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 capsaicinsoids like antitumor and anti-obesity. However, because the characteristic of capsinoids is non-pungent contrary to capsaicinoids, it has been studied to investigate genetic factor related to biosynthesis of capsinoids and to breed papper variety producing capsinoids. Two pathway are known to be involved in capsinoids synthesis, phenylpropanoid and pungent pathway. Capsinoids biosynthesis pathway is common to capsaicinoids, but pungent-antimicrobial peptide (pAMP) gene mutation in phenylpropanoid pathway cause capsinoids production instead of capsaicinoids. ‘SNU11-001’ which have pAMP gene mutation produce high level of capsinoids and ‘Shinhong’ is Korean chilli pepper. In previous research, pAMP mutation in ‘SNU11-001’ have been introduced to ‘Shinhong’ to breed novel ‘Shinhong pepper containing high contents of capsinoids by Marker-Assisted Backcrossing (MABC) method. Recessive homozgyous pAMP 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 B’ (SSHC) ‘BCF1’ and ‘SNU11-001 × Shinhong B’ (SSBH) ‘BCF2’ was proceeded. 202 and 102 markers were used for background selection, respectively. 10 SSHC BC F1 progenies showed the highest recovery rate, 99.5%. The range of recovery rate in SSHC BC F1 was 89.6 to 96.7%. SSHC BC F2 was from self crossing of SSHC BC F1-40 and several parent plants were selected. We will develop SSHB BC F2 line which have pAMP mutation allele and high recovery rate of ‘Shinhong’. This SSHB BC F2 progeny will have pungentialy mal霖 stability by being crossed with ‘Shinhong A’. Finally we will be able to breed ‘Shinhong’ F1 hybrid containing high level of capsinoids.

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 antitumor and anti-obesity (Thrall et al., 2008; Xu-Ju et al., 2011). Capsaicin is produced by condensation of branched-chain fatty acids and vanillylamine produced from vanilllin by pAMP (Curry et al., 1999).

Low-pungent capsinoids is more palatable than capsaicinoids. Pepper cultivar ‘CH-19’ sweet containing capsinoids-like substance was first reported by Yawaza in 1969. Biosynthesis of capsinoids, one of the non-pungent capsaicinoids analgesia, is caused by pAMP mutation causing impediment of the formation vanillylamine from vanilllin (Lang et al., 2009; Tanaka et al., 2010a). Instead of vanillyl alcohol is produced in the plant containing a dysfunctional pAMP gene and the CS gene is responsible for biosynthesis of capsinoids using vanillyl alcohol as one of substrates (Tanaka et al., 2010b; Han et al., 2013).

Backcross breeding was first introduced in 1902 and is an effective breeding method for the introgration of one or a few genes to elite lines (Strelauk, 1993). Marker-assisted backcrossing (MABC) method is known to reduce the time and efforts to develop a cultivar (Hospital and Charroscot, 1997).

Capsinoids have 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 development of new pepper cultivars containing capsinoids.

OBJECTIVES
• To introgress the mutated pAMP 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 pAMP mutant, low level of capsaicinoids and high level of capsinoids was used for donor parent. ‘Shinhong B’ and ‘Shinhong C’ containing normal pAMP, high level of capsinoids and low level of capsaicinoids 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 B Capsicum accessions transcriptome. Polymorphism test was done by EPi™ system (Fluidigm, USA).

EPiTM system
Polymorphism analysis and background selection were performed by EPi™ system (Fluidigm, USA). It automatically collects genotypes of 2,304 or 9,216 SNP markers at a time.

RESULTS AND DISCUSSION

Breeding scheme
‘SNU11-001’ was used for as a pamp mutation donor and ‘Shinhong A’, ‘B’, and ‘C’ lines for recurrent parent. Individual plants having the heterozygous genotype for pAMP marker were selected from each populations and then plants showing the most recovered genetic background of recurrent parent were selected by a set of SNP markers evenly distributed in pepper genome. ‘C’ line BC F1, 60 was in place of ‘Shinhong B’ to construct F1, because of difficulty in crossing between ‘Shinhong B’ and SNU11-001.

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 BC F1-69 was used for parental line instead of SNU11-001 to make F1, alleles of SNPs should be distinguished between ‘Shinhong B’ and ‘Shinhong C’.


table 1. Marker selection for capsinoid ‘Shinhong’ B in MABC

<table>
<thead>
<tr>
<th>Population</th>
<th>Total No. of markers</th>
<th>Number of markers</th>
<th>Allele ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shinhong B</td>
<td>108</td>
<td>71</td>
<td>1.1</td>
<td>0.0005</td>
</tr>
<tr>
<td>Shinhong C</td>
<td>102</td>
<td>72</td>
<td>1.1</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Background selection using EPi™ system
A total of 130 BC F1 plants were analyzed by 93 SNP markers. The most recovered plant by ‘Shinhong B’ genetic background, BC F1-7, was selected and another plant was recovered mostly in chromosome 3 that pAMP gene is located in, BC F1-29 (76.1%) was selected.

MABC of ‘Sweet Shinhong’ C
Marker selection for MABC of BC line
SNP markers were selected for four genotype combination of ‘SNU11-001’, ‘Shinhong B’, ‘Shinhong C’ and ‘SNU11-001 × Shinhong B’ (SSBH) ‘BCF’ was proceeded. 202 and 102 markers were used for background selection, respectively. 10 SSHC BC F1 progenies showed the highest recovery rate, 99.5%. The range of recovery rate in SSHC BC F1 was 89.6 to 96.7%. SSHC BC F2 was from self crossing of SSHC BC F1-40 and several parent plants were selected. We will develop SSHB BC F2 line which have pAMP mutation allele and high recovery rate of ‘Shinhong’. This SSHB BC F2 progeny will have pungentialy mal霖 stability by being crossed with ‘Shinhong A’. Finally we will be able to breed ‘Shinhong’ F1 hybrid containing high level of capsinoids.

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