Construction of SSR linkage maps and identification of QTL for resistance to root rot in sweetpotato (*Ipomoea batatas* (L.) Lam.)

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Keywords: sweetpotato, root rot, SSR marker, linkage map construction, QTL analysis

Posted Date: March 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-17591/v1>

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Version of Record: A version of this preprint was published at BMC Genomics on May 15th, 2020. See the published version at <https://doi.org/10.1186/s12864-020-06775-9>.

Abstract

Background: Sweetpotato root rot is a devastating disease caused by *Fusarium solani* that causes significant yield losses of sweetpotato in China. There is currently no effective method to control the disease. The breeding of resistant varieties is the most effective and economic way to control the disease. To date, quantitative trait locus (QTL) for resistance to root rot have not been reported and the biological mechanisms of resistance remain unclear in sweetpotato. Thus, it is necessary and worthwhile to identify resistance loci to help develop disease-resistant varieties.

Results: In this study, we constructed genetic linkage maps of sweetpotato using a mapping population consisting of 300 individuals derived from a cross between Jizishu 1 and Longshu 9 by simple sequence repeat (SSR) markers, and mapped seven QTLs for resistance to root rot. In total, 484 and 573 polymorphic SSR markers were grouped into 90 linkage groups for Jizishu 1 and Longshu 9, respectively. The total map distance for Jizishu 1 was 3,974.24 cM, with an average marker distance of 8.23 cM. The total map distance for Longshu 9 was 5,163.35 cM, with an average marker distance of 9.01 cM. Five QTLs (qRRM_1, qRRM_2, qRRM_3, qRRM_4, and qRRM_5) were located in five linkage groups of Jizishu 1 map explaining 52.6–57.0% of the variation. Two QTLs (qRRF_1 and qRRF_2) were mapped on two linkage groups of Longshu 9 explaining 57.6% and 53.6% of the variation. 71.4 % of the QTLs had a positive effect on the variation. Three of the seven QTLs, qRRM_3, qRRF_1, and qRRF_2, were colocalized with markers IES43-5mt, IES68-6fs**, and IES108-1fs, respectively.

Conclusions: To our knowledge, this is the first report on the construction of a genetic linkage map for purple sweetpotato (Jizishu 1) and the identification of QTLs associated with resistance to root rot in sweetpotato using SSR markers. These QTLs will have practical significance for the fine mapping of root rot resistance genes and play an important role in sweetpotato marker-assisted breeding.

Background

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is the seventh most important food crop in the world and also serves as animal feed, a raw material in food and feed industries, and an energy crop [1]. Sweetpotato root rot, caused by *Fusarium solani* [2], is one of the most widespread diseases in north China. It directly affects sweetpotato production, resulting in yield losses and quality deterioration, and it is a long-term problem plaguing sweetpotato farmers. The disease can lead to yield losses of 10–20%, and even 100% in severely infected fields [3]. There are currently no effective methodologies to control sweetpotato root rot. The breeding of resistant varieties is the most effective and economic way to control the disease. Conventional breeding for root rot resistance in sweetpotato is complicated, with a long cycle length, and generally improves only single traits. Combining molecular techniques with conventional breeding methods is an effective way to overcome the limitations of seasonal and environmental effects, species isolation, and linkage drag existing in conventional breeding. Root rot resistance loci have not been mapped in sweetpotato to date. One study identified a root rot susceptibility locus, but resistance loci were not successfully identified [4].

The construction of a genetic linkage map is important for quantitative trait locus (QTL) identification, gene cloning, comparative genomic research, and marker-assisted selection breeding. However, sweetpotato, as a highly heterozygous, generally self-incompatible, and outcrossing hexaploid species with a large number of small chromosomes ($2n = 6x = 90$), poses numerous challenges for genetic analysis and breeding [5]. As a result, the progress of molecular biology research on sweetpotato lags far behind that in other major crops. For constructing linkage maps in such heterozygous species, Grattapaglia and Sederoff developed a two-way pseudo-testcross strategy, in which linkage analysis is conducted for each parent separately, as dominant markers that are heterozygous in one parent and recessive homozygous in the other parent will segregate in the F_1 generation, resulting in the development of two parental linkage maps [6].

Several genetic linkage maps for sweetpotato have been constructed using various types of molecular markers, such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), sequence-related amplified polymorphism, simple sequence repeat (SSR), inter SSR, expressed sequence tag-SSR (EST-SSR), retrotransposon-based and single-nucleotide polymorphism (SNP) [7–19]. Ukoskit and Thompson constructed the first low-density linkage maps based on 196 RAPD markers from 76 progenies of the cross Vardaman $\times$ Regal [17]. Cervantes-Flores et al. developed genetic linkage maps of sweetpotato cultivars 'Beauregard' and 'Tanzania' using 947 and 726 AFLP markers, respectively. They also conducted the first QTL analysis for root knot nematode resistance, and identified nine chromosome regions associated with root knot nematode resistance. In addition, 13 QTLs for dry matter rate, 12 QTLs for starch content, and eight QTLs for β -carotene content were identified [7, 20, 21]. Zhao et al. developed the first map that included 90 complete sweetpotato linkage groups using 202 individuals derived from a broad cross between 'Xushu 18' and 'Xu 781' based on AFLP and SSR markers, and mapped 27 QTLs for storage root dry matter content [19]. Using this map, Yu et al. and Li et al. identified QTLs and colocalizing markers for starch content and storage root yield [22, 23].

With the development of high-throughput technology, next-generation sequencing (NGS) has been used to analyse genetic linkage in numerous crop species. Shirasawa et al. established the first high-density genetic map for sweetpotato. They used SNPs identified by double-digest restriction site-associated DNA sequencing based on NGS technology to construct a map for Xushu 18 using an S_1 mapping population comprising 142 individuals, which had 28,087 double-simplex SNPs mapped onto 96 linkage groups, and covered a total distance of 33,020.4 cM [15]. Mollinari et al. built an ultradense multilocus integrated genetic map and characterized the inheritance system in a sweetpotato full-sib family using a newly developed software, MAPpoly [12].

In the present study, we used a mapping population of 300 F_1 individuals derived from a cross between Jizishu 1 and Longshu 9 to construct linkage maps using SSR markers and to conduct QTL analysis for resistance to root rot in sweetpotato. The results of this study are expected to provide useful information for developing resistance to root rot based on major QTLs.

Results

Marker data

In total, 400 SSR primer pairs were screened in the parents and 10 progenies. Among these, 155 primer pairs (Additional file 3: Table S1) were polymorphic between the two parents and were selected to analyse the F₁ population. Finally, 839 good-quality polymorphic markers were obtained, with an average of five markers per primer pair. In total, 506 polymorphic SSR markers were obtained for mapping Jizishu 1, including 217 simplex, 47 duplex, 8 triplex, and 234 double-simplex markers, and 567 polymorphic SSR markers were obtained for mapping Longshu 9, including 237 simplex, 76 duplex, 20 triplex, and 234 double-simplex markers. The percentage of simplex markers was 79.8% ($217/(217+47+8)$) and 71.8% ($237/(237+76+20)$) in Jizishu 1 and Longshu 9, respectively, which was in accordance with the theoretical values for an autohexaploid (75% simplex and 25% non-simplex) according to Chi-square test results, and could be used to construct a genetic map of the hexaploid sweetpotato [7, 10, 19].

Genetic linkage map construction

The single-dose markers were used to construct a framework map of each parent at a LOD score of 5.0 using JoinMap 4.0. Subsequently, duplex and triplex markers were inserted into the framework maps to obtain the final genetic linkage maps. Molecular markers were grouped into 90 linkage groups for each parental map. There were 54 major and 36 minor groups of three or two markers for Jizishu 1, and 68 major and 22 minor groups for Longshu 9.

The linkage map of Jizishu 1 was composed of 484 polymorphic markers, of which 186, 137, 30, and 131 were simplex, duplex, triplex and double-simplex markers, respectively. The largest and smallest linkage groups contained 17 and 2 markers, respectively. The total map distance was 3,974.24 cM, with an average marker distance of 8.23 cM. The longest linkage group was 143.52 cM, the shortest linkage group was 0.34 cM, and the average linkage group length was 44.16 cM (Additional file 4: Table S2). The linkage map of Longshu 9 was composed of 573 polymorphic markers, of which 185, 217, 40, and 131 were simplex, duplex, triplex and double-simplex markers, respectively. The largest and smallest linkage groups contained 17 and 2 markers, respectively. The total map distance was 5,163.35 cM, with an average marker distance of 9.01 cM. The longest linkage group was 151.60 cM, the shortest linkage group was 4.07 cM, and the average linkage group length was 57.37 cM (Additional file 4: Table S3). There were 239 distorted markers (49.38%) and 250 distorted markers (43.63%) in Jizishu 1 and Longshu 9, respectively.

For Jizishu 1, 132 duplex and 30 triplex markers divided 39 homologous relationships into 8 homologous linkage groups. The remaining 51 linkage groups could not be classified into any homologous linkage group (Additional file 1: Fig. S1). For Longshu 9, 212 duplex and 39 triplex markers divided 54 homologous relationships into 9 homologous linkage groups. The remaining 36 linkage groups could not be classified into any homologous linkage group (Additional file 2: Fig. S2).

Double-simplex markers were used to detect the homology of the corresponding linkage groups in the two maps. Among them, 100 double-simplex markers revealed that 42 linkage groups in Jizishu 1 map had

homologous linkage relationships with 40 linkage groups in Longshu 9 map (Additional file 6: Table S4). Homology between the two parental maps is an important criterion for consistency of the maps.

QTL analysis

The root rot disease index in the mapping population showed abnormal distributions in 2016 and 2017 (Fig. 1). In the two years, the average disease index of the mapping population ranged from 3.2 to 100, with a population mean of 58.4. The average disease index of Jizishu 1 was 14.4, indicating high resistance to root rot, and the average disease index of Longshu 9 was 84.5, indicating high susceptibility. ANOVA showed that the disease index was significantly different between the two years (Table 1). Therefore, the disease index for each year and the average values were separately analysed for QTL mapping. In addition, transgressive segregation was observed, that is, some progenies showed a higher disease index and others showed a lower disease index than either parent.

Table 1 Anova of the disease index in the mapping population of Jizishu 1 × Longshu 9

Source	<i>df</i>	SS	MS (<i>SS/df</i>)	F value	P value
Year	1	1781466.431	1781466.431	2015.524	.000
Error	267	235993.941	883.827		

df: degrees of freedom

SS: sum of squares

MS: mean sum of squares

Seven stable QTLs were identified for resistance to root rot at the same genomic location in 2016, 2017, and average data (Table 2). Five QTLs for root rot resistance, named *qRRM_1*, *qRRM_2*, *qRRM_3*, *qRRM_4*, and *qRRM_5* were located in five linkage groups of Jizishu 1, JZ1 (02.09), JZ1 (04.19), JZ1 (05.25), JZ1 (06.33), and JZ1 (00.72), respectively, and explained 52.6–57.0% of the variation in root rot resistance (Table 2 and Fig. 2). Among the five QTLs, only *qRRM_4* had a negative effect on resistance to root rot, explaining 57.0% of the variation, whereas the remaining four QTLs exhibited a positive effect on resistance. Two QTLs, named *qRRF_1* and *qRRF_2*, were located in two linkage groups of Longshu 9, L9 (00.64) and L9 (00.74), respectively (Fig. 3). *qRRF_1* had a positive and *qRRF_2* a negative effect on root rot resistance, explaining 57.6% and 53.6% of the variation, respectively (Table 2). These results agree with the fact that Jizishu 1 is highly resistant, whereas Longshu 9 is highly susceptible to root rot.

Table 2 QTLs detected for resistance to root rot in the Jizishu 1 × Longshu 9 mapping population

QTL	Linkage group	Marker	Marker position (cM) ^a	QTL position (cM) ^b	Environment	LOD ^c	R ² (%) ^d
<i>qRRM_1^p</i>	JZ1(02.09)	IES9-8mt*	66.331	67.331	Y2016	5.04	65.0
				66.045	Y2017	10.15	67.1
				66.045	AVERAGE	3.83	55.9
<i>qRRM_2^p</i>	JZ1(04.19)	IES356-2md	60.406	63.406	Y2016	3.59	60.7
				63.406	Y2017	8.22	63.0
				63.406	AVERAGE	5.55	53.4
<i>qRRM_3^p</i>	JZ1(05.25)	IES43-5mt	84.907	84.907	Y2016	3.48	65.2
				84.907	Y2017	8.95	65.5
				84.907	AVERAGE	3.31	56.5
<i>qRRM_4ⁿ</i>	JZ1(06.33)	IES351-4md	1.844	4.844	Y2016	4.51	63.2
				2.844	Y2017	6.29	65.0
				2.844	AVERAGE	3.48	57.0
<i>qRRM_5^p</i>	JZ1(00.72)	IES68-11ds**	34.102	31.000	Y2016	9.58	62.7
				34.102	Y2017	4.12	58.9
				34.102	AVERAGE	3.85	52.6
<i>qRRF_1^p</i>	L9(00.64)	IES68-6fs**	62.342	62.342	Y2016	5.04	65.0
				62.342	Y2017	7.00	65.9
				62.342	AVERAGE	3.00	57.6
<i>qRRF_2ⁿ</i>	L9(00.74)	IES108-1fs	0.000	0.000	Y2016	5.78	65.5
				0.000	Y2017	6.95	63.9
				0.000	AVERAGE	3.77	53.6

^athe closely linked or co-localized markers position

^bLOD peak position

^cthe estimated LOD score at the QTL peak

^dproportion of phenotypic variation explained by the QTL

^pQTL with a positive effect on resistance to root rot

ⁿQTL with a negative effect on resistance to root rot

*the distorted markers indicate significant differences at the 0.05 level

**the distorted markers indicate significant differences at the 0.01 level

At the location with the highest LOD scores, three of the seven QTLs (*qRRM_3*, *qRRF_1* and *qRRF_2*) were colocalized with the markers IES43-5mt, IES68-6fs**, and IES108-1fs. *qRRM_1*, *qRRM_2*, *qRRM_4*, and *qRRM_5* were closely linked to IES9-8mt*, IES356-2md, IES351-4md, and IES68-11ds**, respectively. These QTLs and their colocalized markers could be used for marker-assisted selection of resistance to root rot in sweetpotato.

Discussion

When generating a genetic population, the genetic characteristics and differences among the parents should be thoroughly considered. Within a certain range, a higher level of polymorphism can be detected when the parents are distantly related and have greater genetic differences, and hence, the constructed map will be more accurate and more saturated. Jizishu 1 is a cultivar with purple skin, purple meat, and high starch content. Longshu 9 is a fresh-eating cultivar with red skin, yellow meat, and low starch content. The average disease index of Jizishu 1 was 14.4, indicating high resistance to root rot, whereas that of Longshu 9 was 84.5, indicating high susceptibility. The genetic variation between these two cultivars is high, and the cross was suitable for constructing a mapping population. As the difference in disease resistance was significant, the QTLs for root rot resistance could be located.

Only four genetic linkage maps of sweetpotato based on SSR markers have been reported, two of them are constructed by EST-SSR markers. Tang et al. constructed the first EST-SSR-based genetic linkage maps with a mapping population of 189 progenies. In total, 74 linkage groups for the female parent, constructed based on 215 loci, were placed on the genetic linkage map. The linkage map covered a total length of 3,826.07 cM, with an average inter-marker distance of 17.80 cM. A genetic linkage map for the male parent was constructed using 250 loci distributed on 80 linkage groups. The linkage map covered 3,955.0 cM, with an average inter-marker distance of 15.7 cM. Seventeen QTLs for starch content were identified [16]. Kim et al. constructed a genetic linkage map based on 137 progenies derived from 'Yeseumi' and 'Annoben' and 210 EST-SSR markers. The total length was 1,508.1 cM, and the mean distance between markers was 7.2 cM. In addition, three QTLs for internode length, one QTL for skin thickness, 15 QTLs for bare skin main colour, and two QTLs for bare skin secondary colour were identified in the genetic linkage maps [9]. Zhao et al. constructed a high-density linkage map based on AFLP and SSR markers using an F₁ population comprising 202 individuals derived from a cross between Xushu 18 and Xu 781. The Xushu 18 map included 1,936 AFLP and 141 SSR markers, and the Xu 781 map contained 1,824 AFLP and 130 SSR markers, with the SSR markers accounting for only 6.7% of the total markers [19]. Other high-density linkage map was constructed based on retrotransposon insertion

polymorphism, SSR, and SNP markers by Sasai et al., but there was only 161 and 176 SSR markers in female parent and male parent, respectively [14].

In our study, 484 and 573 SSR polymorphic markers were grouped into 90 linkage groups on each parental map, respectively. The total map distance of Jizishu 1 was 3,974.24 cM, with an average marker distance of 8.23 cM. The total map distance of Longshu 9 was 5,163.35 cM, with an average marker distance of 9.01 cM. Compared with previously constructed maps, these maps have more polymorphic SSR markers, more linkage groups, longer total map distance, and shorter average inter-marker distance. Moreover, this is the first study to construct a genetic linkage map for purple sweetpotato (Jizishu 1) based on SSR markers, which can be used to enrich the genetic maps of and improve the genetic research on different sweetpotato varieties.

Similar to findings reported by Cervantes-Flores et al. [7], Kriegner et al. [10], Li et al. [11], and Zhao et al. [19], 239 distorted markers (49.38%) and 250 distorted markers (43.63%) were detected in genetic linkage maps of Jizishu 1 and Longshu 9, respectively. This suggests that some preferential pairing occurs in sweetpotato. There are many reasons for distorted segregation. Genetic factors such as gamete fertility, non-homologous recombination, gene conversion, and transposons can affect the distribution of marker sites in a population [24]. In addition to genetic factors, environmental factors and human intervention may cause distorted segregation. For example, the possibility of abnormal segregation appeared in population construction [25]. Sweetpotato is a highly heterozygous vegetative reproduction species, and its offspring is genetically unstable. An impure gene locus controlling a certain character may also cause distorted segregation in some lines [26].

Transgressive segregation was also observed for the root rot disease index in the mapping population. This may be due to the high heterozygosity of the parents, which results in the loss or accumulation of some favourable alleles in their offspring [21, 27, 28]. In sweetpotato breeding programs, this is commonly observed, especially in the hybrid offspring of parental materials with significant genetic differences.

The distribution frequencies of the disease index in the F_1 progeny were continuous, but abnormal in this study. A common method used in the analysis of abnormally distributed data is to log-transform the data before QTL analysis. However, Mutschler et al. believed that log-transformation of phenotypic values could result in incorrect estimation of trait differences among individuals by pulling the skewed tails of the distribution toward the centre, thus reducing the accuracy of QTL mapping [29]. Shen et al. obtained similar QTL mapping results when using transformed and non-transformed nematode resistance data [30]. Non-transformed abnormally distributed data have been used for QTL mapping in various studies [9, 23, 31–34]. Therefore, we used the non-transformed data for QTL analysis in this study and mapped seven stable QTLs, which explained 52.6–57.0% of the phenotypic variation in root rot resistance. We speculated that the root rot resistance of sweet potato might be controlled by several major QTLs. Agarwal et al. mapped two major-effect QTLs for early leaf spot, which explained 47.42% and 47.38% of phenotypic variation, two QTLs for late leaf spot, which explained 47.63% and 34.03% of phenotypic

variation in peanut [35]. Kumar et al. revealed three significant QTLs was for resistance to loose smut in tetraploid durum wheat, one of them explained up to 74% of phenotypic variation [36]. Using composite interval mapping and inclusive composite interval mapping, two major QTLs and one minor QTL were validated which had significant effects in reducing stripe rust severity and explained 59.0–74.1% of the phenotype variation in disease response [37]. However, due to the limited number of markers on the genetic map, we need to increase the density of markers on the map in the future to accurately locate the genes against root rot.

Of the seven QTLs, five were mapped on the Jizishu 1 map, 80.0% of which had a positive effect, and two were located on the Longshu 9 map, one of which had a positive effect. These results agree with the fact that Jizishu 1 is substantially more resistant to root rot than Longshu 9. qRRM_3, qRRF_1, and qRRF_2 were colocalized with the corresponding markers IES43-5mt, IES68-6fs**, and IES108-1 fs. The colocalized SSR markers identified in this study will be more useful for marker-assisted resistance breeding. These QTLs will have practical significance for gene cloning, and genome research of sweetpotato. Because of the lack of QTL mapping data for root rot resistance in sweetpotato in the literature, it is difficult to verify these QTLs. Therefore, the trait will be further studied and monitored in future studies to verify the loci identified in this research.

Conclusions

In this study, the first genetic linkage maps of purple sweetpotato (Jizishu 1) were constructed by SSR markers. To our knowledge, this is also the first report on the identification of QTLs associated with resistance to root rot in sweetpotato. These results will have practical significance for the fine mapping of root rot resistance genes and marker-assisted selection breeding for sweetpotato.

Methods

Plant materials

The mapping population was derived from a cross between the female parent Jizishu 1, a cultivar with resistance to root rot and high starch content that is popular in north China, and the male parent Longshu 9, a cultivar that is susceptible to root rot, has high yield and low starch content, and is popular in China. Both the parents and the F₁ generation were collected from the Institute of Cereal and Oil Crops, Hebei Academy of Agriculture and Forestry Sciences (Shijiazhuang, China) and analysed for esterase isozymes, self-bred progeny was deleted. In total, 300 progenies were used for genetic linkage map construction and QTL analysis.

DNA extraction

Genomic DNA was extracted from fresh leaves of Jizishu 1, Longshu 9, and the 300 F₁ individuals using the cetyltrimethylammonium bromide method [38, 39]. DNA concentrations and quality were determined

using an ultraviolet spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA) and 1.0% agarose gel electrophoresis respectively. The DNA was diluted to 50 ng/μL.

Genotyping

PCRs were carried out using 20 μL reaction mixtures containing 1 μL of DNA (50 ng/μL), 0.6 μL of each primer (5 μM, Invitrogen, China), 6 μL of 2× *Taq* PCR StarMix with loading dye (for PAGE, GenStar, China), and 11.8 μL of ddH₂O. Thermal cycles were as follows: initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 40 s, final extension at 72°C for 5 min, and hold at 10°C. 400 pairs of SSR primers were screened for polymorphism between Jizishu 1 and Longshu 9, and 8% acrylamide gels were used for electrophoresis detection. The polymorphic primers were used to characterise the F₁ segregating population.

Marker recording

Specific bands were read from top to bottom according to the molecular weights in comparison with a standard DNA marker (50 bp DNA Ladder, Tiangen, China). Clear, high-quality and high-resolution bands with a size of 100–700 bp were selected to improve the recording accuracy and reliability. Polymorphic markers were recorded as 1 or 0 according to their presence or absence, respectively, in the parents and the F₁ individuals, and vague or missing bands were recorded as 2. All polymorphic markers were divided into three categories (maternal, paternal, and double-simplex markers) according to their presence in the two parents (Table 3).

Table 3 Marker types in sweetpotato

	Female parent (Jizishu 1)	Male parent (Longshu 9)	Type of marker
□	Presence / 1	Absence / 0	f (maternal)
□	Absence / 0	Presence / 1	m (paternal)
□	Presence / 1	Presence / 1	ds (double-simplex)

Marker dosage was determined as the segregation ratio of markers (presence:absence) in the mapping population. Four cytological hypotheses proposed by Jones in 1967 were used to classify marker dosages without considering strict tetraploid isolation [40]. Based on the goodness-of-fit to the expected segregation ratios for all markers determined using the Chi-square test, we divided markers into four groups on the basis of their segregation ratios: (1) simplex or single-dose markers exist in one of the parents in the form of a single copy, and the segregation ratio in the progeny is 1:1 (presence:absence), (2) duplex or double-dose markers are present in one of the parents in the form of two copies, and the segregation ratio in the progeny is 4:1 for hexasomic, 5:1 for tetrasomic, or 3:1 for disomic or tetrasomic inheritance, (3) triplex or triple-dose markers are present in one of the parents in three copies, having a ratio of 19:1 (hexasomic), 11:1 (tetradisomic) or 7:1 (disomic); (4) double-simplex markers exist in both parents in a single-dose condition and segregate in a 3:1 ratio in the progeny [7, 10]. According to the Chi-

square test results, if the segregation ratio did not conform to Mendelian segregation, it was considered as a distorted marker.

Marker names were determined by considering the following four points: (1) the polymorphic primer names (e.g., IES87), (2) the corresponding specific band number, usually with a large-molecular-weight band in front (e.g., 05), (3) the type of marker, f, m, or ds (Table 3), (4) the dosage of the marker, s, d, t, or ds, which represented simplex, duplex, triplex or double-simplex, respectively. For example, IES295-1fs represents the first polymorphic band from SSR primer IES295, and its marker type is a Longshu 9 single marker. For the distorted markers, * and ** as a suffix indicate significant differences at the 0.05 and 0.01 levels, respectively.

Linkage map construction

The genetic linkage map was constructed using the JoinMap 4.0 software [41] and the pseudo-testcross mapping strategy [6, 42]. Genotype codes of the 300 F_1 individual plants were recorded using the standard genotype analysis method in JoinMap 4.0. When a band was present only in Jizishu 1, progeny with the same band pattern as that of Jizishu 1 was marked 'lm', and offspring with a different band pattern was marked 'll'. When a band was present only in Longshu 9, progeny with the same band pattern as Longshu 9 was marked 'np', otherwise, it was marked 'nn'. When a band was present in both parents, but it was segregated in the progeny, progeny with bands was marked 'h-', progeny without bands was marked 'kk'. Only clear bands were recorded, missing and ambiguous bands were represented as '-'.

Using the outbreeder full-sib family analysis model, the map was constructed in two steps: (1) single-dose markers were used to construct the framework map of each parent at a logarithm of odds (LOD) of 5.0, (2) duplex and triplex markers were inserted into the framework map to obtain the final genetic linkage map [7, 10]. Linkage groups containing the same duplex or triplex markers were considered homologous and divided into corresponding homologous linkage groups in the same parental map. Linkage groups with the same double-simplex markers in the two maps were considered to be homologous.

Names of linkage groups are mainly composed of three parts: (1) the names of the corresponding parents, JZ1 (Jizishu 1) or L9 (Longshu 9); (2) a number between 1 and 15 (written as 01–15) indicating the sequence number of the homologous linkage group; (3) a number between 1 and 90 (written as 01–90) referring to the sequence number of the linkage group. For example, JZ1 (01.01) indicates that the linkage group belongs to the first linkage group on the Jizishu 1 map, and to the first homologous linkage group. JZ1 (00.66) indicates that the linkage group is the 66th linkage group on the Jizishu 1 map, and is not included in any homologous linkage group.

Identification of resistance to root rot

The two parents and the 300 F_1 individuals were planted in the natural disease nursery of Xiong County, Hebei, China (39°06'43"N, 116°14'56"E), the main sweetpotato producing area, on May 16, 2016 and

2017. The experiment was completely randomly arranged, with ridge spacing of 0.85 m and plant spacing of 0.25 m. For each of the F₁ individual, five plants were planted, with three repeats. Each parent as the control were planted in each ridge with five. Forty days after planting and in mid-October, the disease index of aboveground and underground was investigated and calculated, respectively. The final disease index was determined according to the average value of disease index of aboveground and underground. Identification standard of aboveground of sweetpotato resistance to root rot is as follows, '0' the plants grow normally, no disease can be seen; '1' the leaves colour are yellow slightly, others are normal; '2' the branches are few and short, the leaves colour are yellow obviously, and the plant bud or flower; '3' the plant significantly dwarfed without branched, old leaves fall down from bottom to top; '4' the plant die. Identification standard of underground of sweetpotato resistance to root rot is as follows, '0' The fibrous roots and tuberous roots are normal without any disease spots; '1' a few fibrous roots turn black (the number of diseased fibrous roots accounts for less than 10% of the total number of roots), and there are no disease spots on the underground stems, which have no significant effect on tuberous roots formation; '2' a few fibrous roots turn black (the number of diseased fibrous roots accounts for 10–25% of the total number of roots), and there are a few diseased spots on underground stems and tuberous roots, which have a slight effect on tuberous roots formation; '3' nearly half of the fibrous roots turn black (the number of diseased fibrous roots accounts for 25.1–50.0% of the total number of roots), and there are many diseased spots on underground stems and tuberous roots, which have a significant effect on tuberous roots formation; '4' most of the fibrous roots turn black (the number of fibrous diseased roots accounts for more than 50% of the total number of roots), and there are many and large diseased spots on the underground stem, without tuberous roots, or the plant die.

$$DI = \frac{\sum(A * B)}{C * 4} * 100$$

DI: disease index of aboveground or underground, A: number of plants at different levels (0, 1, 2, 3 or 4), B: corresponding level (0, 1, 2, 3 or 4), C: total number of investigated plant, 4: the highest level (4).

Mapping of QTLs for root rot resistance

The frequency distribution of the 300 F₁ individual disease index values in 2016 and 2017 and the means were determined using IBM SPSS Statistics 24.0 software. Genetic linkage map data, phenotypic data for each year, and the average values were used to map QTLs for root rot resistance using the MapQTL5.0 software [43]. First, interval mapping analysis was used to determine the initial location of the QTL. Second, a multiple QTL model was used to precisely locate the QTL, in which the nearest marker associated with the QTL was selected as the cofactor. In this study, a LOD score of 3.0 was used as the typical threshold value to determine the location of the QTL. QTLs appearing at the same genomic location in the two environments and average data were considered stable QTLs. The linkage maps of QTL for the resistance to root rot were drawn by Map Chart 2.2 [44].

Abbreviations

AFLP: Amplified fragment length polymorphism; EST-SSR: expressed sequence tag simple sequence repeat; LOD: logarithm of odds; NGS: next-generation sequencing; QTL: Quantitative trait locus; RAPD: Random amplified polymorphic DNA; SNP: Single nucleotide polymorphism; SSR: Simple sequence repeat

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interest.

Funding

This work was supported by the China Agriculture Research System (CARS-10, Sweetpotato), the Modern Agriculture Industrial Technology System Innovation Team Project of Hebei Province (HBCT2018080203), Key Research and Development Project of Hebei Province (19226338D), the Modern Agricultural Science and Technology Innovation Project of Hebei Province (2019-4-2-7), and the Science and Technology Research and Development Plan Project of Hebei Academy of Agricultural and Forestry Sciences (2018060305). The funding bodies were not involved in the design of the study, in the collection, analysis and interpretation of data or in writing the manuscript.

Authors' contributions

ZM and YH contributed to the linkage analysis and wrote the manuscript. WG, LL and ZW analyzed the resistance of parents and F₁ population to root rot of sweetpotato for two consecutive years and acquired the data. ML and NZ revised the manuscript. MH, WJ and ZG extracted and quantified the DNA, did the PCR and electrophoresis detection. YH and QL conceived and designed the experiments. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to Shaozhen He (College of Agronomy and Biotechnology, China Agricultural University) and Hong Zhai (College of Agronomy and Biotechnology, China Agricultural University) for providing the SSR markers. We would like to thank Jun Qin (Institute of Cereal and Oil Crops, Hebei Academy of Agriculture and Forestry Sciences) and Guoqing Liu (Institute of Millet Crops, Hebei Academy of Agriculture and Forestry Sciences) for their suggestions on our manuscript.

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References

1. Zang N, Zhai H, Gao S, Chen W, He S, Liu Q. Efficient production of transgenic plants using the bar gene for herbicide resistance in sweetpotato. *Sci Hortic-amsterdam*. 2009;122(4):649–53.
2. Chen L, Xu Y, Fang Z. Identification of isolates causing root rot of sweetpotato and tests on resistance of varieties of sweetpotato to root rot. *Jiangsu J Agr Sci*. 1990;6(2):27–32.
3. Chen X, Song G. Preliminary analysis on the trend of genetic variation of resistance to sweet potato root rot. *Shandong Agr Sci*. 1988;4:28–9.
4. Su W, Wang L, Lei J, Zhao T, Shuai Q, Yang X. Identification of AFLP markers linked to a locus conferring inducible resistance to root rot in sweetpotato. *Hubei Agr Sci*. 2011;50(24):5254–6.
5. Dhir SK, Oglesby J, Bhagsari AS. Plant regeneration via somatic embryogenesis, and transient gene expression in sweetpotato protoplasts. *Plant Cell Rep*. 1998;17(9):665–9.
6. Grattapaglia D, Sederoff R. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics*. 1994;137(4):1121–37.
7. Cervantes-Flores JC, Yencho GC, Kriegner A, Pecota KV, Faulk MA, Mwanga ROM, Sosinski BR. Development of a genetic linkage map and identification of homologous linkage groups in sweetpotato using multiple-dose AFLP markers. *Mol Breeding*. 2008;21(4):511–32.
8. Chang KY, Lo HF, Lai YC, Yao PJ, Lin KH, Hwang SY. Identification of quantitative trait loci associated with yield related traits in sweet potato (*Ipomoea batatas*). *Bot Stud*. 2009;50(1):43–55.
9. Kim JH, Chung IK, Kim KM. Construction of a genetic map using EST-SSR markers and QTL analysis of major agronomic characters in hexaploid sweet potato (*Ipomoea batatas* (L.) Lam). *Plos One*. 2017;12(10):e0185073.

10. Kriegner A, Cervantes JC, Burg K, Mwanga RO, Zhang DP. A genetic linkage map of sweetpotato (*Ipomoea batatas* (L.) Lam.) based on AFLP markers. *Mol Breeding*. 2003;11(3):169–85.
11. Li AX, Liu QC, Wang QM, Zhang LM, Zhai H, Liu SZ. Construction of molecular linkage maps using SRAP markers in sweetpotato. *Acta Agron Sin*. 2010;36(8):1286–95.
12. Mollinari M, Olukolu BA, Pereira GDS, Khan A, Gemenet D, Yencho GC, Zeng ZB. Unraveling the hexaploid sweetpotato inheritance using ultra-dense multilocus mapping. *G3*. 2020;10:281–92.
13. Monden Y, Hara T, Okada Y, Jahana O, Kobayashi A, Tabuchi H, Onaga S, Tahara M. Construction of a linkage map based on retrotransposon insertion polymorphisms in sweetpotato via high-throughput sequencing. *Breeding Sci*. 2015;65:145–53.
14. Sasai R, Tabuchi H, Shirasawa K, Kishimoto K, Sato S, Okada Y, Kuramoto A, Kobayashi A, Isobe S, Tahara M, Monden Y. Development of molecular markers associated with resistance to *Meloidogyne incognita* by performing quantitative trait locus analysis and genome-wide association study in sweetpotato. *DNA Res*. 2019;26(5):399–409.
15. Shirasawa K, Tanaka M, Takahata Y, Ma D, Cao Q, Liu Q, Zhai H, Kwak SS, Jeong JC, Yoon UH, Lee HU, Hirakawa H, Isobe S. A high-density SNP genetic map consisting of a complete set of homologous groups in autohexaploid sweetpotato (*Ipomoea batatas*). *Sci Rep-UK*. 2017;7:44207.
16. Kim D, Zhang K, Lu C, Xie D, Fu T, Wang J. Genetic linkage map construction based on EST-SSR and analysis of QTLs for starch content in sweetpotato (*Ipomoea batatas* (L.) lam.). *Sci Agr Sin*. 2016;49(23):4488–506.
17. Ukoskit K, Thompson PG. Autopolyploidy versus allopolyploidy and low-density randomly amplified polymorphic DNA linkage maps of sweetpotato. *J Am Soc Hort Sci*. 1997;122(6):822–8.
18. Wu J, Tan W, He J, Pu Z, Wang D, Zhang Z, Zhan F, Yan W. Construct on of SRAP linkage map and QTL mapping for starch content in sweet potato. *Mol Plant Breeding*. 2005;3(6):841–5.
19. Zhao N, Yu X, Jie Q, Li H, Hu J, Zhai H, He S, Liu Q. A genetic linkage map based on AFLP and SSR markers and mapping of QTL for dry-matter content in sweetpotato. *Mol Breeding*. 2013;32(4):807–20.
20. Cervantes-Flores JC, Yencho GC, Pecota KV, Sosinski B. Detection of quantitative trait loci and inheritance of root-knot nematode resistance in sweetpotato. *J Am Soc Hort Sci*. 2008;133(6):844–51.
21. Cervantes-Flores JC, Sosinski B, Pecota KV, Mwanga ROM, Catignani GL, Truong VD, Watkins RH, Ulmer MR, Yencho GC. Identification of quantitative trait loci for dry-matter, starch, and β -carotene content in sweetpotato. *Mol Breeding*. 2011;106(28):201–16.
22. Yu X, Zhao N, Li H, Jie Q, Zhai H, He S, Li Q, Liu Q. Identification of QTLs for starch content in sweetpotato (*Ipomoea batatas* (L.) Lam). *J Integr Agr*. 2014;13(2):310–5.
23. Li H, Zhao N, Yu X, Liu Y, Zhai H, He S, Li Q, Ma D, Liu Q. Identification of QTLs for storage root yield in sweetpotato. *Sci Hortic-amsterdam*. 2014;170:182–8.
24. Jelena V. An impact of environment on segregation ratio of qualitative traits in maize. *Genetika*. 2008;40(2):145–56.

25. Guo J, Wang F, Lin R, Feng J, Zhang Z, Xu S. Genetic analysis of segregation distortion of SNP and SSR markers in wheat RIL population from the Taichung29 × Baimangmai. *J Plant Protect.* 2018;45(4):795–803.
26. Wang Z, Huai D, Zhang Z, Cheng K, Kang Y, Wan L, Yan L, Jiang H, Lei Y, Liao B. *Front Plant Sci.* 2018;9:827.
27. Tanksley SD. Mapping polygenes. *Annu Rev Genet.* 1993;27:205–33.
28. Young ND. QTL mapping and quantitative disease resistance in plants. *Annu Rev Phytopathol.* 1996;34:479–501.
29. Mutschler MA, Doerge RW, Liu SC, Kuai JP, Liedl BE, Shapiro JA. QTL analysis of pest resistance in the wild tomato *Lycopersicon pennellii*: QTLs controlling acylsugar level and composition. *Theor Appl Genet.* 1996;92(6):709–18.
30. Shen X, Van Becelaere G, Kumar P, Davis RF, May OL, Chee P. QTL mapping for resistance to root-knot nematodes in the M-120 RNR Upland cotton line (*Gossypium hirsutum* L.) of the Auburn 623 RNR source. *Theor Appl Genet.* 2006;113(8):1539–49.
31. An C, Johnnie NJ, Wu J, Guo Y, McCarty JC. Use of fiber and fuzz mutants to detect QTL for yield components, seed, and fiber traits of upland cotton. *Euphytica.* 2010;172(1):21–34.
32. Laila R, Park JI, Robin AHK, Natarajan S, Vijayakumar H, Shirasawa K, Isobe S, Kim HT, Nou IS. Mapping of a novel clubroot resistance QTL using ddRAD-seq in Chinese cabbage (*Brassica rapa* L.). *BMC Plant Biol.* 2019;19:13.
33. Lee SB, Hur YJ, Cho JH, Lee JH, Kim TH, Cho SM, Song YC, Seo YS, Lee J, Kim TS, Park YJ, Oh MK, Park DS. Molecular mapping of qBK1WD, a major QTL for bakanae disease resistance in rice. *Rice.* 2018;11(1):3.
34. Sharma S, Sharma S, Kopisch-Obuch FJ, Keil T, Laubach E, Stein N, Graner A, Jung C. QTL analysis of root-lesion nematode resistance in barley: 1. *Pratylenchus neglectus*. *Theor Appl Genet.* 2011;122(7):1321–30.
35. Agarwal G, Clevenger J, Pandey MK, Wang H, Shasidhar Y, Chu Y, Fountain JC, Choudhary D, Culbreath AK, Liu X, Huang G, Wang X, Deshmukh R, Holbrook CC, Bertoli DJ, Ozias-Akins P, Jackson SA, Varshney RK, Guo B. High-density genetic map using whole-genome resequencing for fine mapping and candidate gene discovery for disease resistance in peanut. *Plant Biotechnol J.* 2018;16(11):1954–67.
36. Kumar S, Knox R, Singh AK, DePauw RM. High-density genetic mapping of a major QTL for resistance to multiple races of loose smut in a tetraploid wheat cross. *Plos One.* 2018;13(2):e0192261.
37. Zeng Q, Wu J, Huang S, Yuan F, Liu S, Wang Q, Mu J, Yu S, Chen L, Han D, Kang Z. SNP-based linkage mapping for validation of adult plant stripe rust resistance QTL in common wheat cultivar Chakwal 86. *The Crop J.* 2019;7:176–86.
38. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. *Focus.* 1990;12:13–5.

39. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. *Nucleic Acid Res.* 1980;8(19):4321–5
40. Jones A. Theoretical segregation ratios of qualitatively inherited characters for hexaploid sweetpotato (*Ipomoea batatas*). *USDA Tech Bull.* 1967; doi:10.22004/ag.econ.171479
41. Ooijen JWV. Joinmap 4 software for the calculation of genetic linkage maps in experimental populations. Wageningen: Kyazma B.V.; 2006.
42. da Silva JAG, Sorrells ME, Burnquish WL, Tanksley SD. RFLP linkage map and genome analysis of *Saccharum spontaneum*. *Genome.* 1993;36(4):782–91.
43. Ooijen JWV. Map QTL 5 software for the mapping quantitative trait loci in experimental populations. Wageningen: Kyazma B.V.; 2004.
44. Voorrips RE. MapChart: software for the graphical presentation of linkage maps and QTL. *J Hered.* 2002;93(1):77–8.

Figures

Figure 1

Frequency distribution of disease index of sweetpotato root rot in the mapping population. Black and white arrows indicate the disease index of Jizishu 1 and Longshu 9, respectively.

Figure 2

The QTLs for resistance to root rot identified in the Jizishu 1 linkage groups. QTLs were shown as vertical bars on the right side of the respective linkage groups. The QTL corresponding markers were indicated by underlined text.

Figure 3

The QTLs for resistance to root rot identified in the Longshu 9 linkage groups. QTLs were shown as vertical bars on the right side of the respective linkage groups. The QTL corresponding markers were indicated by underlined text.

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