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Breeding of ‘Sweet Shinhong’ pepper by Marker-assisted backcrossing (MABC)

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ABSTRACT

Capsinoids is unique compound of pepper, which have similar biological effect to capsaicinoids like anticancer and anti-obesity. However, because the characteristic of capsinoids is non-pungency contrary to capsaicinoids, it has been studied to investigate genetic factor related to biosynthesis of capsinoids and to breed pepper variety producing capsinoids. Two pathway are known to be involved in capsaicinoids synthesis, phenylpropanoid and valine pathway. Capsinoids biosynthesis pathway is common to capsaicinoids, but *putative-aminotransferase* (*pAMT*) gene mutation in phenylpropanoid pathway cause capsinoids production instead of capsaicinoids. ‘SNU11-001’ which have *pAMT* gene mutation produce high level of capsinoids and ‘Shinhong’ is Korean chili pepper. In previous research, *pAMT* mutation in ‘SNU11-001’ have been introgressed to ‘Shinhong’ to breed novel ‘Shinhong’ pepper containing high contents of capsinoids by Marker-Assisted Backcrossing (MABC) method. Recessive homozygous *pAMT* allele was selected by genotyping with KASP marker for foreground selection and 8 to 10 plants which recovered by ‘Shinhong’ genome highly were selected by Fluidigm high-throughput genotyping analysis. ‘Shinhong C × SNU11-001 (SSHC)’ BC_2F_1-40 was selected by MABC with 198 SNP markers and recovery rate was 96.3%. In this study, MABC of ‘SNU11-001 × Shinhong C (SSHC)’ BC_3F_1 and ‘SNU11-001 × Shinhong B (SSHB)’ BC_2F_1 was proceeded. 202 and 102 markers were used for background selection, respectively. 10 SSHC BC_3F_1 progenies showed the highest recovery rate, 99.5%. The range of recovery rate in SSHB BC_2F_1 was 89.6 to 96.7%. SSHC BC_2F_2 was from self-crossing of SSHC BC_2F_1-40 and several *pamt/pamt* plants were selected. We will develop SSHB BC_2F_2 line which have *pAMT* mutation allele and high recovery rate of ‘Shinhong B’. This SSHB BC_2F_2 progeny will have cytoplasmic male sterility by being crossed with ‘Shinhong A’. Finally we will be able to breed sweet ‘Shinhong’ F1 hybrid containing high level of capsinoids.

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INTRODUCTION

The unique characteristic of pepper is pungency, which is caused by capsaicinoids in fruits. Capsaicinoids is an alkaloid derived from pepper’s placenta and have many biomedical functions such as anticancer and anti-obesity (Thiele et al., 2008; Xiu-Ju et al., 2011). Capsaicin is produced by condensation of branched-chain fatty acids and vanillylamine produced from vanillin by *pAMT* (Curry et al., 1999).

Low-pungent capsinoids is more palatable than capsaicinoids. Pepper cultivar ‘CH-19 sweet’ containing capsaicinoids-like substance was first reported by Yazawa in 1989. Biosynthesis of capsiate, one of the non-pungent capsaicinoids analogs, is caused by *pAMT* mutation causing impediment of the formation vanillylamine from vanillin (Lang et al., 2009; Tanaka et al., 2010a). Instead of vanillin, vanillyl alcohol is produced in the plant containig a dysfunctional *pAMT* gene and the *CS* gene is responsible for biosynthesis of capsiate using vanillyl alcohol as one of substrates (Tanaka et al., 2010b; Han et al., 2013).

Backcross breeding was first introduced in 1922 and is an effective breeding method for the introgression of one or a few genes to elite lines (Stoskopf et al., 1993). Marker-assisted backcrossing (MABC) method is known to reduce the time and efforts to develop a cultivar (Hospital and Charcosset, 1997).

Capsiate has been used as a functional food, pepper varieties producing capsinoids-rich fruits have not been developed yet. In this report, we show a MABC program for a development of new pepper cultivars containing capsinoids.

OBJECTIVES

- To introgress the mutated *pAMT* gene to *C. annuum* ‘Shinhong’ parental lines from *C. chinense* SNU11-001.
- To develop pepper varieties containing high levels of capsinoids.

MATERIALS AND METHODS

Plant materials

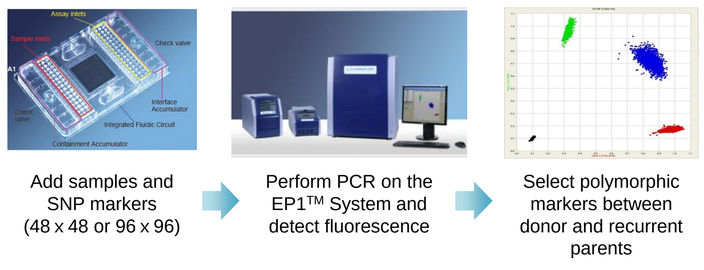
‘SNU11-001’ containing *pAMT* mutant, low level of capsaicinoids and high level of capsinoids was used for donor parent. ‘Shinhong B’ and ‘Shinhong C’ containing normal *pAMT*, high level of capsaicinoids and low level of capsinoids were used for recurrent parent.

SNP markers for background selection

A total of 412 locus specific SNP markers were used for MABC. Markers are evenly distributed in all Capsicum chromosomes. SNPs were mined by 8 *Capsicum* accessions transcriptome. Polymorphism test was done by EP1^{TM} system (Fluidigm®, USA).

EP1TM system

Polymorphism survey and background selection were performed by EP1^{TM} system (Fluidigm®, USA). It automatically collects genotypes of 2,304 or 9,216 SNP markers at a time.



Add samples and SNP markers (48 x 48 or 96 x 96) → Perform PCR on the EP1^{TM} System and detect fluorescence → Select polymorphic markers between donor and recurrent parents

Figure 1. Process of SNP genotyping using the EP1^{TM} system.

KEY REFERENCES

- Lang, Y *et al.,* 2009. Functional loss of *pAMT* results in biosynthesis of capsinoids, capsaicinoids analogs, in Capsicum annuum cv. CH-19 Sweet. Plant J. 59: 953-961.
- Tanaka, Y *et al.,* 2010. Newly mutated putative-aminotransferase in nonpungent pepper (*Capsicum annuum*) results in biosynthesis of capsinoids, capsaicinoids analogues. J. Agric. Food Chem. 58: 1761-1767.
- Tanaka, Y *et al.,* 2010. Novel loss-of-function *putative aminotransferase* alleles cause biosynthesis of capsinoids, nonpungent capsaicinoids analogues, in mildly pungent chili peppers (*Capsicum chinense*). J. Agric. Food Chem. 58: 11762–11767.
- Jeong, H *et al.*, 2015. Marker-assisted backcross breeding for development of pepper varieties (*Capsicum annuum*) containing capsinoids. Mol. Breeding 35.12: 1-10.

RESULTS AND DISCUSSION

Breeding scheme

’SNU11-001’ was used for as a *pamt* mutation donor and ‘Shinhong’ A, B, C lines for recurrent parents. Individual plants having the heterozygous genotype for *pAMT* marker were selected from each populations and then plants showing the most recovered genetic background of recurrent parents were selected by a set of SNP markers evenly distributed in pepper genome. ‘C line BC_1F_1-60’ was in place of ‘Shinhong’ B to construct F_1 because of difficulty in crossing between ‘Shinhong’ B and ‘SNU11-001’.

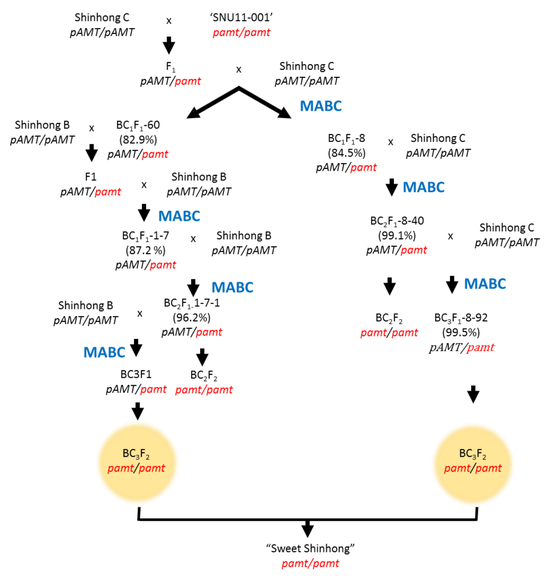


Figure 1. Schematic diagram of breeding ‘Sweet Shinhong’

Development of the *pAMT* KASP marker for the foreground selection

The *pAMT* gene in SNU11-001 had an insertion of transposable element (*Tcc*) on third intron. KASP marker was developed based on a SNP near *Tcc*.

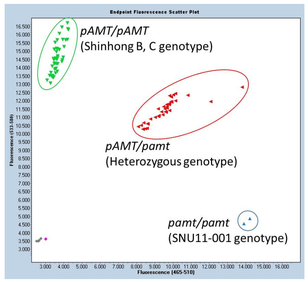


Figure 2. Development of KASP marker to distinguish between *pAMT* and *pamt* mutant.

Polymorphism survey of the SNP markers between parental lines

To select SNP markers polymorphic between ‘Shinhong’ parental lines and ‘SNU11-001’, a total of 412 SNP markers were analyzed. As a result, a total of 144 and 204 SNP markers were polymorphic between ‘Shinhong B’ and ‘SNU11-001’, ‘Shinhong C’ and ‘SNU11-001’, respectively.

Table 1. Number of polymorphic markers and marker density

Chromosome	‘SNU11-001’ x ‘Shinhong B’		‘SNU11-001’ x ‘Shinhong C’	
	Polymorphic marker	Marker density (cM)	Polymorphic marker	Marker density (cM)
1	26	7.03	34	5.25
2	12	7.58	21	4.97
3	18	7.73	32	4.24
4	10	10.73	12	8.68
5	9	5.25	12	6.97
6	16	8.65	21	6.00
7	9	12.92	12	9.15
8	2	13.43	2	6.15
9	10	9.68	12	6.46
10	12	7.83	16	6.30
11	13	7.02	17	5.49
12	7	14.57	13	8.59
Total	144	9.37	204	6.52

MABC of ‘Sweet Shinhong’ C

BC_1F_1-8, BC_2F_1-8-40 and BC_3F_1-8-40-92 was selected as a result of MABC by using EP1^{TM} system, whose recovery rate is 84.8%, 99.1% and 99.5%, respectively. A fragment of the ‘SNU11-001’ in size 45 Mb flanking the *pAMT* locus was inserted in ‘BC_3F_1-8-40-92’.

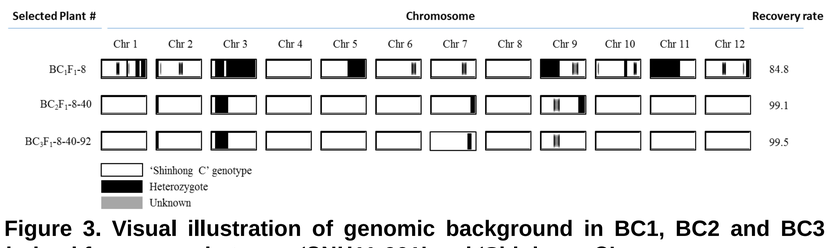


Figure 3. Visual illustration of genomic background in BC1, BC2 and BC3 derived from cross between ‘SNU11-001’ and ‘Shinhong C’.

MABC of ‘Sweet Shinhong’ B

● Marker selection for MABC of B line

SNP markers were selected by four genotype combination of ‘SNU11-001’, ‘Shinhong’ B and ‘Shinhong’ C. Because ‘C line BC1F1-60’ was used for parental line instead of ‘SNU11-001’ to make F1, alleles of SNPs should be distinguish between ‘Shinhong’ B and ‘Shinhong’ C.

Table. 2. Criteria of marker selection for capsiate ‘Shinhong’ B BC1 MABC

	Shinhong B	Shinhong C	SNU11-001	the No. of markers
1	XX	YY	XX	12
2	XX	YY	YY	27
3	YY	XX	XX	25
4	YY	XX	YY	30

● Foreground selection using *pAMT* marker

A total of 294 BC_1F_1 plants derived from ‘Shinhong B’ x ‘SNU11-001’ were used for the first round foreground selection. As a result, 148 plants showed the *pAMT/pAMT* genotype whereas 146 plants showed the heterozygous genotype. 196 BC_2F_1 from BC_1F_1-1-7 and 152 BC_2F_1 from BC_1F_1-1-29 were also used for the second round foreground selection. Among BC_2F_1 plants from two BC_1F_1, 86 and 68 plants showed the heterozygous genotype, respectively.

Table. 1. Genotyping results of the foreground selection in ‘Sweet Shinhong B’ backcross population

Population	Generation	Number of plant			Expected ratio	χ^2	*P-value*
		Total	*pAMT/pAMT*	*pAMT/pamt*			
‘Shinhong B’ x ‘SNU11-001’	BC_1F_1	294	148	146	1:1	0.0671	0.7956
	BC_2F_1 (1-7)	196	110	86	1:1	2.9388	0.0865
	BC_2F_1 (1-29)	152	84	68	1:1	1.6842	0.1944

● Background selection using EP1^{TM} system

A total of 130 BC_1F_1 plants were analyzed by 93 SNP markers. The most recovered plant by ‘Shinhong’ B genetic background, BC_1F_1-1-7 (87.2%) and another plant recovered mostly in chromosome 3 that *pAMT* gene is located in, BC_1F_1-1-29 (76.1%) were selected.

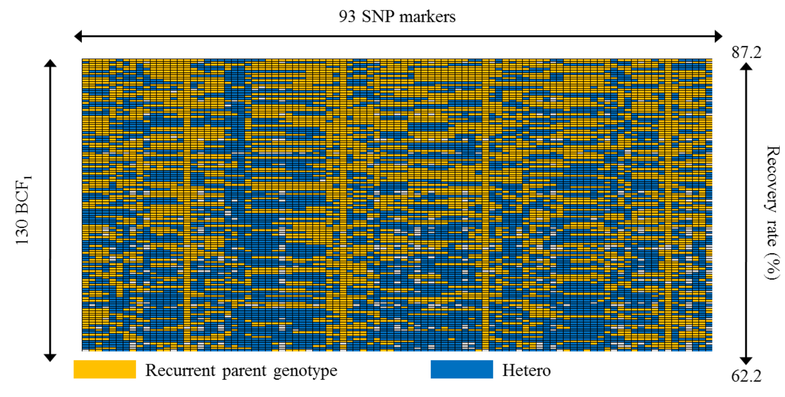


Figure. 4 Genotyping result for background selection in SNU11-001 x Shinhong B BC_1F_1 population.

BC_2F_1 was generated from two BC_1F_1 plants. 22 SNP markers were added for MABC of BC_2F_1 from BC_1F_1-1-7 and 44 SNP markers for BC_2F_1 from BC_1F_1-1-29. In second round of the background selection, the most recovered plants by ‘Shinhong B’ genetic background were selected (BC_2F_1-1-7-1(96.7%), BC_2F_1-1-29-1(95.4%)).

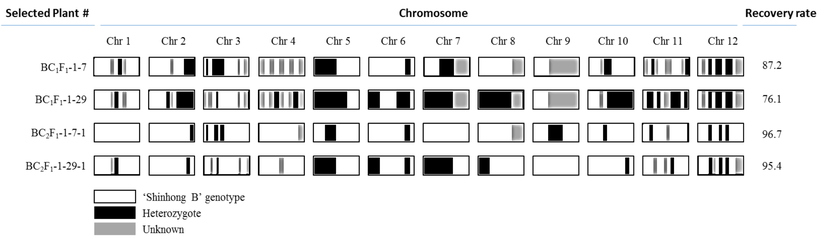


Figure. 5 Visual illustration of genomic background in BC1, BC2 derived from cross between ‘SNU11-001’ and ‘Shinhong B’

ACKNOWLEDGEMENT

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