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Short Communication

Genetic outcomes from a farmer-assisted landrace selection programme to develop a synthetic variety of broccoli

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Abstract

To develop synthetic varieties (Syn) of broccoli for organic agriculture, we initiated a breeding programme from a landrace (LR). A Syn was obtained through a farmer-assisted selection programme that mirrors the original LR. The diversity level of the Syn was assessed using 14 putatively neutral microsatellite markers (simple sequence repeats (SSR)) and seven SSR related to genes involved in flowering control. Four commercial F_1 hybrids were also assessed. Despite the strict selection procedure applied by the farmer to reproduce the LR annually and to obtain the Syn, the detected diversity level was high and similar to that of non-selected LRs. The possible reasons for these genetic outcomes (i.e. SSR position in the genome and farmer selection methods) are discussed here.

Keywords: *Brassica oleracea* L.; dynamic on-farm conservation; farmer-assisted breeding; genetic diversity

Introduction

Broccoli, *Brassica oleracea* L. ssp. *capitata* (L.) DC. convar. *botrytis* (L.) Alef. var. *italica* Plenck (Hammer *et al.*, 2013), is an important crop. The commercial production (with other *Brassica* vegetables) is about 89 million tons worldwide (FAOSTAT, 2011) and 426,000 tons (ISTAT, 2011) in Italy, where part of the production comes from landraces (LRs) (Ciancaleoni *et al.*, 2013; Negri *et al.*, 2013) and is obtained from organic agriculture (OA). All the developed broccoli varieties are essentially F_1 hybrids. To develop synthetic varieties (Syn) of broccoli for OA, we initiated a farmer-assisted breeding programme from a LR. LRs could be the best material for this purpose (Hammer and Gladis, 2001; Falcinelli and Torricelli, 2004; Lammerts van Bueren *et al.*, 2011; Koutis *et al.*, 2012) because they are characterised by (1) adaptation to the proposed environment, since they have developed over time through evolutionary processes including both environmental and farmer selection (Negri *et al.*, 2009; Polegri and Negri 2010), and (2) stability (S. Ciancaleoni, personal communication), since their genetic diversity provides a buffer against environmental fluctuations due to biotic and abiotic stresses. To the best of our knowledge, none has bred broccoli specifically for OA yet.

The aim of this paper was to establish a clear genetic characterisation of the initial material (Syn0) from which the Syn was developed.

Experimental

The LR under study has been cultivated by the 'Vento' family in our region since generations. After the family abandoned the land 9 years ago, the Department

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continued its cultivation by annual reproduction of the seed and applying the mother plant (MP) selection procedures suggested by the farmer: the most vigorous two or three MPs among the 25 usually grown are chosen and intercrossed, while the field is cleared from other *B. oleracea* plants. In addition, the Department assured spatial isolation from nearby cultivated fields.

While starting the breeding programme, we asked the farmer to choose 17 MP among the 25 grown instead of the usual numbers (we were worried about restricting the genetic base too much). The following year, ten plants for each of the 17 MP half-sib progenies (MPHS) were grown in a trial (five plants for each MPHS in each of two replicates, for a total of 170 plants). Among them, the farmer was asked to select, according to his personal opinion, the best eight MPHS and then, within each of them, the best five plants. Accordingly, a Syn0 made of 40 plants by eight MPHS (8C, i.e. eight components), which mirrors the original LR, was obtained (hereafter Syn0_8C).

The genetic diversity of the 40 plants was assessed using 14 putatively neutral simple sequence repeats (SSR) (Love *et al.*, 2004; Cheng *et al.*, 2009; Li *et al.*, 2011) and seven SSR related to genes involved in flowering control [expressed sequence tag (EST)-SSR and the *Bo_FRI*–gene-derived SSR] (Aksoy *et al.*, 2013) (Table 1). The genetic diversity of 32 plants, eight for each of four commercial F_1 hybrids (Ironman, Marathon, Packman and Santee) used as controls, was also assessed. It should be noted that the variation in the F_1 hybrids is generally very low and, consequently, eight individuals are sufficient to test the heterozygosity.

DNA extraction and marker amplification was carried out as reported in Ciancaleoni *et al.* (2013). The number of alleles and band range were recorded for each marker used. The number of successfully analysed genotypes (N), observed (N_a) and effective (N_e) alleles, observed (H_o) and expected (H_e) heterozygosity and fixation index (F) were also calculated for each accession and each marker using the GENALEX software (Peakall and Smouse 2006). The same software was used to estimate a genetic distance (GD) matrix between individuals following Nei (1978). A GD-based neighbour joining (NJ) tree was drawn using the MEGA5 software (Tamura *et al.*, 2011).

Results

The number of alleles per locus ranged from 2 to 11; the total number of alleles were 96 (Table 1), of which over one-fourth (26) were found in the Syn0_8C only. In the Syn0_8C, N_a ranged from 1 to 6 and from 2 to 3 for SSR and EST-SSR/gene-derived SSR, respectively (Table S1, available online). The average N_a, N_e, H_o and H_e values of the Syn0_8C (Table S1, available online) were similar

Table 1. Simple sequence repeat (SSR) marker codes, marker name/genebank entry, repeated motif, linkage group (LG), band range (in base pairs (bp)) and number of observed alleles (N_a), relative to the 21 microsatellites used

SSR	Marker name/ entry	Repeat motif	LG	Band range (bp)	N_a
SSR6[a]	Ol12G04b	(TC)29	8	100–152	11
SSR10[a]	Na12C08	(CT)50	1	268–319	9
SSR20[a]	Ol12F02a	(TC)29	5	148–207	6
SSR22[a]	Ol10B01	(CT)29	6	118–220	9
SSR31[b]	BoGMS1042	(TC)14	1	178–190	4
SSR35[c]	BnGMS633	(AT)18	2	344–356	3
SSR39[b]	BoGMS0702	(AT)18	3	280–285	4
SSR42[b]	BoGMS1465	(GAA)8	3	295–299	4
SSR45[b]	BoGMS0560	(GAA)14	4	282–303	7
SSR47[c]	BnGMS681	(GT)8	4	142–143	2
SSR49[b]	BoGMS0949	(TC)15	5	163–184	6
SSR54[b]	BoGMS0354	(TGT)6(GTT)4	8	106–109	3
SSR58[b]	BoGMS1570	(AATA)6	9	192–193	2
SSR59[b]	BoGMS1467	(CTG)8	9	267–276	4
Bo_FLC1-SSR1[d]	AM231517	(TA)13		135–181	2
Bo_FLC3-SSR3[d]	AY306125	(TC)11		206–208	2
Bo_LFY-SSR7[d]	FJ529019	(TC)15		182–184	3
Bo_FT-SSR9[d]	FJ848914	(TCT)4		200–221	3
Bo_CRY1-SSR17[d]	AJ344565	(T)15(T)13		327–334	2
Bo_FRI–gene-derived19[d]	JF318402	–		378–383	3
Bo_GI-SSR20[d]	GQ177484	(AGC)4		263–265	3

[a] Love *et al.* (2004). [b] Li *et al.* (2011). [c] Cheng *et al.* (2009). [d] Aksoy *et al.* (2013).

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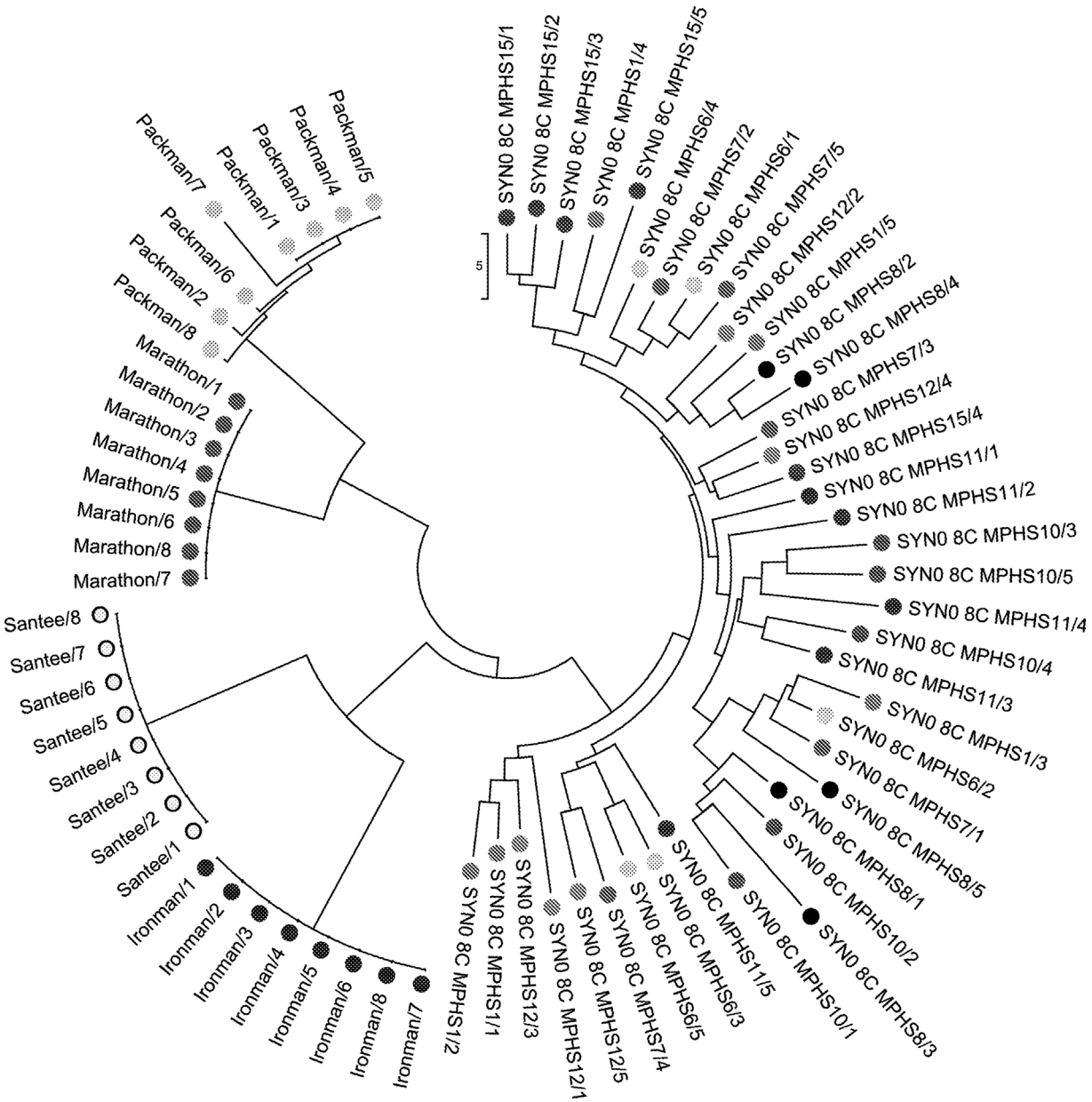

Fig. 1. Neighbour-joining tree of genetic distances. In the tree, genotypes belonging to the same hybrid/family are identified with the same black and white pattern.

to those of LRs and synthetics that had not undergone the same selection procedure (Ciancaleoni *et al.*, 2013), while the H_e values were lower than the H_o values for the hybrids (Table S1, available online). The *F* value was generally low and close to 0 for the Syn0_8C (e.g. in a random mating situation), which was unexpected when considering the selection applied. All the hybrids showed an excess of heterozygosity, as it is expected for varieties developed for this purpose (Table S1, available online).

The high diversity of the Syn0_8C and the uniformity of the hybrids are well depicted in the NJ tree (Fig. 1). All of the plants of the Syn0_8C, even those belonging to the same MPHS, are different from each other; in addition, plants belonging to the same MPHS are not clearly grouped in the NJ tree.

Discussion

The diversity of the Syn0_8C seems to be large enough to guarantee that the derived Syn will also have a diversity level that might be sufficient to buffer environmental fluctuations due to biotic and abiotic stresses that occur under OA. LR selection carried out over generations

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by the farmer has maintained a substantial diversity both in putatively neutral and EST-SSR/gene-derived SSR.

There are at least two hypotheses that can justify these findings. We might have chosen markers that did not probe parts of the genome where significant uniformity was obtained by selection. It can also be supposed that, by choosing the most vigorous plant per generation, the farmer unconsciously selects those plants that have the highest heterozygosity, and different alleles are consequently maintained across generations. This has already been suggested as a possible mechanism for maintaining diversity in a celery LR (Torricelli *et al*., 2013).

To our knowledge, very few data are available for LRs that could explain their diversity. The long term effects of on-farm conservation strategy on LRs diversity deserve to be better investigated.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262113000592

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