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Restorer breeding in sweet pepper: Introgressing *Rf* allele from hot pepper through marker-assisted backcrossing

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ABSTRACT

Lack of a strong and stable restorer-of-fertility (*Rf*) allele in sweet pepper (*Capsicum annuum* L.) has been a hurdle in commercial exploitation of cytoplasmic male sterility (CMS) system for cost-effective production of sweet pepper hybrid seeds. A known sequence characterized amplified region (SCAR) marker (CRF-S_{870}) associated with a fertility restoration phenotype (*Rf* locus) in hot pepper was validated in a strong restorer hot pepper inbred line (AVPP9905). The CRF-S_{870} marker was successfully used in marker-assisted backcrossing (MAB) to transfer *Rf* allele from hot pepper line AVPP9905 to several sweet pepper genotypes. The fertility restoration ability of 21 BC_4F_2 sweet pepper plants obtained through marker-assisted backcrossing was found to be normal, as CMS based plants derived from the backcrossed male plants upon selfing produced a normal amount of fruit with sufficient selfed seeds. The development of restorer lines in sweet pepper is expected to open commercial exploitation of CMS hybrid technology in sweet pepper.

1. Introduction

Among five domesticated *Capsicum* species, *Capsicum annuum* is the most commonly cultivated for its pungent (chili pepper) and non-pungent (sweet pepper) fruits (Kumar et al., 2006). Pepper hybrids are becoming more popular with farmers for their higher yield and fruit quality compared to open pollinated varieties. Although cytoplasmic male sterility (CMS) is used commercially to develop and produce hot (syn. chili) pepper hybrid seed, CMS for sweet pepper hybrid development has not yet been successful. This is primarily because most of the sweet pepper genotypes either lack a restorer-of-fertility (*Rf*) allele (Kumar et al., 2007; Mulyantoro et al., 2014) or restorer lines are unstable with weak fertility restoration ability, therefore cannot be used as the male parent to develop male fertile CMS hybrids, which can produce normal marketable fruits. Thus, identification of sweet pepper restorer lines with a strong and stable *Rf* allele, or alternatively, introgression of an *Rf* allele from hot pepper into sweet pepper (restorer breeding) is a prerequisite for the commercial success of the CMS hybrid technology for sweet pepper. Molecular marker-assisted backcrossing (MAB) can facilitate rapid introgression of the *Rf* allele by early identification of the restorer genotype (*Rf*-) without developing and evaluating test cross progenies.

The effective deployment of markers for the *Rf* allele for MAB requires markers that are tightly linked with the fertility restoration locus (*Rf* allele). At AVRDC—The World Vegetable Center, nine sweet pepper restorer lines were identified by screening 100 inbred lines (Lin et al., 2007). However, fertility restoration ability of these sweet pepper restorer lines was very poor (Lin et al., 2007). Hence, we initiated the introgression of a strong *Rf* allele into sweet pepper, using a sequence characterized amplified region (SCAR) dominant marker (CRF-S_{870}) reported to be linked with the *Rf*-phenotype in hot pepper (Gulyas et al., 2006). The study aimed to validate the CRF-S_{870} marker to correctly predict the fertility restoration phenotype (*Rf*-phenotype), and making progress on introgressing the *Rf* allele from hot into sweet pepper through marker-assisted backcrossing.

2. Materials and methods

2.1. Parents and crosses

During the fall season of 2009 (October 2009–February 2010), a hot pepper inbred line (9955-15/AVPP9905) known to possess a

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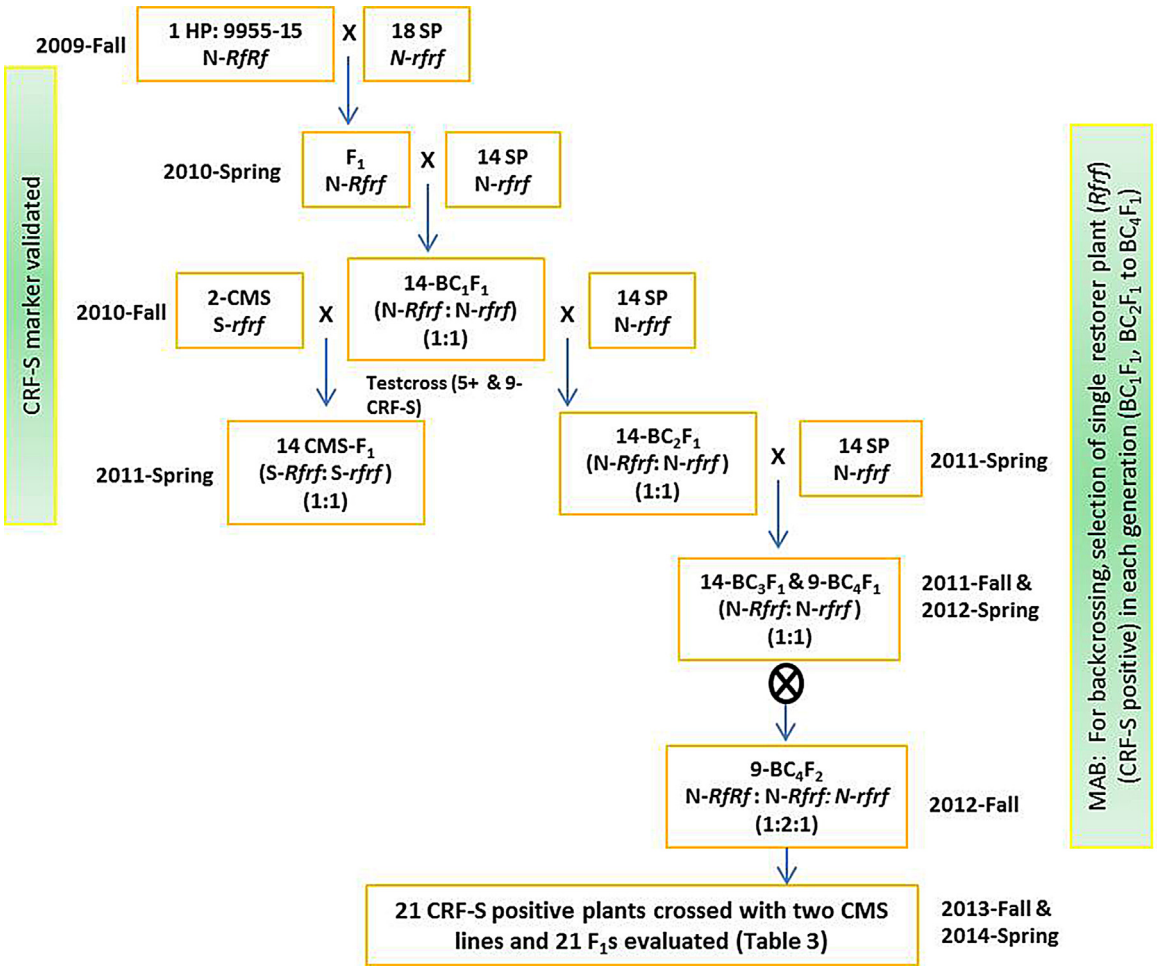

Fig. 1. Steps involved in employing CRF-S_{870} (validation and MAB) to introgress *Rf* allele from hot pepper into sweet pepper (HP = hot pepper, SP = sweet pepper; N = normal cytoplasm, S = male sterile cytoplasm; and *Rf* = restorer-of-fertility).

Table 1
Segregation of CRF-S_{870} in BC_1F_1 plants derived from a common hot pepper seed parent and 18 sweet pepper pollen parents.

Cross#	Crosses[a]	CRF-S_{870} (# of plants)			Yates χ^2 *P* value (1:1)
		Present	Absent	Total	
1	9955-15/2*9950-5700	2	3	5	1.00
2	9955-15/2*0007-2530	2	8	10	0.11
3	9955-15/2*0407-7069	3	5	8	0.72
4	9955-15/2*C00167-3	8	2	10	0.11
5	9955-15/2*C03796	7	3	10	0.34
6	9955-15/2*C05464-B	4	6	10	0.75
7	9955-15/2*0537-7007	5	5	10	0.75
8	9955-15/2*0537-7009	8	2	10	0.11
9	9955-15/2*0537-7010	4	6	10	0.75
10	9955-15/2*0537-7012	3	7	10	0.30
11	9955-15/2*0537-7032	5	5	10	0.75
12	9955-15/2*0537-7033	4	6	10	0.75
13	9955-15/2*0537-7058	7	3	10	0.30
14	9955-15/2*PBC447	5	5	10	0.75
15	9955-15/2*PBC205	10	0	10	0.00
16	9955-15/2*PBC273	2	8	10	0.11
17	9955-15/2*PBC349	8	2	10	0.11
18	9955-15/2*PI601110	4	6	10	0.75
Pooled		91	82	173	0.54

[a] Single plant of hot pepper line (9955-15/AVPP9905) was used to cross with single plant of 18 sweet pepper maintainer lines.

strong restorer-of-fertility (N-*RfRf*) allele for Peterson's male sterile cytoplasm (Lin et al., 2007) was crossed with 18 sweet pepper inbred lines possessing the maintainer (N-*rfrf*) allele (Table 1). Two plants of each parent and 18 F_1s were raised in a greenhouse and F_1s were backcrossed with 18 sweet pepper parents on a single plant-to-plant basis to generate 18 BC_1F_1 combinations in the 2009 spring season (April–August). During the fall season of 2010, due to practical and cost considerations, only 10 plants of each of the 18 BC_1F_1 were raised along with the parents in greenhouse for backcrossing (Table 1). One plant from each of the successfully obtained 14 BC_1F_1 plants was backcrossed to the respective sweet pepper recurrent parent to obtain BC_2F_1 plants. Backcrossing was continued until the BC_4F_1 generation (Fig. 1). Fourteen BC_1F_1 plants were used as pollen parents to develop 14 test cross progenies on two sweet pepper CMS lines possessing Peterson's CMS cytoplasm (Table 2). The test cross populations were grown in the field from August 2011 to March 2012. The presence of the *Rf* locus-associated CRF-S_{870} dominant marker (Gulyas et al., 2006) was examined in both the parents, all 18 F_1s and in all the 18 BC_1F_1 plants. During the fall season of 2011, all the test cross (CMS/BC_1F_1) plants were analyzed for CRF-S_{870} and evaluated for the *Rf*-phenotype to determine the linkage between marker and phenotype (Table 2). For the purpose of examining the restoration ability of the BC_4F_2 plants that were advanced through MAB, 21 plants with CRF-S_{870} were selected (Fig. 1 and Table 3) and crossed as male parent with CMS sweet pepper lines. The test cross progenies were evaluated for their fertility restoration ability.