Practical use of molecular markers of pungency in breeding program of pepper (*Capsicum annuum* L.)

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INTRODUCTION

Marker-assisted selection (MAS) is a useful additional tool in modern plant breeding programs to optimize selection efficiency especially when monogenic traits are involved. For breeding purposes, molecular markers enable analysis of a large number of samples, detection of desired alleles and haplotypes at early stage of plant development, and reduction substantially the breeding period when biennial crops are involved.

Pungency of pepper (*Capsicum* spp.) is due to accumulation of capsaicinoids, of which capsaicin is the most pungent compound. The capsaicin biosynthesis is controlled by the *Pun1* locus, which encodes a putative acyltransferase. The recessive allele of this locus (*pun1*) contains a deletion that results in non-pungency. The co-dominant PCR-based markers developed by Stewart et al., (2005; 2007) were used for distinguishing of these alleles in segregating population of pepper (*Capsicum annuum* L.)

MATERIAL AND METHODS

Pungent (P1) and non-pungent (P2) pepper lines (*Capsicum annuum* L.) were crossed. A total of 25 F_1 and 106 F_2 individual plants were analyzed for determination of *Pun1* allele(s). Total DNA was isolated using "Genomic DNA Purification Kit" (Fermentas) according to the manufacturer's instruction.

A PCR-based, co-dominant marker based on the following primers Pr1F - ATGTCAACCGGCCAGCAGCAT, Pr2R - CTGATTCTTCTGCCACCTTCAATCCC (Stewart et al., 2007), and (Pun1F TCATTAGAAGGTCATACCGCTCCACG (our design) was used to determine the allelic state at *Pun1* for all individuals. The primer combination (Pun1F+Pr2R) amplifies the *pun1* allele (non-pungent). The primer combination (Pr1F+Pr2R) amplifies the *Pun1* allele (pungent).

Three primers were used for multiplex PCR. PCR reactions were performed in 20-µl reaction mixtures containing 2µl 10x PCR buffer, 0.6 µl each of primers (10 µM), 2 µl dNTP's (10mM), 0.1 µl Taq DNA polymerase (1.0U) (Fermentas), and 50 ng of template DNA. The amplifications were performed using a MyCycler thermal cycler (Bio-Rad) with the following cycle conditions: initial activation at 94°C for 4 min; 35 cycles of 94°C for 40 sec, 62°C for 40 sec, and 72°C extension for 1 min; and a final extension at 72°C for 10 min. A total of 15µl of each amplification reaction was analyzed by electrophoresis using a 1.5% ethidium bromide-stained agarose gel.

RESULTS

Two pepper lines (P1-pungent and P2-non-pungent) were crossed. F_1 and F_2 populations were developed. Dou to dominancy of pungency, homozygous (*pun1/pun1*) and heterozygous (*Pun1/pun1*) plants cannot be distinguished by conventional phenotypic screening.

The co-dominant PCR-based markers were used for screening of F1 and F2 populations of pepper. The additional PCR primer Pun1F was designed based on sequences from NCBI of the *pun1* gene of sweet pepper (Acc.num. AY819032.1) and chili pepper (Acc.num. AY819029.1). For the multiplex PCR, the primer Pun1F was added to the primers Pr1F and Pr2R, that resulted in amplification of 450 bp fragment in *pun1*-allele and 1200 bp fragment in *Pun1*-allele.

All F1 plants obtained from cross between pungent (P1) and non-pungent (P2) pepper lines were pungent. PCR screening with co-dominant markers confirmed hybrid purity of obtained F1 hybrids (Fig.1).

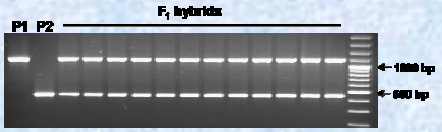

Fig.1. Hybrid purity test by multiplex PCR with the primers for *Pun1*- and *pun1*-alleles. P1 –female parent (pungent- *Pun1/Pun1*), P2 – male parent (non-pungent – *pun1/pun1*). F_1 hybrids – pungent heterozygous plants (*Pun1/pun1*)

At the seedling stage, 106 F_2 individual plants were analyzed for determination the allelic state at Pun1 for all individuals. Homo- and heterozygous genotypes were revealed (Fig.2). Out of 106 tested plants, 22 genotypes contained *Pun1/Pun1* alleles, 57 genotypes – *Pun1/pun1*, 27 genotypes - *pun1/pun1* (Tab.1). The observed segregation ratio was consistent with the expected ratio of pungent and non-pungent genotypes, confirming monogenic inheritance of these trait in *C. annuum* L.

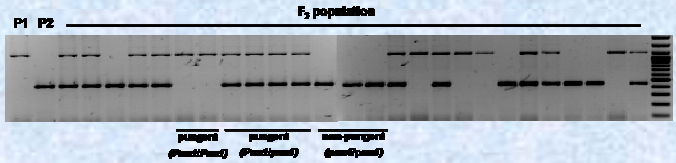

Fig.2. Segregation of the Pun1 allele in a F_2 population originated from the cross between pungent (*Pun1/Pun1*) parent (P1) and non-pungent (*pun1/pun1*) (P2) parent.

Tab. 1. Chi-square test of the segregation ratio of pungency in the F2 population originated from the cross between pungent (*Pun1/Pun1*) parent (P1) and non-pungent (*pun1/pun1*) (P2) parent

	Popul. Size, n	*Pun1/Pun1* (pungent)	*Pun1/pun1* (pungent)	*pun1/pun1* (non-pungent)	χ2
P1	10	10	0	0	
P2	10	0	0	10	
F_1 (P1 x P2)	25	0	25	0	
F_2 [(P1 x P2) x (P1 x P2)]	106	22	57	27	0.012

Only homozygous (*Pun1/Pun1*) seedlings were selected for next step of breeding process aimed at selection of the large-fruited pungent genotypes for development of new pepper cultivar.

So, single-plant selection carried out at the seedling stage by DNA markers is the one of the advantages of marker assisted selection that can be exploited by breeders to accelerate the breeding process.

References:

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2) Stewart C.J., Mazourek M., Stellari G.M., O'Connell M.A., Jahn M. Genetic control of pungency in *C. chinense* via the *Pun1* locus. J. of Exp. Bot., 2007, vol. 58, No 5, pp. 979-991.