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PROPOSAL OF A BREEDING METHOD PREREQUISITE TO CULTIVAR IDENTIFICATION IN ALLOGAMOUS CROPS, E.G. BUNCHING ONION

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Abstract

The genetic uniformity of eight bunching onion accessions were evaluated with 14 SSR markers. The number of alleles per locus ranged from 3 to 7 among the 14 SSR loci, and polymorphism information content from 0.41 to 0.76. All the accessions examined showed very low degrees of uniformity at these polymorphic loci; the average proportion of individuals showing the prevailing genotype at each locus was no higher than 75% in any of the accessions examined. Thus highly heterogeneous are bunching onion cultivars—not only landraces and OP cultivars but also commercially leading F_1 cultivars. Parental lines of bunching onion F_1 cultivars are therefore thought far from pure. It seems impossible to determine an appropriate identification genotype for any of existing cultivars of bunching onion. To make cultivar identification easy and accurate, we propose a breeding procedure, that is, the screening of plants in foundation seed field for individuals homozygous at a small number of SSR loci. The present method will be efficient for cultivar identification and F_1 purity test in any allogamous crop in which inbreeding depression is as severe as in bunching onion.

1. From a genomic library of bunching onion (*Allium fistulosum* L.) more than 60 dinucleotide SSR markers were developed. Of them, 14 markers were used to evaluate the genetic uniformity of eight bunching onion accessions (six F_1 cultivars, one open-pollinated [OP] cultivar and one landrace) and one accession of *A. altaicum* L., the closest wild relative of *A. fistulosum*. In each accession 33 individuals were genotyped. In bunching onion accessions the number of alleles per locus ranged from 3 to 7 among the 14 SSR loci with an average of 4.3, and polymorphism information content (PIC) ranged from 0.41 to 0.76 with an average of 0.59 (Table 1).

2. All the accessions examined showed very low degrees of uniformity at these SSR loci; the proportion of loci polymorphic at 95% level (Pl[95%]: if, in a given accession, one or more individuals per 20 show different genotypes than the prevailing one at given locus, then the locus is judged polymorphic in the accession) was 86% (12/14) in two F_1 cultivars and 100% (14/14) in the other accessions (Table 2). The average proportion over the 14 SSR loci of individuals showing the prevailing genotype at each locus (Pr) was no higher than 75% in any of the accessions.

3. Thus highly heterogeneous are bunching onion cultivars—not only landraces and OP cultivars but also commercially leading F_1 cultivars. Parental lines of bunching onion F_1 cultivars are therefore thought far from pure. The present results have revealed how seriously bunching onion breeders pay attention to avoiding inbreeding depression. It seems impossible to determine an appropriate identification genotype for any of existing cultivars of bunching onion.

4. To make cultivar identification and F_1 purity test easy and accurate in bunching onion and other allogamous crops, we propose a breeding method as follows:

1. Select a small number of highly polymorphic SSR loci that do not link tightly with each other. In F_1 breeding the loci must be appropriately chosen so that the major alleles in the parental lines be different with each other. Two or three SSR loci may be enough for a given cultivar, though we have not collected any simulation data yet.
2. Screen plants in foundation seed field or in earlier generation for individuals homozygous with the prevailing allele at all the SSR loci selected.
3. Harvest foundation seed from the individuals selected or their progeny. In F_1 breeding one parental line must have one allele homozygous at each SSR locus, and the other parental line another allele homozygous.
4. Produce stock seed and then market seed. OP cultivar will be homozygous and uniform at the selected SSR loci, and F_1 cultivar heterozygous and uniform. OP cultivars and parental lines thus bred will exhibit no further inbreeding depression than those bred without the SSR marker selection do, since most loci are not forced into homozygous state by the marker selection itself. As for linkage drag, the selected SSR alleles, if they are prevailing in the mother population, are unlikely to link closely with deleterious genes in coupling phase.

5. Cultivar identification can efficiently be conducted by examining a small number of individuals as to the genotype at a few SSR loci. In addition, the purity of F_1 cultivar can accurately be evaluated by the degree of uniformity at the SSR loci. The present method will be efficient for cultivar identification and F_1 purity test in any allogamous crop in which inbreeding depression is as severe as in bunching onion.

Table 1. Polymorphisms in 14 SSR losi in eight cultivars of bunching onion

Locus	No. of alleles	Allele length in bp	PIC
AFS039	5	273-328	0.57
AFS096	5	165-199	0.66
AFS099	5	235-290	0.57
AFS104	3	186-206	0.64
AFS105	5	246-293	0.66
AFS110	3	232-243	0.54
AFS111	4	208-223	0.47
AFS123	7	180-228	0.62
AFS131	3	148-175	0.60
AFS142	5	239-299	0.76
AFS145	3	197-225	0.41
AFS146	4	184-206	0.54
AFS149	5	176-226	0.68
AFS152	3	213-234	0.54
Mean	4.3		0.59

Table 2. Average of the proportion of individuals showing the prevailing genotype at each locus (Pr) and proportion of polymorphic-at-95% loci (PI[95%]) over 14 SSR markers in bunching onion (*Allium fistulosum*) and its wild relative, *A. altaicum*

Species	Accession	Pr (%)	PI[95%]
A. fistulosum	Cultivar 1 (F_1)	55.9	14/14
	Cultivar 2 (F_1)	69.5	12/14
	Cultivar 3 (F_1)	63.2	14/14
	Cultivar 4 (F_1)	55.8	14/14
	Cultivar 5 (F_1)	48.7	14/14
	Cultivar 6 (F_1)	73.3	12/14
	'Yoshikura' (OP)	50.3	14/14
	'O jima' (landrase)	44.5	14/14
Mean for 8 bunching onion cultivars		57.7	96.4%
A. altaicum	JP138870	49.0	14/14

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