Available online at www.sciencedirect.com
ScienceDirect
Agricultural Sciences in China
2011, 10(8): 1177-1187
August 2011

Population Improvement of Resistance to Late Blight in Tetraploid Potato: A Case Study in Combination with AFLP Marker Assisted Background Selection

YAO Chun-guang[1*], SONG Bo-tao[1*], LIU Jun[1], WU Cheng-jin[2], CHENG Qun[2], LI Da-chun[2] and XIE Cong-hua[1]

[1] *Key Laboratory of Horticultural Plant Biology, Ministry of Education/National Center for Vegetable Improvement (Central China)/Potato Engineering and Technology Research Center of Hubei Province/Huazhong Agricultural University, Wuhan 430070, P.R.China*
[2] *Southern Potato Research Center of China/ Enshi Academy of Agricultural Sciences, Enshi 445000, P.R.China*

Abstract

Cultivated potato with high level of horizontal resistance against late blight is one of the most important goals of potato breeding. The recurrent selection has been adopted to increase the level of potato horizontal resistance and a B3C1 population without *R1-R11* dominant genes has been released by the International Potato Center at the short-day condition of Peru. The present research was carried out to further improve the resistance and the agronomic traits of B3C1 population under long-day condition of Hubei, China, with maximized retention of its genetic diversity. Twenty-seven individual clones of B3C1 were used to generate population B3C2 by in-population crossing with the bulk pollens aiming to elevate the frequency of late blight resistance genotypes and to improve the adaptation to local long-day conditions. The late blight resistance and the main agronomic traits including the maturity, the plant characters and the tuber traits were evaluated for the foreground selection in three years, by which 130 pedigrees were maintained as the basic population of B3C2 for further selection. A total of 312 polymorphic loci detected by 9 AFLP marker combinations were used to monitor the genetic diversity of the populations for the background selection. The B3C2 population of 51 clones was finally selected, of which the frequency of resistant genotypes increased by 23.8% points and the genetic diversity was maintained by about 96% as referred to B3C1. Our results strongly suggested that combination of the foreground selection for target traits and the background selection for the genetic diversity is an efficient strategy in the recurrent selection of tetraploid potato to improve quantitative traits.

Key words: potato, recurrent selection, late blight, horizontal resistance, genetic diversity

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world, but its production is severely threatened by late blight disease caused by *Phytophthora infestans* (Mont.) De Bary (Garelik 2002; Cooke and Lees 2004). Dominant *R* genes, known as *R1-R11* from *S. demissum*, have been introgressed into most modern cultivars, but their race-specific feature makes the resistance unstable because of a rapid evolution of the pathogen (Goodwin *et al*. 1995; Fry and Goodwin 1997). Some new *R* genes thought to be broad-spectrum resistant to *P. infestans* were identified from other sources in recent years such as *RB*/$R_{pi\text{-}blb1}$ from *S. bulbocastanum* (van der Vossen *et al*. 2003; Song *et al*. 2003) and $R_{pi\text{-}ber}$ gene from *S. berthaultii* (Rauscher *et al*. 2006), but the functions of these *R*

Received 3 October, 2010 Accepted 14 December, 2010
Correspondence XIE Cong-hua, Professor, Tel: +86-27-87280969, Fax: +86-27-87286939, E-mail: xiech@mail.hzau.edu.cn
* These authors contributed equally to this study.

doi:10.1016/S1671-2927(11)60108-9

genes in a variety remain ambiguous.

Incorporation of the horizontal resistance governed by multigenes has been expected to create durable resistant varieties of potato to address the challenge of late blight (Collins *et al*. 1999; Oberhagemann *et al*. 1999; Ewing *et al*. 2000; Gebhardt and Valkonen 2001; Jansky and Peloquin 2006). The horizontal resistance has been proved by the quantitative trait loci (QTL) analysis showing the QTLs with various contributions distribute on almost each chromosome (Leonards-Schiper *et al*. 1994; Marczewski *et al*. 2001, 2010; Simko 2002). Low level of the horizontal resistance in a parental material becomes a bottleneck for its use in a breeding program. Particularly, when the horizontal resistance is blurred by the vertical resistance controlled by dominant genes, it is very difficult to distinguish between them and it is almost impossible for evaluation of the horizontal resistance in the offspring. A strategy employed in the International Potato Center (CIP) is to eliminate the known genes (*R1*-*R11*) by which the recurrent selected population B and its succession B3C1 with better adaptation have been developed (Landeo *et al*. 1995, 2000). The population B3C1 unified the local *Andigena* germplasm, ancient *R*-gene-free *tuberosum* varieties, *R*-gene-free clones from population A, as well as the varieties evolved from some wild species of South America and Mexico (Landeo *et al*. 2000; Pérez *et al*. 2000). This fundamental work at CIP has thrown a light on and also built up a base for potato breeding on durable resistance to late blight through its worldwide materials transfer, although further selections are necessary to improve both the resistance level and the adaptability of the population to derive better parental materials and to wipe off unwanted characters of the materials such as extremely long maturity, too many branches and stems, too long (or even barely) stolon tightly to the tubers, too many small tubers, etc.

The recurrent selection is generally adopted to increase the frequency of a desire genotype in a population by phenotypic screening (known as the foreground selection) and it has been successfully used in potato late blight resistance and other traits (Bradshaw *et al*. 2009). Taking the case of population B3C1, it brings not only the horizontal resistance to late blight but also a wider genetic background and some other potential valuable traits (Landeo *et al*. 2000). A maximum retention of its genetic diversity (the background selection) along with the improvement of the resistance is prospectively valuable to compensate for the narrowness of the genetic background of modern varieties. Molecular genetics are aware of useful in the improvement of agricultural populations (Dekkers and Hospital 2002), however, little information is available for the background selection of a population through molecular approaches in tetraploid potato.

Here we report the construction of a recurrent selection population based on partial of population B3C1 aiming at to further elevate the horizontal resistance to late blight by the phenotypic selection and to maximize the retention of the genetic diversity *via* the AFLP marker assisted selection. The method in combination of foreground and background selections is firstly developed and proved efficient in current research.

MATERIALS AND METHODS

Parental materials

The parental materials used for the recurrent selection were from population B3C1, which was a recurrently selected population created in CIP with its resistance to late blight built upon the elimination of *R1*-*R11* genes (Landeo *et al*. 2000) (Table 1).

Cross design

All the parental plants were grown in the plastic greenhouse in the research station of Southern Potato Research Center of China (SPRCC), Enshi, Hubei (30°20′N, 109°39′E, 1 150 m asl) in 2004. Use of the bulk pollens, a mixture of the pollens collected from the paternal plants, is a strategy to maximally retain the genetic diversity of the parents in offspring population and this method has been used in the recurrent selection of potato populations (Landeo *et al*. 2000). Approximately equal amount of pollens from 27 B3C1 clones were mixed to produce the bulk pollen and pollinated to the flowers of maternal plants which were emasculated at bud stage. There were 17 B3C1 clones successfully crossed with the bulk pollen and, in total, 4 650 seeds were produced and used in further selec-

Table 1 27 clones of B3C1 used in present research as the original population[1)]

Parental material	Resistance to late blight	Resistance type[2)]
391002.6	Resistant	Horizontal
391004.18	Resistant	Horizontal
391047.34	Resistant	Horizontal
391058.175	Resistant	Horizontal
391065.69	Resistant	Horizontal
391065.81	Resistant	Horizontal
391585.167	Resistant	Horizontal
391585.179	Resistant	Horizontal
392617.54	Resistant	Horizontal
392633.54	Resistant	Horizontal
392634.52	Resistant	Horizontal
392637.10	Resistant	Horizontal
392639.34	Resistant	Horizontal
392657.171	Resistant	Horizontal
393075.54	Resistant	Horizontal
393077.159	Resistant	Horizontal
393077.54	Resistant	Horizontal
393079.24	Resistant	Horizontal
393079.4	Resistant	Horizontal
393084.31	Resistant	Horizontal
393227.66	Resistant	Horizontal
393242.50	Resistant	Horizontal
393280.57	Resistant	Horizontal
393280.64	Resistant	Horizontal
393284.39	Resistant	Horizontal
393349.68	Resistant	Horizontal
393371.159	Resistant	Horizontal

[1)] The information for B3C1 clones was cited from the CIP's website (http://www.cipotato.org).

[2)] Horizontal, late blight resistance is controlled by multigenes.

tions to form the offspring population B3C2.

Field arrangement for the recurrent selection

Obtained seeds were sowed in the greenhouse of the research station in Huazhong Agricultural University (HAU), Wuhan, Hubei (30°28′N, 114°21′E, 32 m asl) in spring 2005 to produce tubers, which were then used for the field selections in HAU and SPRCC in the following seasons, autumn 2005 in HAU, spring 2006 in HAU and spring 2007 in SPRCC. Single hill for each offspring clones in autumn 2005, 5 hills in spring 2006 and 10 hills with 2 replications in a randomized complete block design in spring 2007 were adopted to evaluate the agronomic traits and the resistance level to late blight for each selected clone. The 5 hill-plots were hand planted with 33 cm within and 60 cm between rows. A late blight susceptible variety Mira was employed as the control and the late blight introducer and planted at two ends of each block and every 5 rows of the tested clones.

Assessment of agronomic traits and resistance to late blight

The agricultural traits were evaluated for judging the adaptation to the local long-day conditions. The maturity was measured as the days from emergence to maturity for each clone in the field. The plant height and diameter of the main stems were measured at the flowering stage for all plants of each clone tested. At the same time, the plant types of each clone were recorded as rosette, prostrate or erect. At harvest time, the length of stolon, number of tubers and tuber weight were taken from all survival plants and the data were presented using a unit of per plant for comparison.

The resistance to foliage blight of all clones was assessed using the detached leaflet assay following the method of Vleeshouwers *et al*. (1999). Five leaflets per clone were collected from the middle part of the plants at the flowering stage, and they were then inoculated with 15 μL of the zoospore suspension of *P. infestans* at a concentration of 5×10^4-10^5 zoospores mL^{-1}. The pathogen isolates were collected from the field grown potato plants in Enshi of Hubei Province and they were considered highly aggressive and tested as a mixture of races 1, 3, 4, 1.3 and probably race 7 (Wang *et al*. 2005). The disease development was monitored from the 2nd to the 7th day after the inoculation by estimation of the percent infection area. The disease score was recorded for each clone as 1 with no incidence of the disease, or just a small necrosis to 9 as almost 100% of the leaflets covered by necrotic lesions, following a 1-9 scale system (Estrada-Ramos *et al*. 1983). The resistance level of each clone was assigned according to the disease scores as very resistant (VR): 1-2.5, resistant (R): 2.6-4.0, moderate (M): 4.1-5.9, susceptible (S): 6.0-7.4, and very susceptible (VS): 7.5-9.0. The disease assessment was carried out independently in three spring seasons of 2006 in HAU, 2007 in SPRCC and 2008 in HAU.

DNA extraction and AFLP analysis

The AFLP analysis was carried out to detect the genetic diversity among the parental materials and the offspring population so as to achieve the marker assisted selection for the genetic background.

The genomic DNA of the field selected clones was extracted from young leaves with the cetyltrimethyl ammonium bromide (CTAB) method (Doyle 1991). The DNA samples were then assessed both by spectrophotometry and gel electrophoresis using DNA standard DL2000 (TaKaRa, Dalian).

The AFLP analysis was conducted using the method of Vos *et al*. (1995) with a little modification. The restricted enzymes used in this research were *Eco*R I and *Mse* I (New England Biolabs, Ltd., UK). Nine primer combinations with great polymorphic expression in a preliminary test were selected (Table 2). The visualization of the bands was carried out in a Thermo EC-160 DNA Sequencing System (Thermo Fisher Scientific, USA) and a silver staining procedure without fixation and pretreatment (An *et al*. 2009).

Table 2 The number of polymorphism bands and percent polymorphism detected by 9 AFLP primer combinations among the populations B3C1and B3C2

Primer combination	No. of bands detected	No. of average bands in one lane	No./% of diversity bands
EAAA+MCAG	31	10.55	23/74.2
EAAC+MCAC	45	23.84	2862.2
EAAC+MCAG	66	39.28	40/60.6
EAAC+MCTG	67	23.92	57/85.1
EATG+MCTA	70	35.50	40/57.1
EATG+MCTC	32	10.19	32/100
EACA+MCCT	57	33.91	40/70.2
EACA+MCGT	44	24.86	26/59.1
EAGA+MCAT	49	24.14	26/53.1
Total	461		312/66.8

Data analysis

Details of the band on the scanned photograph were collected and identified using the Quantity One System (ver. 4.62, Bio-Rad, USA). Bands clearly visible in at least one lane were scored (1 for present, 0 for absent) and entered into a data matrix. All the data were synthesized from two independent AFLP analyses.

The field selected clones of B3C2 were clustered based on the AFLP marker "0/1" data matrix through the DPS software (ver. 9.50) using the "0/1" data systemic clustering program. The Czekanowaki index and UPGMA (unweighted pair-group method arithmetic averages) method were selected before running the program. Then QGA Station software (Chen and Zhu 2003) was used to find the core samples from the basic samples of B3C2 using the "core germplasm" program. Before the final samples were assigned, modifications were carried out based on the clustering map and the performance of the late blight resistance and the agronomic traits so as to maximize the genetic diversity with increasing the genotype frequency of the acceptable resistance and better agronomic traits.

Estimations of the molecular marker variance (*Vm*), the Shannon index of genetic diversity (*Ho*) in the parental materials and the offspring population were carried out based on the marker data matrix. Here, the molecular marker variance (*Vm*) was calculated as the following formula (Smith *et al*. 1997): $Vm=npq/(n-1)$, where n means the total number of individuals in the population, and p and q mean the frequencies of presence and absence of an AFLP fragment in the population, respectively.

The genetic diversity indexes were estimated by using the formulas described by Dong *et al*. (2001):

$Ho=-1/nXi\ln Xi$, where, Xi means the frequency of band i in one population, and n means the number of bands detected.

RESULTS

Assessment of late blight resistance

In the seedling generation, a total of 4 650 B3C2 seeds were sowed in the greenhouse of the research station in HAU and 1 221 B3C2 clones were obtained without selection. In the following generations, these clones were subjected to the adaptation and late blight resistance assessments by growing them in single hill in autumn 2005 in HAU, 5-hill in spring 2006 in HAU and 20-hill in spring 2007 in SPRCC and in spring 2008 in

HAU. The clones which didn't survived due either to disease infection or extremely weak in vigor were naturally eliminated. The size of B3C2 population was reduced gradually also due to the selection for agronomic traits (see next section).

The assessments of late blight resistance were carried out for all parental materials and the field grown B3C2 clones from 2006 to 2008. The distribution of resistance levels in each population is shown in Table 3. Variations among years were found mainly from the changes in very resistant and resistant proportions, which may resulted from various inoculation conditions during the detached-leaflet test. For example, isolates of *P. infestans* prepared from the field infected leaves in 2007 may be more aggressive than those from the storage used in 2006 and 2008. The derived population, B3C2 showed no general trend among years in either proportion of the resistance level or the average late blight score simply because the change in population size approached its final under a successive integrated consideration in both adaptation and the disease resistance.

Table 3 Distribution of late blight resistance levels in B3C1 and in B3C2 populations after the selection based on the disease score from 2006 to 2008 (percentage of each resistance level, %)

Resistance level	Frequency of individuals (%)					
	B3C1			B3C2		
	2006	2007	2008	2006	2007	2008
Very resistant	14.81	7.41	11.11	19.13	12.10	6.29
Resistant	11.11	14.81	3.70	20.94	14.52	11.19
Moderate	18.52	33.33	33.33	18.05	25.00	18.18
Susceptible	29.63	40.74	25.93	13.36	27.42	20.98
Very susceptible	25.93	3.70	25.93	28.52	20.97	43.36
Average late blight score[1)]	5.9	5.3	5.9	5.3	7.1	6.4
Population size[2)]	27	27	27	277	273	130

[1)] The late blight score was assigned to each individual according the 9-scale system with 1, the most resistant, and 9, the most susceptible.

[2)] The numbers of individuals grown in the field for assessments of agronomic traits and late blight resistance.

The late blight scores obtained in three years for 27 B3C1 clones and 130 B3C2 clones (selected in 2007) were averaged for each clone of the two populations and the results are summarized in Fig. 1. It is obvious that the frequency of resistance level showed a normal distribution (chi-square =4.36, *df*=2) in the populations investigated with susceptible the highest in B3C1 while moderate the highest in B3C2 (P_W), suggesting a basic improvement in late blight resistance of B3C2 (P_W). It can be seen further by comparing the late blight scores of the populations which demonstrated that the disease score in B3C2 (P_W, 5.2) was lower than that of B3C1 (5.7). If a clone harboring moderate resistance or above was taken as an acceptable genotype for further use, the frequency of the acceptable clones for each population was accounted and the results indicated that B3C2 had a higher frequency (71.54%) than B3C1 (48.15%). This increase in the B3C2 (P_W) was mainly resulted from the increment of the moderate level in comparison with B3C1 (Fig. 1). The final selection should be implemented for B3C2 by consideration of the adaptation of the offspring and the broadness of their genetic diversity.

Agronomic traits of population B3C2

The main traits associated with adaptation of potato to local long-day conditions are normally considered as maturity, plant growth, number of stems and number of tubers per plant, length of stolons and tuber yield. Since B3C1 is originally from Peru, a short-day region, as well as its complex genetic background (Landeo *et al*. 1995, 2000), maturity, stolon length and the growth vigor should be superior over others for future use of these materials. The main traits of the parental materials and the field evaluated offspring clones in 2008 are shown in Table 4. Comparing to B3C1, significant reduction in the days from emergence to maturity and length of stolons and increase in plant height, stem diameter and number of tuber per plant were observed in B3C2. Although tuber yield was not improved obviously in B3C2, weak offsprings and those with unacceptable plant types (such as rosette) were significantly decreased in comparison to B3C1 (data not shown).

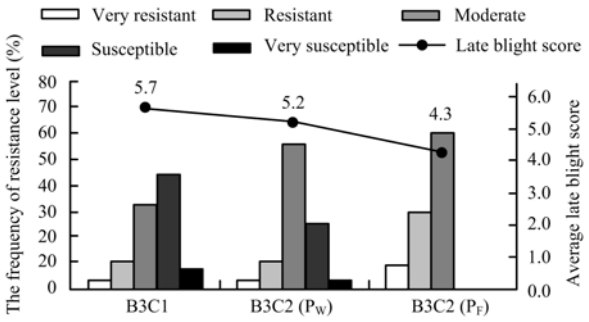

Fig. 1 Distribution of late blight resistance levels and late blight scores in B3C1 and B3C2 based on the means of late blight resistance tested from 2006 to 2008. B3C2 (P_W) represents the population B3C2 based on the 130 field selected clones while the B3C2 (P_F) indicates the final population B3C2 comprising of 51 core clones (see next section).

Table 4 The main agronomic traits of the populations B3C1 and B3C2

Agronomic trait	B3C1	B3C2 (P_W) (field selected)
Days from emergence to maturity (d)[1)]	100.7±8.61 A	92.7±9.45 B
Height of plant (cm)[2)]	49.3±9.6 B	54.0±10.5 A
Diameter of main stem (cm)[2)]	0.84±0.16 B	1.00±0.15 A
Number of stems per plant[2)]	6.7±2.9 A	6.5±2.1 A
Average length of stolon (cm)[2)]	24.9±12.1 A	10.3±4.4 B
Number of tubers per plant[2)]	10.8±4.5 B	16.5±8.1 A
Yield (kg/plant)[1)]	0.35±0.27 A	0.35±0.20 A

[1)] The data are the means±SD of 2007 and 2008.

[2)] The data obtained in 2008.

Different capital letters show significant difference at P=0.01.

AFLP marker assisted selection for maximum retention of genetic diversity

A total of 461 AFLP bands were detected by 9 primer combinations, of which 312 bands were polymorphic, i.e., 66.8% markers showed diversity among the parental materials and the offspring clones (Table 2).

130 field selected B3C2 clones grown in 2008, clustered by DPS software with a systemic cluster program based on the AFLP "0\1" date matrix, and the systemic trees are shown in Fig. 2. The dissimilitude coefficients (Dc) among 130 clones of B3C2 were from 0.21 to 0.41, and those B3C2 clones were clustered into 34 groups (stratums) at Dc=0.33.

In order to select the core members of B3C2 which could maximally maintain the genetic diversity of the parental materials, the "core germplasm" program of the soft package QGA Station (ver. 1) was used, which would select one genotype from each stratum and maximize the allele retention (Ghislain *et al*. 1999, 2006) to form the core samples, namely P_A. Considering the performance against late blight, those susceptible clones selected by the program were replaced by resistant ones in the same stratum according to the cluster analysis to form the core samples of P_R. Furthermore, those clones not selected but with good performance against late blight or with better agronomic traits were also chosen from other stratum to complement the final population, assigned as core samples of P_F. Finally, a total of 51 clones were selected for B3C2 population (Table 5).

As shown in Table 5, after the field evaluations for the growth performance in successive crops (Table 5, P_W), the remained 130 clones of B3C2 had *Vm* value of 80.585 which was 97.5% of that detected in B3C1. No significant allele loss occurred in the field selection (3.47% for B3C2) which was also reflected by the Shannon genetic index (0.258 for B3C2 which were 98.9% of that for B3C1). The results indicated that more than 95% of genetic diversity of the parental materials was retained in the field selected population with the corresponding size. However, the resistance to late blight was not obviously improved as indicated by the average disease score (5.7 for B3C1 and 5.2 for B3C2 of P_W).

The selection of most genetic diverse samples based on AFLP markers alone can effectively reduce the sample size down to less than 30 clones (Table 5, P_A). Change in sample size did not affect the value of *Vm* very much (from 79.676 to 80.019 when size varied between 26 and 52 clones in B3C2). It was also true for the effects of the size on the *Ho* value. However, more allele loss was accompanied with reduction of the sample size, and the largest loss was 5.86% for B3C2 when the smallest size was chosen as the core samples by the software. Since the core sample selection was solely based on the polymorphism indicated by the AFLP markers without considering the late blight resistance and agronomic traits, the population size is needed to be adjusted in combination with a foreground selection for late blight resistance and adaptability and a background selection for maximum retention of the genetic diversity.

By replacing late blight susceptible clones with resistant ones from the same stratum of the systematic tree (Table 5, P_R), comparing to the same population in

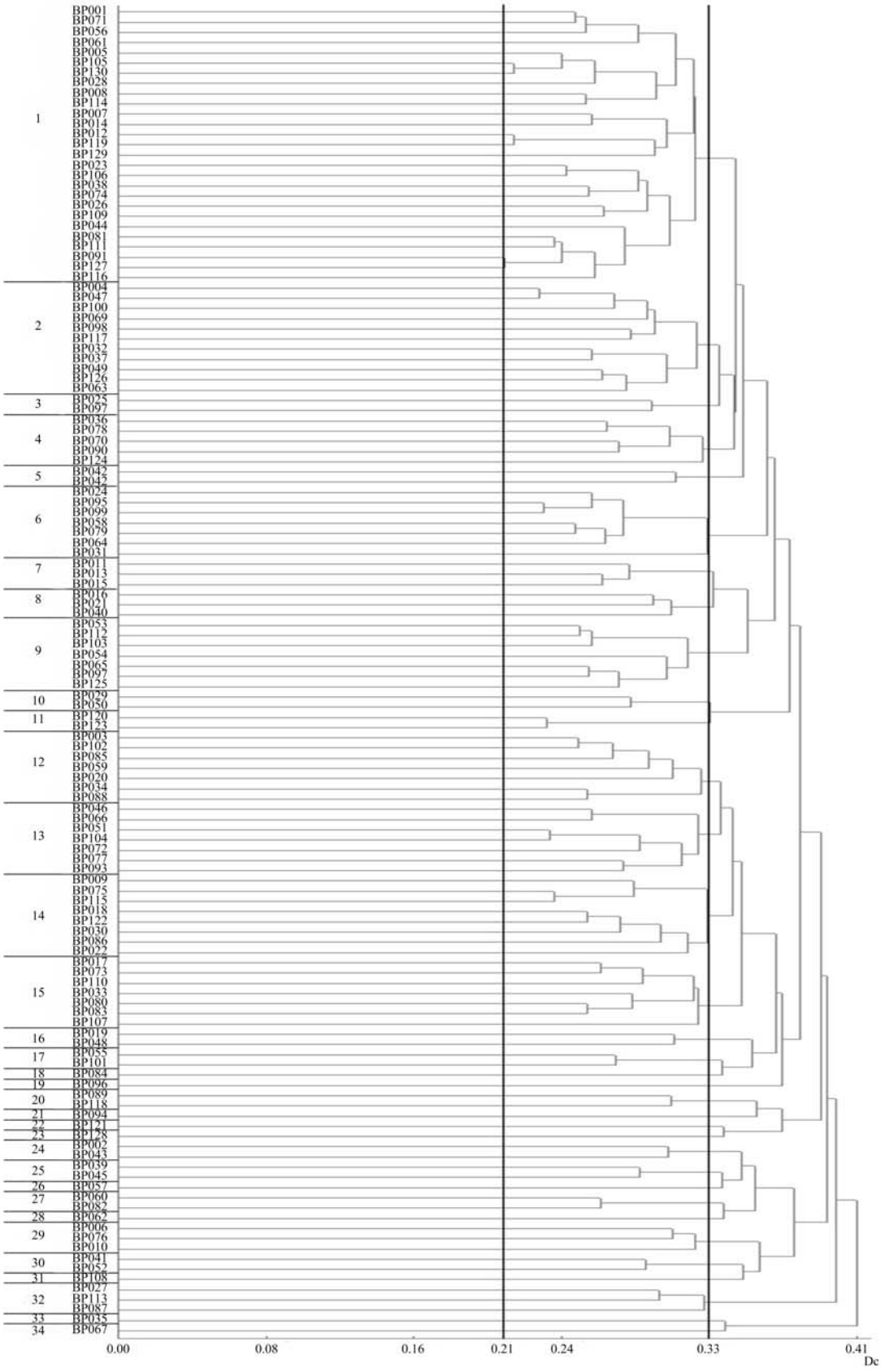

Fig. 2 Systemic trees of 130 field selected B3C2 clones based on the AFLP "0/1" data matrix with the DPS software using a systemic clustering program.

Table 5 Comparison of core sample selections using AFLP markers alone and in combination with late blight disease scores

	Sample size	Molecular marker variance (*Vm*)	Shannon index (*Ho*)	Late blight score	Allele loss (%)
Parental materials					
B3C1	27	82.699	0.261±0.098	5.7±1.57	0
Field selection with general growth performance (P_W)					
B3C2	130	80.585	0.258±0.107	5.2±1.3	3.47
Selection by AFLP markers from P_W for maximum retention of genetic diversity with minimum population size (P_A)					
B3C2	26	79.778	0.249±0.117	4.8±1.6	5.86
	32	79.676	0.251±0.115	4.9±1.5	5.64
	39	79.772	0.251±0.113	5.0±1.4	5.42
	45	80.019	0.252±0.112	5.0±1.3	4.99
	52	79.759	0.253±0.112	5.1±1.3	4.77
AFLP marker assisted selection by replacing late blight susceptible clones with resistant ones from the same stratum of the systematic tree (P_R)					
B3C2	26	79.217	0.248±0.116	4.1±1.2	5.21
	32	79.125	0.250±0.115	4.4±1.2	4.77
	38	79.730	0.253±0.113	4.5±1.1	4.34
	44	79.903	0.253±0.112	4.5±1.1	4.12
	51	79.935	0.254±0.110	4.5±1.1	4.12
AFLP marker assisted selection by adding late blight resistant clones and those with better genetic polymorphism to P_R from non-selected clones to increase the frequency of target genotypes (P_F)					
B3C2	35	80.079	0.251±0.113	3.9±1.2	4.77
	41	79.838	0.252±0.113	4.1±1.2	4.77
	47	79.629	0.253±0.113	4.2±1.2	4.34
	51[1)]	80.347	0.255±0.110	4.3±1.2	4.12
	57	80.355	0.254±0.110	4.2±1.3	4.12

[1)] The final size of the population.

P_A of Table 5, the late blight score in P_R was reduced remarkably from 4.8-5.1 to 4.1-4.5 in B3C2.

To compensate for the loss of genetic diversity in specific size group of a population, some clones, which were not chosen as core samples in P_R but with high polymorphism and late blight resistance, were selected for consideration of the final components of B3C2. As indicated in P_F of Table 5, B3C2 with 51 individuals were obtained. This population maintained about 96% genetic diversity of B3C1 and the average disease score was 1.4 scales lower than that of B3C1.

Considering the distribution of late blight resistance levels in the final B3C2 population, there were no susceptible and highly susceptible clones (Fig. 1), i.e., all the individuals in B3C2 had an acceptable resistance to late blight. The frequency of resistant and very resistant genotypes in this population was 39.2%, which was 23.8 points higher than that of B3C1 indicating a very significant improvement of late blight resistance in B3C2.

DISCUSSION

The recurrent selection has long been applied to plant breeding for improvement of quantitative traits (Haullauer 1985).

Not like the recurrent selection for other crops that there is no segregation of F_1 seeds and each target genotype can be selected from corresponding crosses, the recurrent selection for the tetrasomic inherited potato has been made with the bulk pollen pollination to ensure random crosses within the original population (Sanford and Ladd 1979; Gautney and Haynes 1983; Landeo 1995). Using similar method, 27 clones of the B3C1 population which was developed at CIP with horizontal resistance to late blight from diverse genetic resources (Landeo 1995) were used in present research to promote the resistance and to improve the adaptability of B3C1 to the local long-day condition. Our results showed a remarkable improvement of these two target traits (Tables 3, 4 and Fig. 1). The late blight resistance of B3C2 indicated by the frequency of resistant and very resistant genotypes was up by at least 23% points compared to B3C1, and accordingly, the average disease scores were declined from 5.7 in B3C1 to 4.3 in B3C2. It is noticeable that the late blight resistance level of the B3C1 clones selected were all assigned to 'resistant' at CIP (Table 1) but they showed diverse variation in present research, which may because of different pathogen races or distinct environ-

ments between the two regions.

The agronomic traits, especially maturity, stolon length and number of tubers formed per plant which are most concerned as adaptability when a potato variety introduced from short-day to long-day condition, were also improved with different levels in B3C2 (Table 4) although more cycles of recurrent selection may be needed for improvement of yield.

The final sizes of B3C2 were decided not only on the foreground selection for late blight resistance and adaptability, but also on the background selection for their genetic diversity. It is almost impossible to quantify the genetic diversity of a population by phenotypic assessments until the molecular markers capturing most genetic variations have been involved. The background selection was applied to the backcross breeding for the retained chromosomal segments around the target gene (Young and Tanksley 1989) and the genetic background of the backcross parent (Frisch *et al*. 1999). We first report here AFLP markers have been successfully used to the selection of the core samples of offspring populations with maximum retention of parental genetic diversity, and a procedure of molecular maker assisted selection is established. After three years of the foreground assessment (test for late blight resistance and evaluation for agronomic traits), B3C2 with reasonable size were subjected to the AFLP analysis with 9 primer pairs to detect a total of 312 alleles of the parental materials. By clustering individual clones according to their AFLP genotypes, the core samples were then chosen from each AFLP genotype stratum to ensure a maximum retention of the genetic diversity. In combination with the resistance level, the clones for offspring population were decided. While the target traits were improved, over 95% of parental genetic diversity were retained in B3C2, implying a great potential of these populations as a source of high horizontal resistance to late blight with a broad genetic background. Our results demonstrate that the strategy of foreground selection for the target trait in combination with background selection for the genetic diversity is applicable to potato recurrent selection. In addition to AFLP markers, those showing polymorphism in a population could be also useful in detecting the genetic diversity.

CONCLUSION

Focusing on high level horizontal resistance against late blight and better adaptability to local long-day conditions, we generated a potato populations B3C2 based on the B3C1 released by CIP. In combination of the foreground selection for agronomic traits and late blight resistance and the background selection for genetic diversity assissted with ALFP markers, the offspring population was obtained with increase in late blight resistant genotypes by over 20 points in percentage and the genetic diversity was maintained by about 96% in three years. Our results demonstrated that combination of the foreground selection for target traits and the background selection for the genetic diversity is an efficient strategy in the recurrent selection of tetraploid potato to improve quantitative traits.

Acknowledgements

Funding from the National High Technology Research and Development Program of China (2003AA207130) is gratefully acknowledged.

References

An Z W, Xie L L, Cheng L, Zhou Y, Zhang Q, He X G, Huang H S. 2009. A silver staining procedure for nucleic acids in polyacrylamide gels without fixation and pretreatment. *Analytical Biochemistry*, **391**, 77-79.

Bradshaw J E, Dale M F B, Mackay G R. 2009. Improving the yield, processing quality and disease and pest resistance of potatoes by genotypic recurrent selection. *Euphytica*, **170**, 215-227.

Chen G, Zhu J. 2003. *QGA Station 1.0. Software for Classical Quantitative Genetics*. Institute of Bioinformatics, Zhejiang University, China. [2006-01-24]. http://ibi.zju.edu.cn/software/qga/index.htm

Collins A, Milbourne D, Ramsay L, Meyer R, Chatot-Balandras C, Oberhagemann P, de Jong W, Gebhardt C, Bonnel E, Waugh R. 1999. QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. *Molecular Breeding*, **5**, 387-398.

Cooke D E L, Lees A K. 2004. Markers, old and new, for examining *Phytophthora infestans* diversity. *Plant Pathology*, **53**, 692-704.

Dekkers J C M, Hospital F. 2002. The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews Genetics*, **3**, 22-32.

Dong Y S, Zhuang B C, Zhao L M, Sun H, He M Y. 2001. The genetic diversity of annual wild soybeans grown in China. *Theoretical and Applied Genetics*, **103**, 98-103.

Doyle J J. 1991. DNA protocols for plants. In: Hewitt G M, Johnson A W B, Young J P W, eds., *Molecular Techniques in Taxonomy*. North Atlantic Treaty Organization, Scientific Affairs Division, No. 57. pp. 283-293.

Ewing E E, Šimko I, Smart C D, Bonierbale M W, Mizubuti E S G, May G D, Fry W E. 2000. Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. *Molecular Breeding*, **6**, 25-36.

Estrada-Ramos N, Pérez-Alvarez O, Henfling J, Malamud O. 1983. Testing CIP and ICA potato selection for late blight resistance and yield at Rionegro (LA Selva) station in Colombia. In: Hooker W J, ed., *Research for the Potato in the Year 2000*. International Potato Center, Lima, Peru. pp. 78-79.

Frisch M, Bohn M, Melchinger A E. 1999. Comparison of selection strategies for marker-assisted backcrossing of a gene. *Crop Science*, **39**, 1295-1301.

Fry W E, Goodwin S B. 1997. Resurgence of the Irish potato famine fungus. *Bioscience*, **47**, 363-371.

Garelik G. 2002. Taking the bite out of potato blight. *Science*, **298**, 1702-1704.

Gautney T L, Haynes F L. 1983. Recurrent selection for heat tolerance in diploid potatoes (*Solanum tuberosum* subsp. *phureja* and *stenotomum*). *American Journal of Potato Research*, **60**, 537-542.

Gebhardt C, Valkonen J P T. 2001. Organization of genes controlling disease resistance in the potato genome. *Annual Review of Phytopathology*, **39**, 79-102.

Ghislain M, Zhang D, Fajardo D, Huamán Z, Hijmans R J. 1999. Marker-assisted sampling of the cultivated Andean potato *Solanum phureja* collection using RAPD markers. *Genetic Resources and Crop Evolution*, **46**, 547-555.

Ghislain M, Andrade D, Rodríguez F, Hijmans R J, Spooner D M. 2006. Genetic analysis of the cultivated potato *Solanum tuberosum* L. *phureja* group using RAPDs and nuclear SSRs. *Theoretical and Applied Genetics*, **113**, 1515-1527.

Goodwin S B, Sujkowski L S, Fry W E. 1995. Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. *Phytopathology*, **85**, 669-676.

Hallauer A R. 1985. Compendium of recurrent selection methods and their application. *Critical Reviews in Plant Sciences*, **3**, 1-33.

Jansky S H, Peloquin S J. 2006. Advantages of wild diploid *Solanum* species over cultivated diploid relatives in potato breeding programs. *Genetic Resources and Crop Evolution*, **53**, 669-674.

Landeo J A, Gastelo M, Beltran G, Diaz L. 2000. Quantifying genetic variance for horizontal resistance to late blight in potato breeding population B3C1. In: *Scientist and Farmer: Partners in Research for the 21st Century Program Report 1999-2000*. International Potato Center, Lima, Peru. pp. 63-68.

Landeo J A, Gastelo M, Pinedo H, Flores F. 1995. Breeding for horizontal resistance to late blight in potato free of *R* genes. In: Dowley L J, Bannon E, Cooke L R, Keane T, O'Sullivan E, eds., *Phytophthora infestans 150*. Bole Press Ltd., Dublin, Ireland. pp. 268-274.

Leonards-Schippers C, Gieffers W, Schäfer-Pregl R, Ritter E, Knapp S J, Salamini F, Gebhardt C. 1994. Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. *Genetics*, **137**, 67-77.

Marczewski W, Flis B, Syller J, Schäfer-Pregl R, Gebhardt C. 2001. A major quantitative trait locus for resistance to potato leafroll virus is located in a resistance hotspot on potato chromosome XI and is tightly linked to N-gene-like markers. *Molecular Plant-Microbe Interactions*, **14**, 1420-1425.

Mihovilovich E, Munive S, Bonierbale M. 2010. Influence of day-length and isolates of *Phytophthora infestans* on field resistance to late blight of potato. *Theoretical and Applied Genetics*, **120**, 1265-1278.

Oberhagemann P, Chatot-Balandras C, Schäfer-Pregl R, Wegener D, Palomino C, Salamini F, Bonnel E, Gebhardt C. 1999. A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. *Molecular Breeding*, **5**, 399-415.

Pérez W, Salas A, Raymundo R, Huamán Z, Nelson R, Bonierbale M. 2000. Evaluation of wild potato species for resistance to late blight. In: *Scientist and Farmer: Partners in Research for the 21st Century Program Report 1999-2000*. International Potato Center, Lima, Peru. pp. 49-62.

Rauscher G M, Smart C D, Simko I, Bonierbale M, Mayton H, Greenland A, Fry W E. 2006. Characterization and mapping of RPi-ber, a novel potato late blight resistance gene from *Solanum berthaultii*. *Theoretical and Applied Genetics*, **112**, 674-687.

Sanford L L, Ladd T L. 1979. Selection for resistance to potato leafhopper in potatoes. II. Progress after two cycles of phenotypic recurrent selection. *American Journal Potato Research*, **56**, 541-547.

Simko I. 2002. Comparative analysis of quantitative trait loci for foliage resistance to *Phytophthora infestans* in tuber-bearing *Solanum* species. *American Journal of Potato Research*, **79**, 125-132.

Smith J S C, Chin E C L, Shu H, Smith O S, Wall S J, Senior M L, Mitchell S E, Kresovich S, Ziegle J. 1997. An evaluation of

the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLP and pedigree. *Theoretical and Applied Genetics*, **95**, 163-173.

Song J Q, Bradeen J M, Naess S K, Raasch J A, Wielgus S M, Haberlach G T, Liu J, Kuang H H, Austin-Phillips S, Buell C R, Helgeson J P, *et al*. 2003. Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences of the USA*, **100**, 9128-9133.

Vleeshouwers V G A A, van Dooijeweert W, Keizer L C P, Sijpkes L, Govers F, Colon L T. 1999. A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation. *European Journal of Plant Pathology*, **150**, 241-250.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Friters A, Pot J, Paleman J, Kuiper M, Zabeau M. 1995. AFLP: a new technique for DNA finger printing. *Nucleic Acids Research*, **23**, 4407-4414.

van der Vossen E, Sikkema A, Hekkert B T L, Gros J, Stevens P, Muskens M, Wouters D, Pereira A, Stiekema W, Allefs S. 2003. An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. *The Plant Journal*, **36**, 867-882.

Wang B L, Liu J, Tian Z D, Song B T, Xie C H. 2005. Monitoring the expression patterns of potato genes associated with quantitative resistance to late blight during *Phytophthora infestans* infection using cDNA microarrays. *Plant Science*, **169**, 1155-1167.

Young N D, Tanksley S D. 1989. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. *Theoretical and Applied Genetics*, **77**, 353-359.

(Managing editor ZHANG Yi-min)