153252	PREDICTED: uncharacterized protein LOC107060450 [Solanum tuberosum]
	Soltu.DM.08G009080.2	24148809	24153153	PREDICTED: uncharacterized protein LOC107060450 [Solanum tuberosum]
2	Soltu.DM.08G009100.1	24353907	24365325	PREDICTED: probable phosphoinositide phosphatase SAC9 [Solanum tuberosum]
3	Soltu.DM.08G009110.1	24382527	24397536	PREDICTED: probable phosphoinositide phosphatase SAC9 [Solanum tuberosum]
4	Soltu.DM.08G009160.1	24513703	24515707	PREDICTED: putative F-box protein At5g55150 [Solanum pennellii]
5	Soltu.DM.08G009180.1	24553914	24555312	PREDICTED: uncharacterized protein LOC102596535 [Solanum tuberosum]
6	Soltu.DM.08G009190.1	24559067	24560647	hypothetical protein T459_07555 [Capsicum annuum]
7	Soltu.DM.08G009200.1	24623005	24623439	
8	Soltu.DM.08G009210.1	24753728	24763107	PREDICTED: methylthioribose-1-phosphate isomerase [Solanum tuberosum]
9	Soltu.DM.08G009230.1	25060164	25064157	PREDICTED: pentatricopeptide repeat-containing protein At5g42310, mitochondrial isoform X1 [Solanum tuberosum]
	Soltu.DM.08G009230.2	25060126	25063995	PREDICTED: pentatricopeptide repeat-containing protein At5g42310, mitochondrial isoform X1 [Solanum tuberosum]
	Soltu.DM.08G009230.3	25060126	25064161	PREDICTED: pentatricopeptide repeat-containing protein At5g42310, mitochondrial isoform X1 [Solanum tuberosum]
10	Soltu.DM.08G009280.1	25807041	25809000	PREDICTED: chlorophyll(ide) b reductase NOL, chloroplastic [Solanum tuberosum]
11	Soltu.DM.08G009290.1	25817451	25836334	chlorophyll(ide) b reductase NOL, chloroplastic isoform X2 [Solanum lycopersicum]
12	Soltu.DM.08G009310.1	25862144	25864160	PREDICTED: probable 3-hydroxyisobutyryl-CoA hydrolase 3 [Solanum tuberosum]
13	Soltu.DM.08G009320.1	25878588	25883250	PREDICTED: probable 3-hydroxyisobutyryl-CoA hydrolase 3 [Solanum tuberosum]
14	Soltu.DM.08G009350.1	26096933	26103061	hypothetical protein PRUPE_3G016800 [Prunus persica]
15	Soltu.DM.08G009370.1	26445239	26448721	PREDICTED: pentatricopeptide repeat-containing protein At1g08070, chloroplastic-like [Solanum tuberosum]
16	Soltu.DM.08G009390.1	26554158	26557390	PREDICTED: uncharacterized protein LOC102580040 [Solanum tuberosum]
17	Soltu.DM.08G009400.1	26558710	26561538	PREDICTED: WAT1-related protein At1g43650-like isoform X1 [Nicotiana tabacum]
18	Soltu.DM.08G009410.1	26563157	26563699	
19	Soltu.DM.08G009420.1	26604407	26608896	PREDICTED: pentatricopeptide repeat-containing protein At3g53170 [Solanum tuberosum]

	Soltu.DM.08G009420.2	26604407	26608896	PREDICTED: pentatricopeptide repeat-containing protein At3g53170 [Solanum tuberosum]
	Soltu.DM.08G009420.3	26604407	26608896	PREDICTED: pentatricopeptide repeat-containing protein At3g53170 [Solanum tuberosum]
	Soltu.DM.08G009420.4	26604407	26608896	PREDICTED: pentatricopeptide repeat-containing protein At3g53170 [Solanum tuberosum]
	Soltu.DM.08G009420.5	26604407	26608896	PREDICTED: pentatricopeptide repeat-containing protein At3g53170 [Solanum tuberosum]
	Soltu.DM.08G009420.6	26604407	26608896	PREDICTED: pentatricopeptide repeat-containing protein At3g53170 [Solanum tuberosum]
20	Soltu.DM.08G009430.1	26663840	26664515	
21	Soltu.DM.08G009450.1	26720893	26729103	ADP-ribosylation factor GTPase-activating protein AGD2-like isoform X1 [Solanum lycopersicum]

2

Figure 1

Figure 1 The distribution of high-quality credible loci on chromosomes

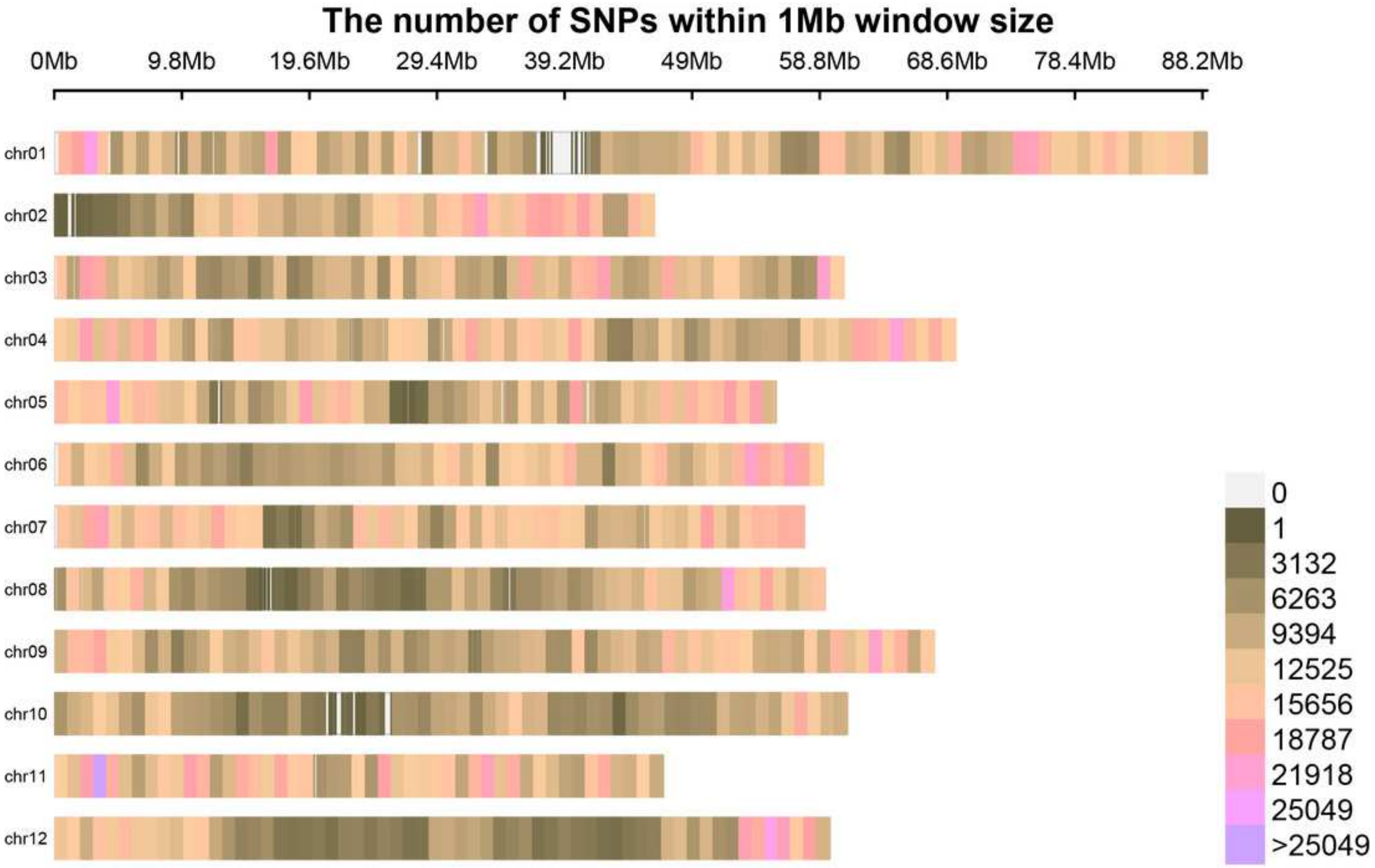

Figure 2

Figure 2 The distribution of ED correlation values on chromosomes