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Construction of Integrated Genetic Map between Various Existing DNA Markers and Newly Developed P450-related PBA Markers in Diploid Potato (*Solanum tuberosum*)

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We have generated estimates of the genomic distribution of newly developed molecular markers, which are P450-based analogues (PBAs), in diploid potato (*Solanum tuberosum*, 2n = 2x = 24) based on functional genomics. A total of 401 markers, including 111 SSR, 33 RFLP, 87 RFLP-STS, 45 CAPS, 94 RAPD, 15 PBA, 9 AFLP, 3 RGL and 4 ISSR markers, were employed in a parental polymorphism survey. A total of 127 out of 401 markers (172 loci) displayed polymorphisms between parents and were confirmed to show segregation in partial progenies. These selected 127 markers were then tested for their possible use for a whole mapping population. The subsequent results of genetic mapping of the PBAs revealed that they were distributed on at least 8 chromosomes, suggesting that they have a significant potential not only as tools for assessing genetic diversity but also as effective markers to construct more detailed genetic maps of potato, in conjunction with existing identified genetic loci. To integrate the information from existing maps into our mapping study, we performed a comparative analysis between two representative maps (RFLP and SSR-based) with our PBA map. Based on the subsequent results, we predict that our map will be useful as a bridge between the existing genetic maps of potato and will enable to integrate information about different markers.

Key Words: potato genetic map, map integration, PBA markers, diverse marker systems.

Introduction

The construction of genetic maps enables to conduct comparative genomic studies between related genera in the Solanaceae family e.g., tomato-potato (Tanksley *et al.* 1992), tomato-pepper (Livingstone *et al.* 1999), tomato-eggplant (Doganlar *et al.* 2002). Such maps also enable to evaluate and utilize genetic resources of different crops for breeding programs, and indeed, marker-assisted selection (MAS) of useful genes has become a powerful tool in breeding programs of potato (Watanabe 1994). In potato, in addition to the first genetic linkage map constructed by Bonierbale *et al.* (1988), several genetic maps have been reported, based on different marker systems such as RFLPs (Gebhardt *et al.* 1989, Tanksley *et al.* 1992), AFLPs (van Eck *et al.* 1995, Meyer *et al.* 1998) and SSRs (Milbourne *et al.* 1998). A list of previous studies in which representative genetic maps of potato were generated is presented in Table 1. Because each genetic map was constructed by using different marker systems for different populations, integration of the information from various genetic maps of potato has not yet been conducted. Therefore, it is necessary to combine different DNA markers on the same genetic map.

In the present study, we attempted to construct a unified genetic map of potato, by employing a series of DNA markers for the same population. We have recently demonstrated that genetic analyses of cytochrome P450 gene-analogues are effective to compare functional genes among plant genomes (Yamanaka *et al.* 2003). Because P450 genes occur as a multi-gene family and their products are involved in various important physiological pathways, a small number of primer-sets against conservative domains of the P450 mono-oxygenase gene could be useful as universal tools for the assessment of the genome-wide diversity of P450-related functional genes in different plant species. We designated these PCR-based DNA markers as PBAs (P450-based analogues) that are considered as functional genetic markers, compared with neutral genetic markers, such as RFLPs, RAPDs, AFLPs and SSRs (Yamanaka *et al.* 2003). Thus, we also attempted to incorporate the PBA markers into our new genetic map of potato. In the case of functional gene-related markers, RGLs (Resistance gene-like fragments, Leister *et*

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Table 1. Representative potato genetic maps reported previously

Population	Mainly used marker (s)	Coverage[1] (cM)	No. of loci mapped	Res. Grp. (based information)	Reference
S. phureja×(*S. tuberosum* ×*S. chacoense*)	RFLP	606	134	Cornell	Bonierbale *et al.* 1988
S. tuberosum	RFLP	690	141	Max-Planck	Gebhardt *et al.* 1989
S. tuberosum	RFLP	1034	304	Max-Planck, Cornell	Gebhardt *et al.* 1991
S. tuberosum ×*S. berthaultii*	RFLP	684	260	Cornell	Tanksley *et al.* 1992
S. tuberosum	RFLP, Isozyme, transposon, morphology	1120	193	Wageningen	Jacobs *et al.* 1995
S. tuberosum	AFLP	1170	264	Wageningen	van Eck *et al.* 1995
S. tuberosum	RGL	706 (m) 733 (p)	82 101	Max-Planck	Leister *et al.* 1996
S. tuberosum	SSR	879	89	Max-Planck	Milbourne *et al.* 1998
S. tuberosum	AFLP	991 (m) 485 (p)	231 106	Scottish Crop Res. Inst., Max-Planck	Mayer *et al.* 1998
S. tuberosum	CAPS	718	147	Max-Planck	Chen *et al.* 2001

[1] (m) and (p) denote maps based on maternally and paternally derived genomes, respectively.

al. 1996) have also been reported, and experiments designed to refine and utilize RGLs for practical analyses of the potato genome have been conducted (Hämäläinen *et al.* 1998, Kasai *et al.* 2000, Watanabe *et al.* 2003).

To estimate the genomic distribution of PBA markers in diploid potato, the genetic mapping of PBAs with known genetic markers was carried out in the present study for their potential not only as tools for the assessment of genetic diversity but also as new genetic markers in mapping analysis of the potato genome.

Materials and Methods

Plant material and DNA extraction procedures

A diploid potato (*Solanum tuberosum*, $2n = 2x = 24$) population consisting of 152 F_1 individual plants, derived from a cross between 86.61.26 and 84.194.30 (Watanabe *et al.* 1994), was used as the mapping population. Total DNA was extracted from each individual plant specimen according to the method of Fulton *et al.* (1995).

DNA markers

To compare information from previously reported genetic maps of potato using different marker systems, various marker types were employed in the present study. A total of 401 molecular markers, including 111 SSRs (Provan *et al.* 1996, Milbourne *et al.* 1998, Ghislain *et al.* 2003, Solanaceae Genomics Network (SGN) HP, http://www.sgn.cornell.edu/index.html), 33 RFLPs (Tanksley *et al.* 1992), 87 STSs (constructed in this study), 45 CAPS (Chen *et al.* 2001), 94 RAPDs (Williams *et al.* 1990), nine AFLPs (Vos *et al.* 1995), 15 PBAs (P450-based analogues, Yamanaka *et al.* 2003), three RGLs (resistant gene-like fragments, Leister *et al.* 1996, Hämäläinen *et al.* 1998, Watanabe *et al.* 2003) and four ISSRs (Prevost and Wilkinson 1999), were used for a parental polymorphism survey (Table 2).

STS primer sets were designed from RFLP probe sequence information (SGN database, http://www.sgn.cornell.edu/index.html) using Primer3 software (http://www-genome.wi.mit.edu/genome_software/other/primer3.html). Each primer was tested in a PCR reaction prior to undertaking the mapping study. The primers that amplified relevant fragments are listed in Table 3. A total of 127 out of 401 markers showed polymorphisms between the parental lines (Table 2) and also a segregation among several sample F_1 progenies. This set of markers was therefore selected for subsequent population mapping. The segregation analysis procedures for each marker basically followed the original reports already cited herein, except for the procedures which are outlined below.

RFLP analysis

A parental polymorphism survey was conducted using

Table 2. Number of polymorphic loci detected using different marker systems

Marker	No. of tested markers (A)	No. of polymorphic markers[1]	No. of polymorphic loci (B)	Frequency (B/A)
SSR	111	37[2]	49[2]	0.44
RFLP	33	12	12	0.36
RFLP-STS	87	15	15	0.17
CAPS	45	3	3	0.07
RAPD	94	33	42	0.47
AFLP	9	9	16	1.50
PBA	15	15	27	1.80
RGL	3	1	2	0.67
ISSR	4	2	6	1.50
Total	401	127	172	

[1] Markers showing polymorphism between parental lines

[2] Including multi-locus SSR markers (Milbourne *et al.* 1998, Ghislain *et al.* 2003)

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Table 3. List of the RFLP-STS markers generated and tested in the present study

RFLP locus[1)]	Chr. No.	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Ann. temp.	Fragments amplified	Polymorphism observed
TG301	1	GCAATTGAAAAGTGTCGAATGG	GCCAGGAAAACGAATTAATAGGG	55		
TG59	1	GGTAGTTTCCCACCATTTTACCC	GTCATAGTGTGAGTAGATTCC	55	○	
TG161	1	CAGCTCTTTACTGGTGATGG	CCACCTTAGAATTACTACATTCGG	55		
TG158	2	GTGCCCATGAAGAAGTATTACCC	AACCTAAGCAACCCGAAACC	60	○	
TG14	2	GGCCAACTGATTGCCTATCC	ACAAGTCGTTTAAGCTTAGACTACC	60		
TG34	2	GCGCAGACAAAAGATGAAGG	CAGATCGACTAGCAACATGAGG	60		
TG48	2	GTTCTTGCCGGTGGCAGATTGG	CCGCCAAAGGTGAAAGCAGAAGG	65		
TG74	3	CACTGAAGTGCTTCAACTATCC	GGCTTGCAAGTGTATTACATCC	55	○	
TG242	3	AGTAATCGTTGAGGATGTAGG	CTCGTTGAAAGATGTGATGG	55	○	○
(STS 6)	(11)					
TG65	4	GGACTTGAACCATTGGATCTGG	CGTCAGCAATGTAGGAAGAAGG	60		
TG22	4	GACCACCATTAGCAGCCTTCTCC	CCGGTCATCGATGTAGCATGTCC	60	○	○
(STS 9)	(3)					
TG441	5	AGTGGACTATCCGAAAACATGG	CCTATGGCGACCTTATAACGG	60	○	
TG318	5	GGGTAGAATGAAGGATGATTTCCC	GACAGAGTACGTGTACATATGTCC	60		
TG69	5	GCAATGAGGTCCATCATAGCC	AACCCTTGAGTCAACAACTCC	60	○	
TG118	6	TCCGGAACCAACTAGTTTGGG	CACAGTACGAGCGACACAAGG	60	○	
TG482	6	GTCAAAGTCATTCTGACTTCC	GTATAAGTCTCGTTGAAAGCC	55		
TG438	7	GAGGCCCTTTAACAAATGTCC	CTACACAAATTTACCTCGTTGTACC	60		
TG176	8	GAAGATACAAGAGTGTCAATGG	AGTCTCGGGTTATAGATTGG	55		
TG35	9	CCTGCAAGTGTCAACTTAAAAGCC	CTTGAAAGGTCCCTCGTACAACC	60		
TG52	10	CTTTAACCCGTCAGATCTGG	CTGTACTGGAAAGGTGATTGG	55		
TG63	10	GAGCACTCTAGGTAAATCGG	CTGAAGACAGGAAGTGATGG	55	○	○
(STS 10)	(1)					
TG403	10	GTTCCCTTATGCAGCTTTGG	ATGTATGGCCGACTTCTAGG	60	○	
TG47	11	TGTTGGTTACTCGTAATCCC	GAACGTTCGTTTGTTTCACC	55		
TG26	11	GCCAAAAGCTTTCTAACTGG	CATCCTGAAGCTTTTCTAAGG	55	○	
TG360	12	TAAGATAGCCCCAGAACACC	GGTGGTAATACCTGTTTTCCC	55	○	
TG68	12	CGTAAGGGTTGTGATGTAGG	CGACGTGACTTGAAGAAAGG	55	○	
TG296	12	CGGTTGGTTTGATTTGTAGG	GTACCTCAGGTCAAAACTGG	55		
TG24	1	GATTGTTGAAACGATGTAAGGG	TTCACTCCTCTTAGTTTGTGG	55		
TG70	1	TCTTGATGCTTGGTTATATGGG	TGGAGAAAAGAGGGAGTAGG	55		
TG129	3	GGCATTTCAAACTTAGAGTTGG	GAAGTCGGATTTAGAGAAGAGG	55		
TG652	4	CCTTAACTGAAGAACACAACC	GAGACGTAGGATGTAAACCC	55	○	○
CT194	4	GTTCCAATGAAGCAATCTGG	CACTGGCTTGACTATAAGGG	55	○	
TG62	4	GCATGAGGAAAGGTCATGTAGG	GGTTGACGAAGAGGAACAGG	55	○	
TG163	4	GTTTCCAGTCCTTGATCTCC	CTGAAGACTCGGTTAAAGTCC	55	○	
TG351	5	ATCACACTGGGTATAACTAGG	AAACCGGAACTTTAAACTGG	55		
TG23	5	CGAGGTATATACCATCCTTCTCC	GTAGACTATGGACCAGTACACC	55		
TG499	7	AACTGAGTGGAGATCTCAAGG	TGACTAACTTTTGCACTTACGG	55	○	
TG218	7	CAGTTGTATTCCAGAAATGCC	CTCTCGATTCCATCGTAACC	55	○	○
(STS 2)	(5)					
TG572	7	TGCTGAGAATGATAGAGG	CGTAGTCTTGATTCATGG	50	○	
TG128	7	TCAGAAAGAGTATCCTCATCC	ATGGATTCAATTCCAACACC	50	○	○
TG61	9	AGTCATCTGATACACACTAGAAAGG	AATTCAGTCGAGGGACAAGG	60		
TG9	9	GTACATGGTCTAATAGCC	TCTAGAGTACTCATGTCC	50	○	
TG18	9	CATCTAACTTGCAGCTATCC	CTAGTCGGTAATTTCTTGACC	55	○	○
TG254	9	CTTAGGCTTGGTGGATAGAGG	GACAGGTTTACCATTTCTTACGG	55	○	○
TG230	10	ATCGCTCGAACCTTATTGATGG	TGATGTACTGTGGTTCTAAACGG	55		
TG303	10	CTATGCATGCTAGATATTGCC	AAGAAAGGGAGAGACATACC	55	○	
TG508	11	GGGGTATATTATGTTCTCAAACTCC	GTGACCCCTATAGACATACTACC	55		
TG57	11	GGTGCTTCCTGAAGATGG	GAAGGTGGTAACGACTAAGG	55	○	
TG497	11	GGAGAGTGAAGATGCATTGG	CAGTCAAACGTAGAGACAAGG	55	○	
TG46	11	GAAGGTTGGAACATGACAAAGG	ACAAGCTGGAAGTGTTAGGG	55	○	
TG36	11	AGTGCTCTGCAACTAATGGG	TTTAGAGGACTTTACCTTCTAACGG	55	○	
CT233	1	GGCTTACGACGAAAAAGCTG	TTTGTTACATCCGGTGCTTG	55	○	
TG67	1	TGCACCACAAGAGTCAAAGC	GGGGTAATGCATCGTGATCT	55	○	○

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Table 3. (continued)

RFLP locus[1]	Chr. No.	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Ann. temp.	Fragments amplified	Polymorphism observed
TG245	1	TGGAGTGGACTGAGCAACAG	GCAAGACCAAGAGCCTGAAC	55	○	
TG273	1	TTGAAGATTGCAGCGAACAC	TTTGCTGGGGAACTGGTATC	55	○	
TG460	1	CGGCATAGTGTCTTTTGATCC	CCCAACAGACAAAGGGAAGA	55	○	
TG465	1	TCTCCCGGCTCTAAATCTGA	TATGCCCAAGCTTTGAATCC	55	○	
TG151	2	GGGGTAAGGTCTGTGTGCAT	CGCCTCTTGGTTTGAATCTC	55	○	
TG165	2	ATGCTCCTAAGCCGTGAAAA	CATCCAAAAAGCCTCCAAAA	55	○	
TG308	2	GGGTTGGAGGAGACAAAAGG	GGTGGTTTGAGCTGCTTGAT	55	○	
TG492	2	TGGCAAACTTACAAGGGACA	TAATTGCAGCCATTTGTGGA	55		
TG114	3	ATAGCTGACCGCGACTTGTT	CAACCCTCCGACGAGAAATA	55	○	○
TG246	3	TGAGGCAATGAGTTCCTATCTG	ATCCGAAAAGCAACAAGTGG	55	○	○
(STS 11)	(1)					
TG388	3	CCACTCCTGACAACTGCAAA	TGAGGAAGAGGGTAGCAATCA	55	○	
TG525	3	AGTTCACCTCCCAGCATCAC	CCTCCTGGGCTTAGAAAACC	55	○	
TG272	4	AAGCCGTGCACTGAAGAGTT	TCAGCTGAAGAACAGCGAGA	55	○	
TG483	4	CCCCATCTCCACAAGCTAAA	TTATGCCTCCGTGGTACTCC	55		
TG500	4	TGTTCACCAACTTGGGTGTG	GGGGTAGAATGATGGCAGGT	55	○	
TG574	4	TGTTTTTGCAGAATCAACACG	CTACTTGACCCAGCATGCAC	55	○	
TG279	6	CGAGATTTGCAGGAACCATT	GGCACTCAACACATAAAACCA	55	○	
TG356	6	CCGCAAAATTAAGACACCAAA	AAAGCTTGCCTTGTCTGCAT	55	○	
TG365	6	CGTTAGCTCGGAGGAGAAAT	TTCCTATGCAAGCCTTTTCC	55	○	○
(STS 12)	(3)					
TG590	6	AGCACGCCTTAACATGAACC	TTGTTCAACGTCAATTGAAAGG	55		
TG174	7	AATTCCAAGGCCAACAAGG	TAAGGAATGCTGGCCACCTC	55	○	
TG183	7	GCCCCTGTGTGGATTCTAGT	TGAGGGGATAGGAACTTCCAG	55	○	
TG342	7	TTTGGTTGATCTCAGCTTGAC	TCGTTGCCGGCTAAAGTTAT	55	○	
TG302	8	GGACTCTCCCGCGAATTTAT	TGGGACTCCTCCTTTTCTTTC	55	○	○
TG349	8	GGGAATTGGGGTTCTTGACT	CCATTTACCAAGGCATTACCA	55	○	
TG505	8	TCAATCTGCCATTCCCTTCT	TATGTTCATGTGGCATTTGG	55	○	
TG291	9	CACAAGTAAGGGGAGCGTCT	GTTGCCCATTAGCATTCACA	55	○	
TG551	9	CCATATGCTGCTCATCTCACA	CCATAAATGCATGTGATCTTTGA	55	○	
CT182	11	TACATCACCGGTCACCTTCA	GGAGCTGCTAGTGCCAATTC	55	○	
TG147	11	TGTTTGGTCCAAGATTCGTTC	AACTCAAAATAAGATCACGATCCA	55	○	
TG384	11	TGTGCTGGGTGTTGGTAAAA	CAAGGGCAGAAACCTGGTAA	55	○	○
TG111	12	TTCTACCCTGATCCTCGACCT	ACAATTCTTTGTCCGGGTTG	55	○	○
(STS 8)	(11)					
TG180	12	CATGCATTCTAATCGGGTGA	GGCTCTGCAAGTCGAAAATAA	55	○	
TG565	12	TTGGGGTCGCTAAAAATGAA	AGGTTTGGTTTCTGGGGTTT	55		

[1] Markers that were mapped to a different chromosome from the originally determined RFLP locus are indicated by a new marker name, (STS ##).

2 μg of extracted DNA, digested with each of the five restriction enzymes, *Dra* I, *Eco*RI, *Hin*dIII, *Msp*I and *Xba*I. Probes indicating polymorphisms between parental lines were used for subsequent segregation analysis. Southern blotting was conducted according to standard procedures and hybridization and detection of these blots were performed using the AlkPhos Direct Labeling and Detection System (Amersham), according to the manufacturer's instructions.

STS analysis

PCR amplification of the STS markers constructed in this study (Table 3) was carried out in a total reaction solution of 25 μl containing 1 × *Ex Taq* buffer (TaKaRa), 200 μM of dNTPs, 1 μM of each forward and reverse primer, 0.5 U of *Ex Taq* polymerase (TaKaRa) and ca. 20 ng of template DNA. A total reaction of 35 cycles was programmed for 30 sec at 94°C, 30 sec at each annealing temperature (Table 3) and 1 min at 72°C. PCR products were then electrophoresed in 1.5% agarose gels.

Linkage analysis and map construction

For the preliminary linkage analysis, the data set was split into subsets corresponding to parental meiosis, and these two subsets of data were employed for linkage analysis based on F_1 pseudo-testcross strategy (Grattapaglia and Sederoff 1994) using MapMaker 3.0 with the backcross option at a LOD score of 3.0. Then, the integration of both maternally and paternally based maps was carried out using JoinMap 3.0 (van Ooijen and Voorrips 2001), with the parameter set for the progeny derived from cross-pollinaton (CP).

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The linkage groups were separated using a LOD score of 4.0. Comparative analysis was then conducted between our map and two existing representative maps, an RFLP-based map from a Cornell University group (Tanksley *et al.* 1992) and an SSR-based map from a Max-Planck Institute group (Milbourne *et al.* 1998).

Results and Discussion

Efficiency of detection of polymorphic loci in different marker systems

In the present study, we attempted to test various marker systems in a single potato population. The efficiency of detection of polymorphic loci for each marker is summarized in Table 2 and ranged from 0.07 (CAPS) to 1.80 (PBA). In total, 127 markers detected polymorphisms successfully at 172 loci between both parental lines. The considerably low detection frequency of CAPS seemed to be due to the lack of availability of the original PCR primers and also to the reported restriction enzymes. Indeed, it is sometimes difficult to apply originally published genomic mapping information to other populations with different genetic backgrounds, because both the reproducibility of the PCR and applicability of restriction enzymes can be problematic, depending on the materials used. In contrast to CAPS, the PBA markers seemed to have a distinct advantage over the other marker types and showed a much higher efficiency in the polymorphism survey. Additionally, in previous studies of genetic diversity between plant species, a small number of primer sets (combinations of three forward and five reverse primers) could detect large numbers of polymorphic fragments, both within and between plant species (Yamanaka *et al.* 2003). Furthermore, in the present study, it was found that all the primer sets used could detect polymorphisms between parental lines, indicating that the PBA markers are effective for the detection of polymorphisms in a mapping population (Table 2). It was also advantageous for the subsequent mapping procedures that our PBA markers would reduce laborious preliminary marker selection, such as parental polymorphism survey.

Refinement of existing markers

We attempted to convert existing RFLP markers into new PCR markers (RFLP-STS), and a list of 87 such markers that we constructed is shown in Table 3. Out of 87 primer-sets of these PCR markers, 61 successfully amplified fragments in preliminary PCR testing, prior to initiation of the polymorphism survey. Polymorphisms between parental lines were then observed when 15 out of the 61 primer-sets were used, and these primer-sets were then applied to construct a genetic map (Table 3). All the RFLP-STS polymorphisms observed were based on the presence or absence of the amplified fragments. These RFLP-STS markers are useful tools to conduct mapping studies because they do not involve laborious and complex RFLP analyses and there is no requirement for a large quantity of DNA sample . In addition, it was revealed that the original RFLP markers could be applied for other Solanaceae species as well as potato (Tanksley *et al.* 1992, Livingstone *et al.* 1999, Doganlar *et al.* 2002). It was suggested that our RFLP-STS markers could also be used for various Solanaceae species such as eggplant, tomato, pepper, etc.

Map construction and distribution of PBA markers in the potato genome

For preliminary linkage analysis, two tentative maps related to both parental polymorphisms were constructed. As for the maternally (86.61.26) based map, 14 linkage groups and 14 unlinked loci were found. Out of 14 linkage groups, 12 corresponded to each chromosome. In the remaining two groups, one was assigned to a part of chromosome XII, and another was an unknown linkage group because of the absence of known marker. As for the paternally (84.194.30) based map, 12 linkage groups were found and assigned to each chromosome, with 16 unlinked loci. Then the integration of both maternal and paternal maps was conducted. The integrated map is shown in Figure 1. All the 12 linkage groups were assigned to each corresponding chromosome, with a coverage of 673 cM and an average marker distance of 8.2 cM. A total of 82 loci were mapped within this population, including 13 PBA, 4 ISSR, 27 RAPD, 22 SSR, 7 RFLP and 9 RFLP-STS loci. Segregation distortion of the mapped loci was estimated by chi-square test to the hypothesis of the absence of difference of segregation ratios from 1 : 1 and 1 : 2 : 1. The segregation ratios of 35 loci showed a distortion ($P < 0.05$), including five PBA, three ISSR, sixteen RAPD, five SSR, one RFLP and five RFLP-STS loci. Seven STS loci derived from RFLP markers were

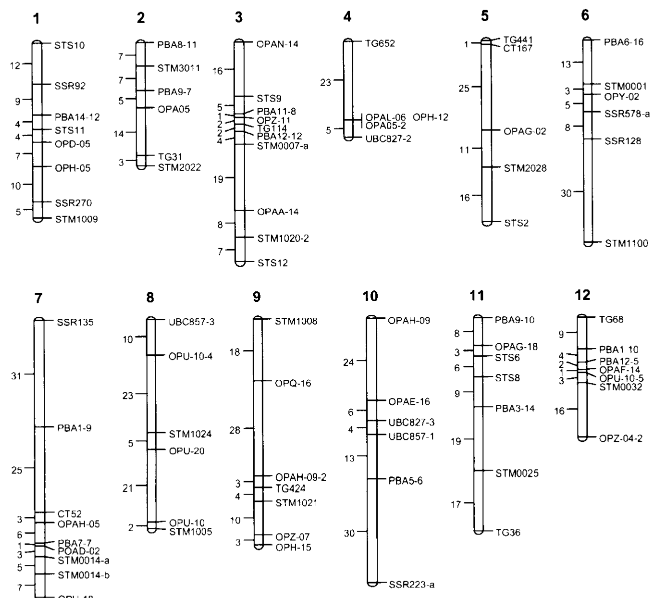

Fig. 1. Genetic map constructed in the present study and distribution of PBA loci in the diploid potato population (86.61.26. × 84.194.30.).

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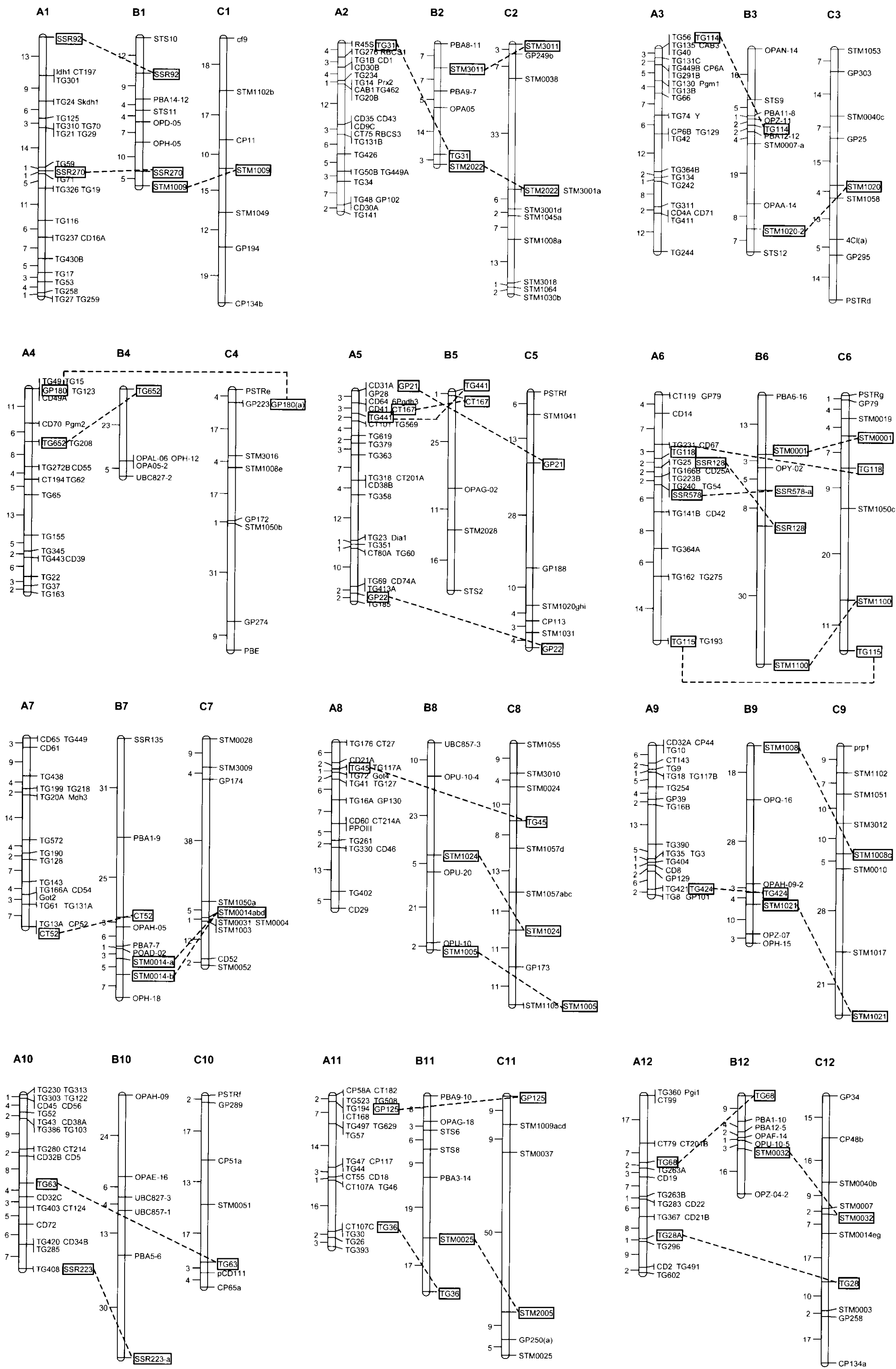

Fig. 2. Integration of information from two representative maps (Tanksley *et al.* 1992 and Milbourne *et al.* 1998) and the map constructed in the present study. Corresponding loci between maps (indicated by open boxes) are connected by broken lines. A: RFLP-based map generated by the Cornell University group (Tanksley *et al.* 1992). B: Map constructed in the present study. C: SSR-based map generated by a group in a Max-Planck laboratory (Milbourne *et al.* 1998).

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mapped on unexpected chromosomes (designated as "STS ##" in Fig. 1 and Table 3). In the case of PBAs, 13/27 polymorphic loci between the parental lines were distributed on at least eight chromosomes (chromosomes I, II, III, VI, VII, X, XI and XII), indicating that these markers could detect polymorphisms widely in the potato genome by using only a small number of primer sets. It was suggested that PBAs could be used as genetic markers in mapping studies, and as tools for the assessment of genetic diversity previously reported (Yamanaka *et al.* 2003).

Since RAPDs are efficient molecular markers for the construction of genetic maps, they had been used in many previous studies. However, it was reported that RAPDs tended to be located in specific regions of the genome (Monna *et al.* 1994). Also, in our study, the distribution of the RAPD loci was concentrated in specific regions of the potato genome (Fig. 1). Both RAPDs and PBAs are convenient for use, even in the absence of relevant genetic information about the plant materials. However, when taking into account the characteristics of RAPD, such as the requirement for dozens of primers to identify an adequate number of polymorphisms and their biased distribution in the genome, we considered that PBAs, with only 15 primer-pairs in combination of eight primers, had a distinct advantage over RAPDs as effective genetic markers for mapping studies.

Integration of map information based on different marker systems

To enable us to successfully integrate information from existing maps into our own map, we first demonstrated that an effective bridging comparison could be made between our map and two representative maps, an RFLP-based map constructed by Tanksley *et al.* (1992) and an SSR-based map developed by Milbourne *et al.* (1998) (Fig. 2). For each of the chromosomes, some associations of genetic markers were obtained among the three maps with commonly used markers, and it was therefore possible to estimate roughly the positions of the markers in different genetic maps. Based on these results, it was considered that our map showed a high probability of acting as a bridge between different existing maps, and would therefore enable successful integration of information from different maps.

The first potato genetic linkage map constructed by Bonierbale *et al.* (1988) was based on RFLP analysis using both cDNA and genomic clones from tomato as probes. This group of Cornell University used a population derived from an inter-specific cross *S. phureja* × (*S. tuberosum* × *S. chacoence*) (2n = 2x = 24). A second potato RFLP map was then independently constructed by a group in a laboratory from the Max-Planck Institute using an intra-specific backcross population derived from diploid potato breeding lines (Gebhardt *et al.* 1989). These two groups have been in the forefront of the development of genetic maps of potato, and most of the maps that were subsequently constructed were based on the seminal work of these two groups (see Table 1).

One of the representative maps generated by Tanksley *et al.* (1992) was a high-density RFLP-based map. This map covered 684 cM of the potato genome using 260 RFLP markers, and it still remains one of the most informative maps with the highest density. Recent technical developments, however, have enabled us to utilize PCR-based molecular markers for mapping studies such as SSRs (simple sequence repeats), which are one of the established PCR-based marker systems with distinct advantages such as the requirement for only simple PCR procedures, high levels of polymorphism, co-dominance and affordability, among others. Taking these benefits into account, 112 primer-sets of SSR markers were developed and an SSR map has been constructed by a group from the Max-Planck Institute (Milbourne *et al.* 1998). Additional SSR markers for the potato genome have also been developed (Ghislain *et al.* 2003). Although the information from these marker studies including, inter alia, informative high density RFLPs and convenient SSRs, is indeed helpful for other potato mapping studies, it is difficult to immediately use the information from these representative maps, because of the differences in the populations used and in the mapping systems. Thus, integration of both informative and convenient maps enables us to evaluate and utilize genetic resources more efficiently for breeding programs, such as marker-assisted selection (MAS) of useful genes.

Based on the background of potato mapping studies, we present a framework of our study to bridge the previous independent genetic map information gleaned from different marker systems, and we set about to combine this analysis with the information previously reported in our mapping experiments. Although this work is still of a preliminary stage, we predict that the concept of integration of map information will become much more important for procedures such as MAS, in breeding programs, comparative genomics, etc. in the near future.

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