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ORIGINAL PAPER

A major QTL located on chromosome V associates with in vitro tuberization in a tetraploid potato population

Jun Zhou · Hui Fang · Jianwei Shan · Xiaoxi Gao · Lin Chen · Conghua Xie · Tingting Xie · Jun Liu

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Abstract The cultivated potato (*Solanum tuberosum* L.) is an autotetraploid species. The complexity of tetrasomic inheritance and the lack of pure lines increase the difficulty of genetic analysis of the inherited characteristics. Tuberization is the determinant step for economic yield of potato. To understand the complex genetic basis of tuberization of the cultivated potato, we developed linkage maps for a tetraploid population (F1) of 237 genotypes and mapped QTLs for the percent of in vitro tuberized plantlets (% IVT). The paternal map for E108 (well tuberized) covered 948 cM and included 12 linkage groups, all of which contained all four homologous chromosomes. The maternal map for E20 (nontuberized) covered 1,286 cM and included 14 linkage groups, 12 of which contained all four homologous chromosomes. All 12 chromosomes of potato were tagged using the SSR markers. A major QTL (MT05) with additive effect was detected on chromosome V of E108 which explained 16.23 % of the variation for % IVT, and two minor QTLs (mt05 and mt09) displaying simplex dominant effects were located on chromosome V and chromosome IX of E20 which explained 5.33 and 4.59 % of the variation for % IVT, respectively. Based on the additive model of MT05, the segregation ratio of the gametic genotypes ($Q-$: qq = 5:1) matched the ratio of the tuberized genotypes to the nontuberized genotypes in the population suggesting that the segregation of in vitro tuberization in this population is controlled by a major-effect gene or genes. The mapping results of three important candidate genes indicated that the QTL causal genes detected in our study are new. In this study, we developed the almost complete linkage maps of a tetraploid population, identified a major QTL on chromosome V affecting in vitro tuberization, suggested a major-effect gene with minor modifiers model controlling this trait and found that the QTLs identified here correspond to new tuberization genes. Our work provides new and useful information about the genetic basis for tuberization of this autotetraploid crop.

Keywords Potato · QTL · In vitro tuberization · Tetraploid

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J. Zhou · H. Fang · J. Shan · X. Gao · L. Chen · C. Xie · T. Xie · J. Liu (✉)
National Center for Vegetable Improvement (Central China), Wuhan, China
e-mail: liujun@mail.hzau.edu.cn

J. Zhou · H. Fang · J. Shan · X. Gao · L. Chen · C. Xie · T. Xie · J. Liu
Key Laboratory of Horticultural Plant Biology, Huazhong Agricultural University, Ministry of Education, Wuhan, China

J. Zhou · H. Fang · J. Shan · X. Gao · L. Chen · C. Xie · T. Xie · J. Liu
Potato Engineering and Technology Research Center of Hubei Province, Huazhong, China

J. Zhou · H. Fang · J. Shan · X. Gao · L. Chen · C. Xie · T. Xie · J. Liu
Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China

Introduction

Potato originates from the Andes and has become one of the most important food crop grown in more countries than any other crops just after maize (http://faostat.fao.org). As

a storage organ and a vegetative propagation system, the tuber has the most important economic impacts on potato crop. To describe the status of the plant when it becomes capable of forming tubers, it is common to say that the plant has become induced to tuberize. The tuber formation process (tuberization) is the determinant step for economic yield and/or propagation rate of potato (Ewing and Struik 1992). But tuberization is a complex developmental process, which differentiates specialized underground propagation organs or tubers under favorable conditions (Rodríguez-Falcón et al. 2006), and the genetic basis of this process is far away from being fully understood due to the highly heterozygous nature of potatoes. Especially in cultivated potatoes (*Solanum tuberosum* L.) which display tetrasomic inheritance, the genetic analysis of tuberization is difficult and lacking.

Tuberization in potatoes has often been studied using an in vitro system based on single-node stem cuttings, in which tuberization can be synchronously induced in response to day length (Ewing and Wareing 1978) or high levels of sucrose in the media (Hussey and Stacey 1984; Garner and Blake 1989). Such system has become an established method for virus-free certified seed potato production, as well as for germplasm conservation and exchange (Roca et al. 1979; Dodds et al. 1991), and was used in researching the molecular mechanisms underlying potato tuberization (Bachem et al. 1996; Ronning et al. 2003; Hannapel 2010; Inui et al. 2010).

Different pieces of evidence indicated that photoperiodic control of tuberization shared several common elements with that of flowering regulation (Rodríguez-Falcón et al. 2006). In the last decade, more and more data supported that several important genes, such as *Phytochrome*, *CONSTANS* (*CO*), *FLOWERING LOCUS T* (*FT*), LOV blue light receptors and transcription factor *CDF*, involved in the long-distance signaling pathway of Arabidopsis flowering, also played a prominent role in seasonal tuberization control (Yanovsky et al. 2000; Martínez-García et al. 2002; Inui et al. 2010; Navarro et al. 2011; González-Schain et al. 2012; Kloosterman et al. 2013). Three potato homologs of these important genes have recently been demonstrated to be involved in day-length tuberization control, including *FT* homolog *StSP6A* (Navarro et al. 2011), *CO* homolog *StCO* (González-Schain et al. 2012) and *CDF* homolog *StCDF1* (Kloosterman et al. 2013). Although much progress has been made, the molecular mechanism underlying day-length tuberization control is still not fully understood.

In addition to the efforts made in the molecular aspects, the genetic basis of tuberization has been extensively studied in recent years. It is probable that tuberization and time to plant maturity are related physiological traits, which are controlled by genetic factors as well as day length. Based on the work with cultivated potatoes and heritability studies, Mendoza and Haynes (1977) hypothesized that the initiation of tuberization (or early maturity) was controlled by one dominant major gene with modifiers and that short critical photoperiod was dominant to long. Van den Berg et al. (1996) identified 11 distinct loci on seven linkage groups affecting tuberization in reciprocal backcrosses between *S. tuberosum* and *Solanum berthaultii*. Most of the loci had small effects, but a QTL on chromosome V explained 27 % of the variance (tuberization in long days could be interpreted as early maturity). In the recent two decades, more than ten studies have focused on the QTL of maturity and about 70 maturity QTLs of potato have been reported. These maturity QTLs spread on the 12 potato chromosomes and were mapped in diploid or tetraploid populations. Several researchers found major QTL for maturity on chromosome V (Collins et al. 1999; Visker et al. 2005; Malosetti et al. 2006; Bradshaw et al. 2008) and the functions overlap of these regions was also documented. Recently, a potato homolog of transcription factor *CDF* (*StCDF1*) was cloned from a major-effect QTL for the plant maturity mapped to a 110 kilobases (kb) interval on the north arm of chromosome V (Kloosterman et al. 2013). This gene was demonstrated to regulate tuberization and plant life cycle length, by acting as a mediator between the *CO*-dependent pathway and the *StSP6A* mobile tuberization signal. However, the genetic basis of tuberization is not clear enough due to the highly heterozygous nature of potatoes. To understand the genetic basis of tuberization is not only an important aspect of theoretical research, but also critical to improve potato yield and quality.

In recent years, several linkage maps developed by the software package TetraploidMap for Windows (Hackett et al. 2007) for the tetraploid potato have been published (Bradshaw et al. 2008; Khu et al. 2008; McCord et al. 2011; Groth et al. 2013) and the statistical methods for tetrasomic inheritance have been established. In the present study, our objectives were to construct a genetic linkage map and identify QTLs in a tetraploid potato population segregating on in vitro tuberization. The results provide useful information about the genetic basis for tuberization of this autotetraploid crop.

Materials and methods

Plant materials and conditions for induction of tuberization

A tetraploid potato ($2n = 4x = 48$) F1 population, MT I, consisted of 237 individuals from a cross between 395049.20 (E20) and 395049.108 (E108) which was made in 2008. E108 and E20 were progenies of the population 395049, derived from 393075.54 × 391679.12 (seeds were offered by the International Potato Center).

The parents (E108 and E20) of the tested population MT I were selected from over 100 genotypes based on the performance of in vitro tuberization. The paternal parent E108 always tuberized well and quickly, both in 8/16 and 16/8 h photoperiod, but the maternal parent E20 could not tuberize in either photoperiod. They were evaluated repeatedly eight times to ensure that the phenotypes were stable. Segregation for tuberization was observed in their offspring in both photoperiods.

Seeds of the population MT I were surface sterilized and germinated in vitro in a plant growth regulator-free and sucrose-free MS medium (Murashige and Skoog 1962). Offspring and parents had been maintained vegetatively as in vitro grown plantlets and multiplied though single-node cuttings on semisolid (7 g L^{-1} ager) MS basal medium with 4 % sucrose and incubated at 22 ± 1 °C with 16/8 h day/night photoperiod (light intensity 83 µmol m^{-2} s^{-1}).

For each genotype, the second or the third (from the apical) single-node cutting, leaf included, was transferred to the microtuber induction medium supplemented with 8 % sucrose, 0.7 % agar and 0.2 % activated carbon, and then incubated at 18 ± 1 °C with two different photoperiods (8/16 h and 16/8 h day/night). After 8 weeks, the percentage of the in vitro tuberized plantlets (% IVT) was measured by using 45 plantlets for each genotype in each photoperiod. The proportion of the tuberized genotypes to the nontuberized genotypes was tested by the Chi-square test.

DNA extraction and marker generation

Young leaves of in vitro plantlets were used for DNA extraction according to the CTAB protocol as described by Dellaporta et al. (1983). The parents of the population MT I were screened with 164 simple sequence repeat (SSR) primers from the CIP database (http://research.cip.cgiar.org/IPD/SSRprimer) and Feingold et al. (2005). Only SSRs with polymorphism and good band quality were used to genotype the entire mapping population. The amplification of SSR markers was performed according to Feingold et al. (2005). Primer sequences and location information of the SSRs used to identify linkage groups are detailed in online supplementary (Table S1: SSR primer sequences and map location for screening of tetraploid potato population MT I).

AFLP assays were carried out according to the protocol described by Vos et al. (1995), followed by silver staining according to Han et al. (2008). Gels were scored manually, with each band being scored as a locus with dominant (present) versus recessive (absent) allele. Sixty-eight *Eco*R I/*Mse* I combinations and 66 *Pst* I/*Mse* I combinations were used for map construction. Pre-amplification primer sequences of *Eco*R I, *Mse* I and *Pst* I were 5′- GACTGCGTACCAATTC-3′, 5′-GATGAGTCCTGAGTAA-3′ and 5′-GACTGCGTACATGCAG-3′, respectively. AFLP marker names consisted of the three selective bases of the forward primer, three selective bases from the reverse primer and the molecular size of the marker in base pairs.

Three candidate gene loci including *StSP6A*, *StCO* and *StCDF1* were tested in the population MT I. SSR markers for the former two (*StSP6A* and *StCO*) were designed based on the potato genome sequence superscaffold and pseudomolecule information (The Potato Genome Sequencing Consortium, http://www.potatogenome.net/index.php/Main_Page). The gene sequence of the *StCDF1* (Kloosterman et al. 2013) gene was used to design primers specific for this gene. All primer sequences for candidate genes are detailed in online supplementary Table S1.

Map construction

Linkage analysis was performed as described by Bradshaw et al. (2008), using the software program TetraploidMap for Windows (Hackett et al. 2007). This software package was developed for mapping in autotetraploid species and was designed to handle dominant and codominant markers in several dosage configurations scored on two parents and their full-sib offspring.

For map construction, we selected four types of markers that did not significantly differ from the expected segregation ratios: simplex dominant markers (segregating 1:1) with a p value >0.001 from a Chi-square test for goodness of fit, duplex dominant markers (segregating 5:1) with a p value >0.01, double-simplex dominant markers (present in both parents and segregating 3:1) with a p value >0.01 and all SSR (multiallelic) markers. Double-simplex dominant markers segregating 11:1 or 35:1, which are extremely uninformative about recombination, were omitted from the linkage analysis.

Firstly, the most likely parental genotype was identified using the approach of Luo et al. (2000) based on the marker phenotype and segregation ratios in the progeny. Then the dosage of each marker was inferred and the program performed a preliminary cluster analysis to identify simplex markers on the same homologous chromosome linked in coupling. All simplex, duplex and multiallelic markers were then combined into linkage groups by cluster analysis. For each linkage group, a simulated annealing algorithm (Hackett et al. 2003) was used to identify the order of the markers and to calculate map distance between the markers. Homologous chromosomes or linkage groups were identified via simplex markers linked in repulsion, duplex markers and multiallelic markers. Double-simplex markers, which are present in both parents but segregate in their progenies, were used along with SSR markers to align the parental maps.

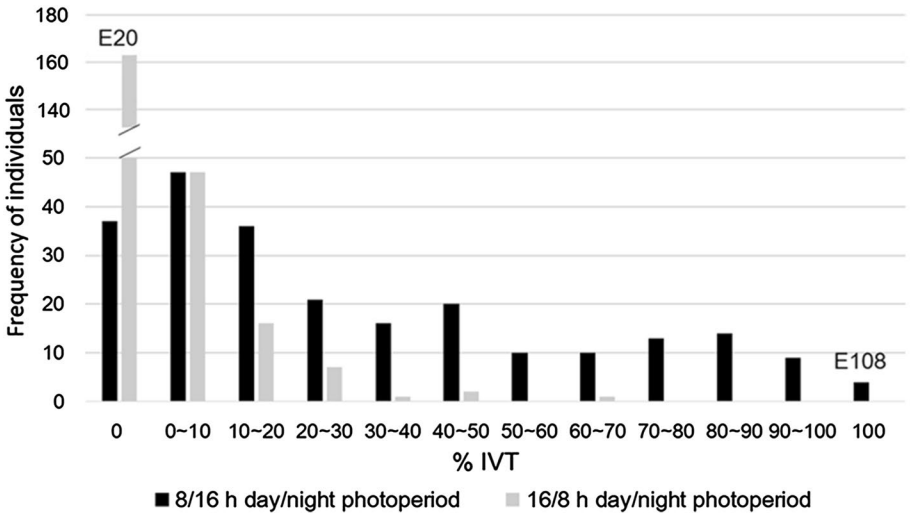

Fig. 1 Histogram showing the frequency distributions of the percentage of the in vitro tuberized plantlets (% IVT) calculated using 45 plantlets for each genotype in the population MT I (total of 237 genotypes) in the 8 and 16 h photoperiod. The parents E20 and E108 are indicated on the top of the class to which they belong

Marker–trait associations and QTL analysis

To obtain a first idea about marker–phenotype associations, a single marker analysis of variance was performed with the one-way ANOVA analysis of SPSS 20.0. At the same time, the TetraploidMap also ran a preliminary analysis of variance (single-point ANOVA) to identify linkage groups likely to be associated with each trait, and then QTL interval mapping for selected linkage groups. Only markers with associated p values <0.01 in two tests were reported.

QTL analysis was performed with the interval mapping (IM) routine of TetraploidMap for Windows (Hackett et al. 2007). The full regression model consists of six QTL genotypes, i.e., gametes Q1Q2, Q1Q3, Q1Q4, Q2Q3, Q2Q4 and Q3Q4 derived from the parent Q1Q2Q3Q4, reflecting the six combinations in which any two parental alleles can be transmitted to offspring. If a QTL was found to be significant, ten simpler models (four simplex models and six duplex models) were compared to the full model using a likelihood ratio test. Each simplex model tests one simplex allele (Q) on each homologous chromosome in turn, therefore making four simplex models, through comparing the trait values between genotypes Qq and qq to test for the simplex allele (e.g., compare Q12 + Q13 + Q14 vs. Q23 + Q24 + Q34 to test for simplex allele on homolog 1). Duplex models test dominant duplex allele (QQ) on pairs of homologous chromosome in turn, so there are six duplex models (e.g., compare Q12 + Q13 + Q14 + Q23 + Q24 vs. Q34 to test for duplex allele on homologs 1 and 2 and absence from homologs 3 and 4). In the case that a simpler model represented the data as well as the full model, the results of the simpler model were reported. Permutation tests (Churchill and Doerge 1994) with 1,000 iterations were performed to determine the significance threshold of the presence of a QTL.

Results

Phenotypic data

The histogram of the percent of the in vitro tuberized plantlets (% IVT) with two photoperiods is shown in Fig. 1. The male parent E108 could tuberize in both the photoperiods. In 8 h photoperiod, it always tuberized in all the plantlets and had the largest % IVT (100 %). In 16 h photoperiod, it tuberized in most of the plantlets and had the % IVT from 67 to 80 % in eight repetitions. In contrast, no plantlet of the female parent E20 had tuberized in 8 h photoperiod or 16 h photoperiod and consequently got the smallest % IVT (0 %). The phenotypes of E108 and E20 were stable in eight repetitions.

The distribution of the phenotypic data in the population MT I was skewed both in the 8 h photoperiod and 16 h photoperiod. In the 8 h photoperiod, there were 37 genotypes with the same tuberization phenotype as the female parent E20 (% IVT = 0 %), while the segregation ratio of the tuberized genotypes to the nontuberized genotypes in the population was 200:37 which was consistent with a 5:1 ratio ($p > 0.05$). The ratio of the well-tuberized (% IVT >20 %) genotypes to the poorly tuberized (% IVT <20 %) genotypes was 117:120, which was consistent with a 1:1 ratio ($p > 0.05$). In the 16 h photoperiod, a large part of the population had not tuberized and the segregation ratio of the tuberized genotypes to the nontuberized genotypes was 74:163. All of the 74 genotypes which tuberized in the 16 h photoperiod also tuberized in the 8 h photoperiod.

Linkage maps

The genetic map was constructed using the SSR and AFLP markers. In total, 65 SSR primers were used to genotype the entire population MT I and resulted in 117 markers,

of which 16 were multiallelic markers, 65 were simplex dominant markers, 21 were duplex dominant markers and 15 were double-simplex dominant markers. A total of 124 AFLP primer combinations resulted in 589 markers. Out of these, only markers that were consistent with the expected segregation ratios were used for map construction.

The map for the male parent E108 (well tuberized) consisted of 276 AFLP markers, 64 SSR markers and 2 candidate alleles (CDF1-1,100 bp and CDF1-1,000 bp), of which 189 were simplex, 62 were duplex, 78 were double-simplex and 13 were multiallelic markers. Finally, 315 markers were resolved into 12 linkage groups and 27 markers were unlinked. Total map length (calculated from the lengths of the overall linkage groups) of the paternal map was 948 cM and the average marker interval was 3.0 cM. All 12 linkage groups could be assigned to chromosome I–XII by SSR markers on the groups. Despite that there was just one marker on the homologs 3 and 4 of chromosome III and homolog 1 of chromosome X, all 12 linkage groups contained all four homologous chromosomes.

A total of 382 markers were used to construct the maternal map. These included 317 AFLP markers and 65 SSR markers, of which 231 were simplex, 57 were duplex, 78 were double-simplex and 16 were multiallelic markers. After analysis, 341 markers were resolved into 14 linkage groups and 41 markers were unlinked. Total map length (calculated from the lengths of the overall linkage groups) was 1,286 cM of the maternal map and the average marker interval was 3.8 cM. All 14 linkage groups could be assigned to chromosome I–XII through the use of SSR markers. Both chromosome I and IV included two linkage groups. Twelve of the 14 linkage groups contained all four homologs, the other two (chromosome IV-1 and chromosome X) contained three remaining. The full maps (containing all chromosomes of the two parents) can be seen in supplementary 2 (Fig. S2 and S3: composite maps for the parents E108 and E20 of tetraploid potato population MT I).

Marker–trait associations and QTL analysis

In the one-way ANOVA analysis of SPSS 20.0, ten markers were significantly ($P < 0.01$) associated with the phenotypic data in 8 h photoperiod. Eight of them were also found in the single-point ANOVA analysis performed by TetraploidMap (Table 1). The other two markers were not included in the genetic maps because they were not consistent with the expected segregation ratios. All eight significant markers were simplex. Four of them combined into two coupling groups, located on homolog 2 of chromosome V of E108 and homolog 3 of chromosome IX of E20. Three of the remaining four significant markers were located on homologs 3 and 4 of chromosome V of E108 and homolog 2 of chromosome V of E20, respectively. The other one was unlinked. Of the eight significant markers that were associated with the phenotypic data, four markers were favorable for in vitro tuberization (including 2 markers located on chromosome V of E108, one marker located on chromosome V of E20 and one unlinked marker) and the other four were unfavorable for tuberization (2 located on chromosome V of E108 and 2 located on chromosome IX of E20). Three markers were significantly ($P < 0.01$) associated with the phenotypic data in 16 h photoperiod. One of them was located on homolog 4 of chromosome V of E108 and had positive effect on tuberization. Furthermore, this marker was also significantly ($P < 0.01$) associated with the phenotypic data in 8 h photoperiod.The other two were located on chromosome IX and XII of E20 (Table 1). The results shown in Table 1 can be summarized as follows: there were both positive and negative alleles affecting in vitro tuberization under each photoperiod and the same positive allele was detected both in 8 and 16 h photoperiod. The ten markers associated with the phenotypic data in 8 or 16 h photoperiod were located on chromosome V of E108 and chromosome V, IX and XII of E20.

Results from the single-point ANOVA analysis indicated the putative QTL for in vitro tuberization on chromosome V, IX or XII. This indication was confirmed by the interval mapping method. We utilized the IM routine of TetraploidMap for almost all linkage groups identified. Three QTLs were identified using the phenotypic data in the 8 h photoperiod, but none was detected in the 16 h photoperiod. So the QTLs mentioned hereinafter indicated the QTLs detected in the 8 h photoperiod. One QTL, MT05, was detected with the peak LOD at 88 cM on chromosome V of E108 between marker PCCMCGT-250 and EACGMCGT-630-1, the one-LOD support interval for which was 80–95 cM, and explained 16.23 % of the variation for % IVT (Fig. 2; Table 2). Two QTLs displaying simplex dominant effects were detected with the peak LOD at 110 cM on chromosome V (mt05, favorable for tuberization) and at 82 cM on chromosome IX (mt09, unfavorable for tuberization) of E20 which explained 5.33 and 4.59 % of the variation for % IVT, respectively (Fig. 2; Table 2). The LOD graph of QTL mt05 was flat and resulted in a wide one-LOD support interval (60–110 cM) for it. For mt09, with the peak LOD between marker EAACMCGT-850-2 and PACMCAT-100, the one-LOD support interval was 70–84 cM.

The effect of MT05 could not be explained by a simplex or duplex dominant model. Although alleles 3 and 4 in MT05 had positive effects on tuberization while alleles 1 and 2 had negative effects (Table 2), the difference between the effects of these four alleles were not significant suggesting that the effects of these alleles were additive. The mean % IVT associated with each gametic

Table 1 Significant markers ($p < 0.01$) for % IVT based on the single-point ANOVA analysis

Environment[a]	Marker	Parent	Chromosome	Position (cM)	Homolog	*p* value	Mean (0)[b]	Mean (1)	SE[c]
SD	STI0049-2	E108	V	94.90	4	0.0037	26.30	37.79	3.92
SD	STM5146	E108	V	73.20	2	0.0020	36.65	24.59	3.86
SD	PCCMCTG-520	E108	V	69.67	2	0.0013	36.88	24.33	3.85
SD	PCCMCGT-250	E108	V	89.07	3	0.0072	25.37	35.89	3.88
SD	PACMCAT-100	E20	IX	79.86	3	0.0007	37.09	23.84	3.85
SD	STI0057-2	E20	IX	75.29	3	0.0012	36.92	24.28	3.85
SD	PCCMCAC-67	E20	V	62.50	2	0.0038	25.25	36.52	3.86
SD	STI0005-3	E20	Unlinked	–	–	0.0014	24.53	36.99	3.85
LD	STI0049-2	E108	V	94.90	4	0.0053	2.15	5.13	1.06
LD	PACMCAC-980	E20	IX	78.67	4	0.0019	1.49	4.91	1.09
LD	PATMCGT-700	E20	XII	46.46	2	0.0028	5.00	1.68	1.10

[a] SD (short day) indicates 8 h photoperiod and LD (long day) indicates 16 h photoperiod

[b] Mean (0) and mean (1) refer to trait averages of individuals without (0) and with (1) the marker

[c] SE

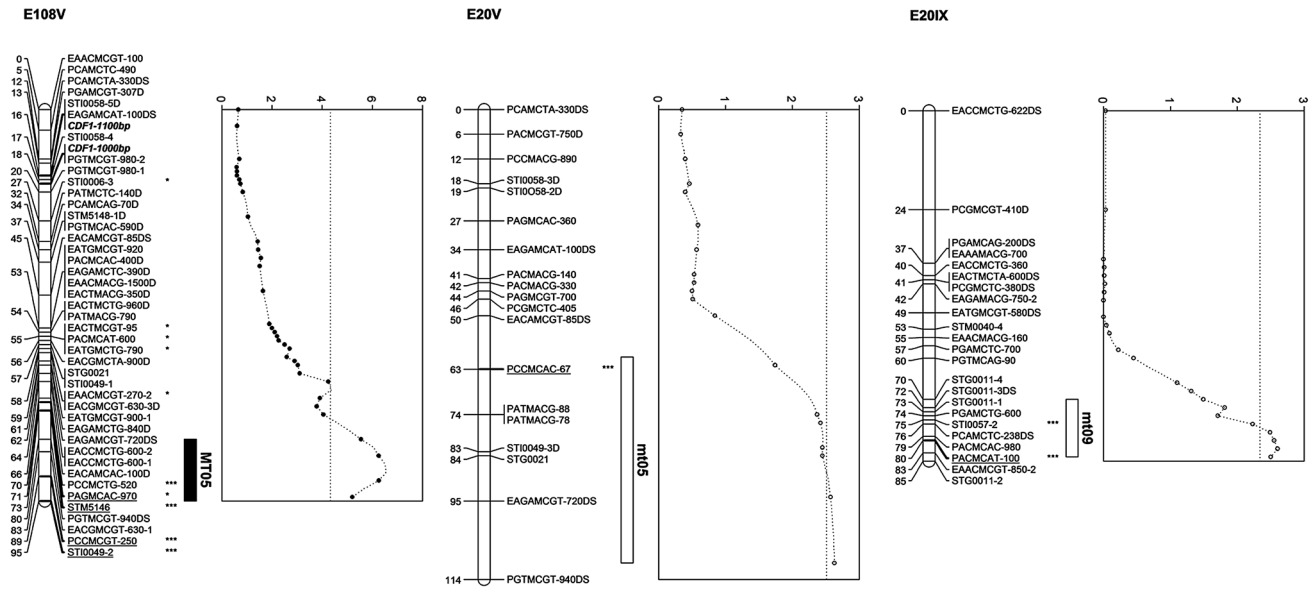

Fig. 2 Locations and LOD graphs of the QTLs detected by interval mapping. The denotation on the top of the linkage group indicates the parent, and *roman numerals* refer to actual chromosomes. The markers are on the *right* of the groups and the cumulative distance in cM on the *left*. SSRs begin with the designation STI, STM or STG. *Duplex* markers are indicated by marker names ending with *letter* "D". *Double-simplex* markers are indicated by marker names ending with *letter* "DS". The markers named CDF1 indicate the alleles of the candidate gene *StCDF1*. Beside the markers, "*asterisks*" shows the ANOVA significance at this locus (***$p < 0.01$; *$p < 0.05$). Locations of one QTL (*black bars*) detected on E108 and two QTLs (*white bars*) detected on E20 are charted by one-LOD support intervals. In QTL names beside the *bars*, "MT" means QTL for microtuber, majuscule indicates the QTL detected on E108 and *lowercase* indicates the QTL detected on E20. *Arabic numerals* indicate on which chromosome the QTL were detected. 95 % thresholds (*vertical dotted lines*) are determined by permutation tests on each QTL. Maps were drawn by MapChart 2.2 (Voorrips 2002)

genotype of MT05 is shown in Fig. 3. The genotype Q34 associated with the largest % IVT could be assigned as QQ, then qq = Q12 (the genotype associated with the smallest % IVT) and Qq = Q13 = Q14 = Q23 = Q24 (the genotypes associated with the trait values at the midpoint of the largest and smallest % IVT). In other words, the segregation ratio of the gametic genotypes Q− to qq is 5:1.

Four of the eight markers significantly ($P < 0.01$) associated with the phenotypic data in the 8 h photoperiod were located on the QTL threshold intervals, including STM5146 (located on homolog 2 of MT05), PCCMCGT-250 (located

Table 2 QTL detected for % IVT via interval mapping

QTL	Parent	Chromosome	Position (cM)	LOD	R^2	Model	QTL present mean (SE)	QTL absent mean (SE)
MT05[a]	E108	V	88	6.55	16.23	Additive effects[b]	H34 49.37 (4.30) QQ	H12 13.94 (4.31) qq
mt05	E20	V	110	2.63	5.33	Simplex dominant effects[c]	+H2 37.59 (2.60)	−H2 23.12 (2.82)
mt09	E20	IX	82	2.49	4.59	Simplex dominant effects	+H3 23.64 (2.84)	−H3 37.13 (2.60)

[a] QTL name "MT05" means the QTL detected on chromosome 5 for microtuber formation. Majuscule "MT" indicates the QTL detected on E108 and lowercase "mt" indicates the QTL detected on E20. The Arabic numerals indicate on which chromosome the QTLs were detected

[b] For QTL with additive effects, there are six means, one for each QTL genotype. We listed the highest mean (QQ) and the lowest mean (qq). H12 is the presence of homologous chromosome 1 and 2; H34 is the presence of homologous chromosome 3 and 4

[c] If a simpler model is not significantly different from the full model, we reported the simpler model where +H2 is the presence of homologous chromosome 2 (Q) and –H2 is the absence of homolog 2 (q)

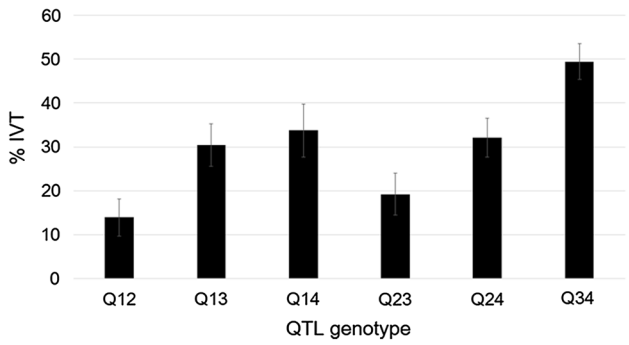

Fig. 3 Allelic effects of the major QTL MT05 for % IVT, detected on chromosome V of E108. *Error bars* represent the SE for each QTL genotype. Q refers to the putative QTL; 1–4 refer to the four homologs of each chromosome

on homolog 3 of MT05), STI0049-2 (located on homolog 4 of MT05) and PACMCAT-100 (located on homolog 3 of mt09) (Fig. 2). At the same time, two markers, PCCMCAC-67 (located on homolog 2 of mt05 and associated with the phenotypic data with a p value <0.01) and PAGMCAC-970 (located on homolog 1 of MT05 and associated with the phenotypic data with a p value <0.05) were included in the analysis of the cumulative effects of the alleles (6 markers with underline in Fig. 2). With an increasing number of positive alleles, the % IVT continuously increases as shown in Fig. 4. This result suggests that these alleles are all related to in vitro tuberization in this population.

Finally, we tested the segregation of three candidate genes in our population. There was no polymorphism in the candidate gene loci *StSP6A* and *StCO*. Only candidate gene *StCDF1* was mapped on the north arm of chromosome V of E108. It resulted two alleles which were located at 16 and 18 cM of chromosome V (Fig. 2). The location of *StCDF1* in our population was near the position reported in the potato genome sequence superscaffold and pseudomolecule information (between 4,316 and 4,320 kb of chromosome V), but out of the one-LOD support interval (80–95 cM) of MT05. These results indicate that the QTLs detected in our population are not caused by these candidate genes. In another word, the QTLs we identified in this tetraploid population might be new for tuberization.

Discussion

Tetraploid linkage maps

In recent years, significant progress has been made in developing the theory of linkage analysis and QTL mapping in autotetraploid species (Luo et al. 2001; Hackett et al. 2001, 2007) and several linkage maps for tetraploid potatoes have been published (Bradshaw et al. 2008; Khu et al. 2008; McCord et al. 2011; Groth et al. 2013). Based on the total map length, integrity of the linkage group and the member of the identified chromosomes, the linkage maps developed by Bradshaw et al. (2008) are the most comprehensive ones for tetraploid potatoes. They obtained the total length of 1,202 cM for the maternal map (12601ab1) and 1,234 cM for the paternal map (Stirling). Ten of the 13 maternal groups and 11 of the 12 paternal groups contained all four homologous chromosomes. Using SSR markers and the comigrating markers from the ultrahigh-density (UHD) linkage maps of diploid potato (van Os et al. 2006), they identified (in one or both parents) eight potato chromosomes, while the other four chromosomes remain unaligned. Khu et al. (2008) obtained longer distances for their tetraploid maps using a small population (92 individuals), 2,940 and 1,929 cM for the female and male parents, respectively. However, the lengths of these maps are based on the cumulative length of each homologous coupling linkage group, not the length of overall linkage groups. These longer distances could not indicate a larger coverage of the total genome.

Our estimates of 948 cM for paternal map (E108) and 1,286 cM for maternal map (E20) compare favorably with the estimates of McCord et al. (2011) which obtained

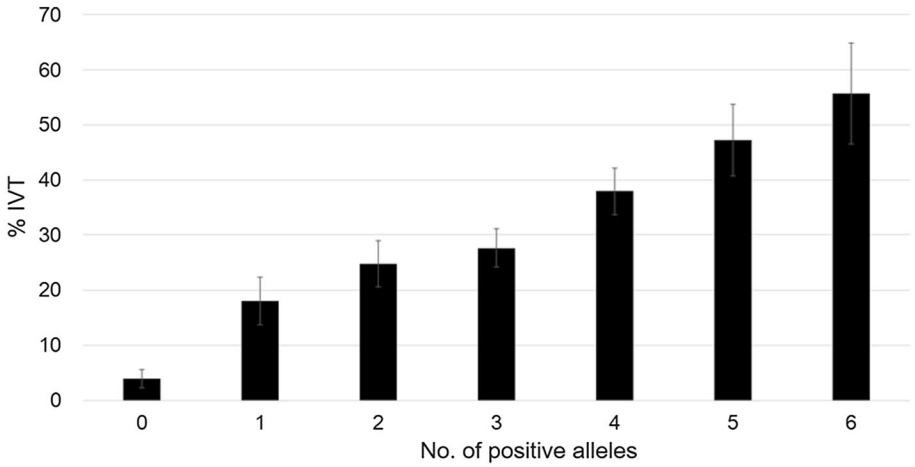

Fig. 4 Cumulative effects of the alleles affecting in vitro tuberization in the population MT I. The six alleles are represented by markers PAGMCAC-970, STM5146, PCCMCGT-250, STI0049-2 (the *four* markers indicated the four alleles *1*, *2*, *3* and *4* of MT05), PACMCAT-100 (indicating the allele *3* of mt09) and PCCMCAC-67 (indicating the allele *2* of mt05). The data for a given number of the positive alleles represent the mean values for all individuals that had the given number, regardless of which of the six possible alleles they were

940.2 cM for male parent B1829-5 and 1,034.4 cM for female parent Atlantic. However, they just tagged nine potato chromosomes using a total of 14 SSR markers and the integrity of the linkage groups in their maps were not satisfactory. Ten of 13 groups of four homologous chromosomes were identified for Atlantic and 5 of 14 groups of four were identified for B1829-5. We constructed the almost complete linkage maps for a tetraploid population using a larger population (237 individuals) and more markers (315 markers for paternal map and 341 for maternal map). All 12 linkage groups identified for E108 contained all four homologous chromosomes. Twelve linkage groups of four homologous chromosomes were established for E20 with two groups of three remaining. Using a total of 104 SSR markers, we tagged all 12 chromosomes of potato.

Reported loci for tuberization and MT05

A major QTL located on chromosome V (MT05) was identified in our tetraploid population affecting in vitro tuberization. The location of MT05 detected here is far away from the north arm of chromosome V, suggesting that it may be a new region for tuberization. The north arm of chromosome V is known to be associated with life cycle length of potato plants because the famous association between QTLs for maturity and for late blight resistance is located in this region (Collins et al. 1999; Visker et al. 2003; Bormann et al. 2004; Bradshaw et al. 2004; Simko et al. 2006; Śliwka et al. 2007) and the gene *StCDF1* is cloned from this region (Kloosterman et al. 2013). Danan et al. (2011) integrated QTL results for late blight resistance and for maturity from 19 QTL publications. Eight individual QTLs for maturity on chromosome V were clustered into one meta-QTL and 18 markers were summarized to associate with maturity and/or late blight resistance on chromosome V. The marker interval GP21–GP179 containing gene *StCDF1* (Kloosterman et al. 2013) was included in these 18 markers. We tested the segregation of gene *StCDF1* in our population. It resulted two alleles, which both were mapped on the north arm of chromosome V (16 and 18 cM), but all out of the one-LOD interval (80–95 cM) of MT05. At the same time, the SSR marker STI0006 included in the 18 markers and tightly linked to GP21 (Danan et al. 2011) was mapped on 27 cM of chromosome V near the alleles of gene *StCDF1* in our population. Bradshaw et al. (2008) identified a QTL for maturity on chromosome V which explained 56 % of the phenotypic variance in a tetraploid population. Although the closest marker STM3179 linked to the LOD peak of this QTL has not been used in our study, the SSR marker STM5148 which was 19 cM south of marker STM3179 (Bradshaw et al. 2008) was mapped on 37 cM of chromosome V in our population and was far away from MT05 (80–95 cM). Therefore, we believe that not only MT05 is not caused by gene *StCDF1*, but also it is different from the maturity QTLs reported previously. It may be a new region on chromosome V for maturity or tuberization.

Besides gene *StCDF1*, we also tested the segregation of candidate genes *StSP6A* and *StCO*, the potato homologs of *FLOWERING LOCUS T* (*FT*) and *CONSTANS* (*CO*). The *CO*/*FT* module is central to the day-length control of plant developmental progresses including floral induction in several species (Turck et al. 2008) and tuber formation in potato (González-Schain et al. 2012). Day length is sensed by leaves in which the signal is transported to the circadian

clock and circadian regulated genes; then a mobile signal ('florigen' or 'tuberigen') is synthesized and transported to the stems to induce flowering or tuberization (Rodríguez-Falcón et al. 2006). In *Arabidopsis thaliana*, the mobile *FT* protein acts as the florigen (Koornneef et al. 1991). *FT* expression is controlled by *CO* which acts downstream of a signaling cascade involving the clock gene *GIGANTEA* (*GI*) (Park et al. 1999; Suárez-López et al. 2001). On the basis of what has been found in *Arabidopsis*, potato homologs of *FT* (*StSP6A*) and *CO* (*StCO*) have been identified and demonstrated to be involved in day-length tuberization control (Navarro et al. 2011; González-Schain et al. 2012). However, the additional circadian regulated genes or genes contributing to the transport or response to the transmissible signal remain to be identified. There was no polymorphism in the candidate gene loci *StSP6A* and *StCO* in our population, indicating that the QTLs detected in the present study are not caused by these genes. In other words, these QTLs may contain new genes controlling tuberization.

Genetic model for tuberization

The population distribution of in vitro tuberization in the 8 h photoperiod suggests that a major QTL in the investigated clones controls this trait. Skewed phenotypic distributions caused by the segregation of a major gene or genes have been demonstrated in potato Corky ringspot disease (CRS) resistance (Khu et al. 2008), glycoalkaloid content in tubers (Sørensen et al. 2008), internal heat necrosis (IHN) of tubers (McCord et al. 2011), late blight resistance (Li et al. 2012) and wart race 1 resistance of tubers (Groth et al. 2013), and in other species, such as flower carotenoids of *Mimulus* (Bradshaw et al. 1995). MT05 detected in our population fits an additive model, which could be assigned as QQ, Qq and qq. The segregation ratio of the gametic genotypes ($Q-$: qq = 5:1) matched the ratio of the tuberized genotypes to the nontuberized genotypes in the population MT I. This observation suggests that the segregation of in vitro tuberization in the 8 h photoperiod in this researched population is controlled by a major-effect gene or genes. A few gene models controlling tuberization have been reported previously. Mendoza and Haynes (1977) evaluated the number of days to tuber initiation at 11, 13 and 15 h photoperiods in hybrids between cultivated potato clones and suggested that the initiation of tuberization was controlled by one dominant major gene with modifiers and that short critical photoperiod was dominant to long. Jansky et al. (2004) proposed a two-gene duplicate dominant epistasis model for tuberization (based on field evaluations) under long photoperiod in hybrids between cultivated and wild species. Also in hybrids between cultivated and wild species, a two-gene model with complementary gene action was suggested by Kittipadukal et al. (2012) based on evaluations of tuberization in the greenhouse.

The results of the present study confirmed the one major gene with modifiers model in hybrids between cultivated potatoes proposed by Mendoza and Haynes (1977). However, the variation for in vitro tuberization explained by MT05 was 16.23 %, which could not compare with other major QTL [for example, Li et al. (2012) detected a conditional QTL which explained 83.3 % of the variation for late blight resistance], but it demonstrated some of the complexities of tuberization and also confirmed the sensitivity of the tuberization response to a given environment. The parents E108 and E20 tested here came from the same family and had identical genetic background. This, to a large extent, minimized the obstructive influences of the observed differences in tuberization that may be caused by different genetic resources used, especially when the tetraploid parents are derived from different ancestors. Therefore, the genetic differences in MT05 between them (E108 and E20) would be the real origin that produces the different ability to tuberize and the phenotypic skew observed.

The effects of minor QTLs

The effect of mt09 unfavorable for tuberization, represented by the presence of marker PACMCAT-100 in each offspring, was dissected into the possible gametic genotypes H12 (containing alleles 1 and 2 in MT05, similar thereafter), H13, H14, H23, H24, and H34, based on the interval between marker PAGMCAC-970 and STI0049-2 in MT05. Offspring making up each gametic genotype were separated into groups with or without marker PACMCAT-100 (Fig. 5). The alleles 1 and 2 in MT05 performed the same effect of mt09 with negative effect on tuberization, but alleles 3 and 4 showed opposite function. As shown in Fig. 5, the % IVT means in all six genotypes were decreased by the presence of marker PACMCAT-100, especially in genotype H13 (the reduction was significant), which indicated that there was interaction (may be epistasis) between mt09 and allele 3 in MT05. For genotypes with the positive allele 4 in MT05 (H14, H24, and H34), the offspring tuberized well (% IVT > 20 %) even with the presence of marker PACMCAT-100, indicating that the positive effect of allele 4 in MT05 was not influenced significantly by mt09. Furthermore, the positive allele 4 (represented by the presence of marker STI0049-2) was detected not only in the 8 h photoperiod, but also in the 16 h photoperiod, confirming the stable effect of this allele again.

The same analysis was performed on mt05 with positive effect on tuberization, represented by the presence of marker PCCMCAC-67 (Fig. 6). With or without the presence of marker PCCMCAC-67, the tendency in the six

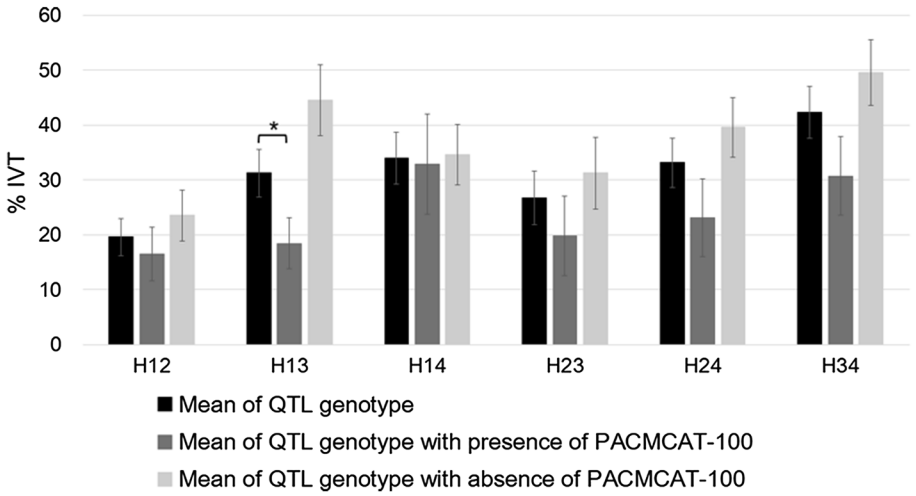

Fig. 5 The effect of the minor QTL mt09, represented by the presence of marker PACMCAT-100 in each offspring. Each QTL genotype for MT05 based on the interval between PAGMCAC-970 and STI0049-2. H12 refers to homologs 1 and 2, and similar thereafter

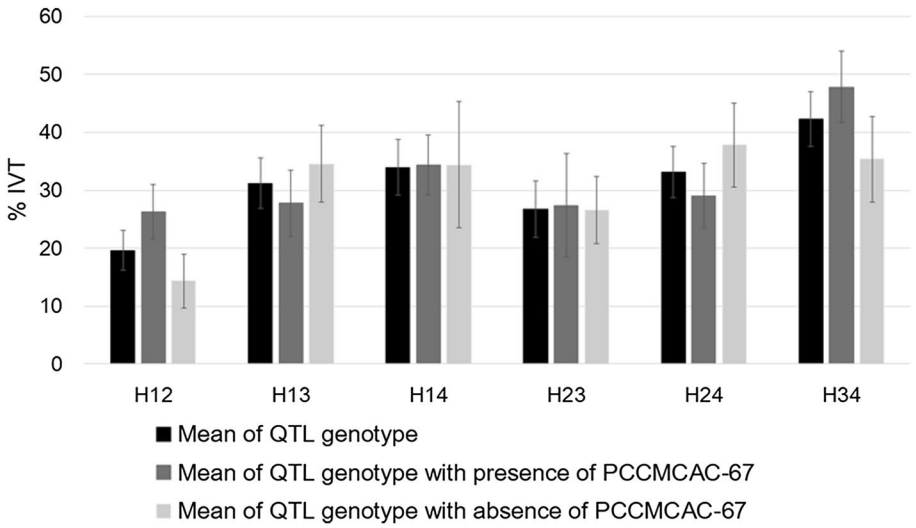

Fig. 6 The effect of the minor QTL mt05, represented by the presence of marker PCCMCAC-67 in each offspring. Each QTL genotype for MT05 based on the interval between PAGMCAC-970 and STI0049-2. H12 refers to homologs 1 and 2, and similar thereafter

genotypes was undefined which indicated that the effect of mt05 was not significant and could be ignored.

Although the present study could not elucidate the minor QTL effect as to whether it is additive, dominant or interactive, the major QTL effect with minor modifiers of in vitro tuberization were clearly confirmed in this population. Furthermore, we could conclude that the major QTL may control individuals of the progeny to tuberize or not, and the minor modifiers could influence individuals to tuberize well or poorly.

In vitro and in vivo tuberization

It has been known that all *S. tuberosum* species and varieties are capable of tuberizing under short days (Rodríguez-Falcón et al. 2006). In the present study, while the paternal parent of the tested population (MT I) E108 tuberized well in both 8 and 16 h photoperiod, the maternal parent E20 tuberized in neither photoperiod. A total of 37 offspring did not tuberize (% IVT = 0) and 120 offspring tuberized poorly (% IVT < 20 %) in the 8 h photoperiod. So, it is possible that in vitro tuberization does not totally reflect the in vivo ability of plants to tuberize, due to the inducing effects of high sucrose.

A lot of researchers (Alsadon et al. 1988; Alsadon 1989; Gopal 1996, 2001; Gopal and Minocha 1998) reported a close relationship between in vitro and in vivo systems and suggested that various tuber characters, such as tuber color, tuber shape, number of eyes, average tuber weight and so on, under the two conditions may be controlled by the same genetic system, which further confirmed the early conclusion (Henry et al. 1994) that there were no special 'tissue culture genes'. In recent years, in vitro tuberization system has been used to research the molecular mechanisms underlying potato tuberization (Bachem et al. 1996; Ronning et al. 2003; Hannapel 2010; Inui et al. 2010). Tuberization

in vitro has facilitated the understanding of the molecular basis for potato tuberization.

On the other hand, the poor correlation between in vitro and in vivo performance for tuber yield was found in different reports (Gopal and Minocha 1998; Naik et al. 1998). Gopal and Minocha (1998) investigated the correlation between in vitro and in vivo performance of 22 potato genotypes (*S. tuberosum*). They found that some genotypes did not microtuberize at all, whereas some gave very good microtuber yield under in vitro conditions. Under in vivo conditions all genotypes formed tubers and the differences in the performance of various genotypes were not so distinct as under in vitro conditions. Furthermore, both working in hybrids between cultivated and wild species, the tuberization data based on field evaluations were fit for a two-gene duplicate dominant epistasis model (Jansky et al. 2004), whereas the evaluations of tuberization in the greenhouse were fit for a two-gene model with complementary gene action (Kittipadukal et al. 2012). That indicates that a higher proportion of cultivated × wild species hybrids tuberize in the field than in the greenhouse. So the tuberization performance in the field was different from the performance in the greenhouse and more greatly different from the in vitro performance. The poor correlation between in vitro and in vivo tuberization is mainly due to differences in cultivation conditions under in vitro vs. in vivo. For example, the transition of photoperiod (both length and intensity) in nature undergoes a process of gradual change, which is difficult to be simulated in the laboratory. On the other hand, the carbon sources for plantlets growing under these two conditions are also different (light under in vivo and sucrose under in vitro). As a result, the differences in phenotypic expressions of genotypes were much enlarged under in vitro conditions compared to under in vivo conditions. That may be another reason why MT05 is different from the QTLs affecting tuberization in-field, and MT05 may lead us to a new understanding of the mechanism of tuberization.

The present study constructed a tetraploid potato population (F1) segregating on the in vitro tuberization, developed almost complete linkage maps based on it and identified a major QTL on chromosome V affecting in vitro tuberization. Furthermore, a major-effect gene with minor modifiers model was suggested to control the trait in this population, and the mapping results of the candidate genes indicated that the QTLs causal genes identified here might be new. Our work provides additional confirmation of previous researches which suggested that few-gene models are responsible for the tuberization process of cultivated potatoes, and we also provide the possibility that there are new genes involved in the tuberization process in tetraploid populations.

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Conflict of interest The authors declare that they have no conflict of interest.

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