Randomly Amplified Polymorphic DNA in Bulb Onion and Its Use to Assess Inbred Integrity

James M. Bradeen
Department of Horticulture, University of Wisconsin, Madison, WI 53706

Michael J. Havey
U.S. Department of Agriculture-Agricultural Research Service, Department of Horticulture, University of Wisconsin, Madison, WI 53706

Abstract. Commercial bulb-onion (Allium cepa L.) growers often complain that hybrids they have grown successfully for a few years fail to perform at the expected level. Inbreds used to produce hybrid-onion seed rarely have been self-pollinated for more than two generations and retain a high level of heterozygosity. Over time, selection, drift, or contamination of inbreds may contribute to disappointing hybrid performance. We identified randomly amplified polymorphic DNA (RAPD) markers among two inbred onion lines, demonstrated their Mendelian inheritance, and tried to distinguish among and examine changes in independently maintained, publicly released inbred lines of onion. We observed poor agreement between data sets based on genetically characterized and uncharacterized RAPD markers. Our analyses used only genetically characterized RAPD markers and revealed that contamination, in addition to drift and/or selection, likely contributed to differences among independently maintained, publicly released inbreds. However, RAPD markers were not able to distinguish confidently among four related inbreds. RAPD markers will be useful in Allium genetics and breeding, but identifying and characterizing reliable polymorphisms is critical.

Commercial bulb-onion growers often complain that a hybrid they have grown successfully for a few years fails to perform at the expected level. Presumably, changes in hybrid performance may be attributed to changes in the inbreds used to generate the hybrid. Because of severe inbreeding depression, inbred onion lines are generally developed by self pollination of a single bulb for one or two generations followed by cross-pollination among a few selected bulbs (Pike, 1986). The inbred line remains quite heterozygous, allowing changes in genetic constitution over time by drift or selection.

Randomly amplified polymorphic DNA (RAPD) has been proposed as a substitute for or a complement to isozymes and restriction fragment-length polymorphisms (RFLPs) for many applications in plant genetics and breeding (Williams et al., 1990). RAPD markers require only small amounts of DNA, are potentially cheaper and faster, and require less technical expertise than RFLPs. Disadvantages of RAPD markers include dominance (Williams et al., 1990), difficulties with repeatability, and skewed segregation ratios (Echt et al., 1992; Heun and Helentjaris, 1993; Reiter et al., 1992; Riedy et al., 1992; Thomann et al., 1994; Tulsieram et al., 1992; Weeden et al., 1992). There have been two reports of RAPD markers in Allium, assessing variability among species (Wilkie et al., 1993) and cultivars (Roxas and Pefley, 1992). However, neither presented inheritance data. In this study, we identified 12 normally segregating RAPD markers and evaluated changes in independently maintained, publicly released inbreds due to contamination, drift, or selection.

Materials and Methods

Plant material and DNA extraction. ‘Alisa Craig’ (AC) is an European cultivar grown as a show onion in the United Kingdom that has been selected for large size and possesses correlated traits of low pungency, low soluble solids, and poor longevity in storage (Bedford, 1984; Hosfield et al., 1977a, 1977b). ‘Brigham Yellow Globe’ (BYG) is a cultivar grown in the eastern United States primarily as a storage onion and is characterized by high pungency, high soluble solids, and good longevity in storage (Magruder et al., 1941). AC43 and BYG 15-23 are inbreds developed by self-pollinating single bulbs for one and two generations, respectively. These two inbreds and an F1 family from self-pollination of a single F1 plant were the gift of J.F. Watson, SunSeeds, Brooks, Ore.

Green leaf tissue was harvested from at least 20 seedlings each of BYG15-23, AC43, 59 F, families from self-pollination of F1 bulbs from BYG15-23 x AC43 (seed lots listed by Bradeen, 1994), and four publicly released inbred lines (Table 1) maintained at five separate locations [U.S. Dept. of Agriculture (USDA) and one company in Wisconsin, two companies in Idaho, and one in Oregon]. Harvested tissue was frozen in liquid nitrogen and lyophilized. DNA was extracted and purified through CsCl gradients (Murray and Thompson, 1980). Concentrations were determined spectrophotometrically and adjusted to 1 ng DNA/µl with sterile distilled water.

RAPD amplification conditions and procedures. Amplifications were carried out in a 15-µl volume with 50 mM KCl, 20 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2 mM MgCl2, 100 µM each of dATP, dCTP, dGTP, and dTTP (dNTPs), 5 ng template DNA, 0.33 µM decamer primer, and 0.5 unit Taq polymerase (Promega, Madison, Wis.). A total of 580 decamer primers (Operon, Alameda, Calif.) was examined, sets A-E and AA-AX (each set contains 20 numbered primers; sequences listed by Bradeen, 1994). Thermal cycler (9600 PCR machine; Perkin-Elmer, Norwalk, Conn.) con-
The first consisted only of bands segregating at the expected 3:1 ratio (subsequently referred to as genetically characterized). The second set consisted of polymorphisms not segregating in BYG15-23 x AC43 (referred to as genetically uncharacterized). The matrix correlation was calculated for the simple matching (SM; Euclidean distance) matrices of the two data sets (NTSYS-pc 1.70, Exeter Software, Setauket, N.Y.). The SM matrix of the genetically characterized data set was used for UPGMA cluster analysis (NTSYS) and the raw genetically characterized data set for principal component analysis (PCA) (SAS Institute, Cary, N.C.).

Results and Discussion

Identification and segregation of RAPD markers. Of 580 primers examined, 527 failed to amplify reliably a polymorphism between BYG15-23 and AC43. Of the 527 primers, 22 did not amplify any clearly defined bands, 30 amplified a polymorphism that was ambiguous or could not be repeated, and 475 amplified distinct, repeatable bands but detected no polymorphisms. This low level of putative polymorphism may be due, in part, to the highly heterozygous nature of onion and the dominance of RAPD markers. Of the remaining 53 primers that revealed repeatable polymorphisms among the parents (9.1% of the total number evaluated), 10 amplified putative RAPD markers that did not segregate, possibly due to heterozygosity in one parent, and 28 were eliminated due to ambiguities in the presence of polymorphic bands among F1 families; 15 primers (2.6% of the original 580) revealed one storable segregating RAPD each. Twelve of the RAPD markers segregated at the expected 3:1 ratio (P > 0.05) for a dominant marker (Fig. 1, Table 2). No codominant RAPD markers were detected. Linkage analysis of these 12 polymorphisms revealed three associations: DO3 and D12 at a recombination level of 0.18, AD19 and AE09 at 0.09, and Cl5 and AF12 at 0.07. Decamer primers were less efficient at detecting polymor-
Table 2. Observed segregation ratios and chi-square goodness-of-fit values to expected 3:1 segregation ratios for 1.5 randomly amplified polymorphic DNA markers.

| Primer | A-:aa | $\chi^2$ | $P$ |
|--------|-------|----------|-----|
| C15    | 46:13 | 0.28     | 0.60|
| D03    | 41:18 | 0.95     | 0.33|
| D10    | 42:17 | 0.46     | 0.50|
| D12    | 40:19 | 1.63     | 0.20|
| AB14   | 36:23 | 6.15     | 0.01*|
| AB16   | 50:9  | 2.99     | 0.08|
| AB20   | 50:9  | 2.99     | 0.08|
| AD19   | 43:16 | 0.14     | 0.71|
| AE09   | 46:13 | 0.28     | 0.60|
| AF07   | 51:8  | 4.12     | 0.04*|
| AF12   | 42:17 | 0.46     | 0.50|
| AG19   | 42:17 | 0.46     | 0.50|
| AJ14   | 54:5  | 8.59     | 0.00*|
| AK20   | 45:14 | 0.05     | 0.82|
| API2   | 46:13 | 0.28     | 0.60|

*Significantly deviating from expected ratios at $P < 0.05$.

Phenotypes in onion than hybridizations of random cDNAs. Only 9.1% of the primers detected a reliable polymorphism between AC43 and BYG15-23 and 2.1% of all primers revealed polymorphisms segregating at expected Mendelian ratios. Bark and Havey (1995) observed that 67% of cDNA probes detected RFLPs between AC43 and BYG15-23 digested with four restriction enzymes.

Although we have not established segregations for all of the RFLPs, their frequency in onion suggests that RFLPs may provide more numerous and useful codominant markers for map development. However, to identify RAPD markers more efficiently in a highly heterozygous crop such as onion, a better approach would be to evaluate F$_3$ family DNAs to reveal segregating RAPD markers that appear monomorphic between heterozygous parents.

**Inbred integrity.** For this study, we specifically requested N-cytoplasmic lines as determined by pedigree. Chloroplast clones differentiating N and S cytoplasms (Havey, 1993) confirmed that all USDA accessions possessed only N cytoplasm. S cytoplasm was detected in MSU611B from companies 1, 2, and 4; in MSU826B from companies 1 and 2; and trace amounts in MSU4535B and MSU5718B from company 1 (Fig. 2). Tatebe (1968), Corriveau and Coleman (1988), and Havey (1995) established maternal inheritance of the chloroplast DNA in *A. cepa*. Therefore, contamination of the N-cytoplasmic inbreds with S cytoplasm most likely occurred via seed or bulb mixtures due to human error in maintaining the inbred lines. Because the S-cytoplasmic lines are male-sterile, it is not possible for S cytoplasm to be transferred to the N-cytoplasmic maintainer line by infrequent paternal or biparental inheritance of the cpDNA.

Each of the 12 primers generated at least one polymorphism among or within inbred lines and revealed 11 genetically characterized and 11 uncharacterized RAPD markers [C15, D03, D12, AB16, AB20, AD19, AE09 (two polymorphisms), AF12, and AG19 (two polymorphisms)] (Fig. 3; Table 3). All primers except AB16 detected polymorphisms among inbred lines for the geneti-

![Fig. 2. Autoradiograph of inbred DNA digested with *Bgl II* and hybridized with orchid chloroplast clones 3, 4, and 6a differentiating male-fertile (N) and male-sterile (S) cytoplasms.](image)

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![Fig. 3. Randomly amplified polymorphic DNA for AC43, BYG15-23, and inbred lines generated using primer AG19. The genetically characterized band (GC) is polymorphic within and across inbred lines. Additionally, other bands that were not genetically characterized (numbers 1 and 2) were polymorphic within and among inbred lines.](image)

Fig. 3. Randomly amplified polymorphic DNA for AC43, BYG15-23, and inbred lines generated using primer AG19. The genetically characterized band (GC) is polymorphic within and across inbred lines. Additionally, other bands that were not genetically characterized (numbers 1 and 2) were polymorphic within and among inbred lines.
Table 3. Size and presence (1) or absence (0) of genetically characterized (C) and uncharacterized (U) randomly amplified polymorphic DNA (RAPD) markers for parents of segregating family and inbred lines.

| Inbred  | Source | Primer  |
|---------|--------|---------|
|         |        | C15 | C15 | D03 | D03 | D10 | D12 | D12 | AB16 | AB16 |
|         |        | 750* | 780 | 720 | 670 | 650 | 550 | 570 | 1160 | 1070 |
| AC43    |        | 1   | 1   | 1   | 1   | 1   | 1   | 0   | 0    | 0    |
| BYG15-23|       | 0   | 1   | 0   | 1   | 0   | 0   | 1   | 1    | 0    |
| MSU611B| Company 1 | 0   | 1   | 0   | 1   | 1   | 0   | 1   | 1    | 0    |
|        | Company 2 | 0   | 1   | 0   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | Company 3 | 0   | 0   | 0   | 1   | 1   | 0   | 1   | 1    | 0    |
|        | Company 4 | 0   | 0   | 0   | 1   | 1   | 0   | 1   | 1    | 0    |
|        | USDA     | 0   | 0   | 0   | 1   | 1   | 0   | 1   | 1    | 0    |
| MSU826B| Company 1 | 0   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 1    |
|        | Company 2 | 0   | 1   | 1   | 1   | 1   | 0   | 1   | 1    | 0    |
|        | Company 3 | 1   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | Company 4 | 1   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | USDA     | 0   | 1   | 0   | 1   | 1   | 0   | 1   | 1    | 0    |
| MSU4535B| Company 1 | 0   | 1   | 1   | 0   | 0   | 0   | 0   | 1    | 0    |
|        | Company 2 | 0   | 1   | 1   | 0   | 1   | 0   | 0   | 1    | 0    |
|        | Company 3 | 0   | 1   | 0   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | Company 4 | 0   | 1   | 0   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | USDA     | 0   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
| MSU5718B| Company 1 | 0   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | Company 2 | 0   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | Company 3 | 0   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | Company 4 | 0   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
|        | USDA     | 0   | 1   | 0   | 1   | 1   | 0   | 0   | 1    | 0    |
| MSU4535B| Company 1 | 0   | 1   | 1   | 0   | 0   | 0   | 0   | 1    | 0    |
|        | Company 2 | 0   | 1   | 1   | 0   | 0   | 0   | 0   | 1    | 0    |
|        | Company 3 | 0   | 1   | 1   | 1   | 0   | 0   | 0   | 1    | 0    |
|        | Company 4 | 1   | 1   | 1   | 1   | 0   | 0   | 0   | 1    | 0    |
|        | USDA     | 0   | 1   | 1   | 1   | 1   | 0   | 0   | 1    | 0    |
| MSU5718B| Company 1 | 0   | 1   | 1   | 0   | 0   | 0   | 0   | 1    | 0    |
|        | Company 2 | 0   | 1   | 1   | 0   | 0   | 0   | 0   | 1    | 0    |
|        | Company 3 | 0   | 1   | 1   | 1   | 0   | 0   | 0   | 1    | 0    |
|        | Company 4 | 0   | 1   | 1   | 1   | 0   | 0   | 0   | 1    | 0    |
|        | USDA     | 0   | 1   | 0   | 1   | 1   | 0   | 0   | 1    | 0    |

*Mostly characterized markers; however, the genetically characterized polymorphism from primer AF12 could not be unambiguously scored for all inbreds and was excluded. Two genetically characterized (AB14 and AP12) and three uncharacterized (D03, AD19, and AF07) RAPD markers were polymorphic among inbred lines but monomorphic within an inbred line (Table 3). Differences were detected among sources of the same inbred lines for pairs of linked genetically characterized RAPD markers, e.g., MSU5718B from companies 1 and 4 for primers AD19 and AE09 (Table 3). The matrix correlation between the genetically characterized and uncharacterized SM matrices was only 0.38, indicating substantial disagreement between the two data sets. The 11 genetically characterized and 11 uncharacterized polymorphisms identified
Table 3 (continued).

| Primer | AG19 C | AG19 U | AG19 U | AK20 C | AP12 C |
|--------|--------|--------|--------|--------|--------|
| AC43   | 1      | 0      | 1      | 1      | 1      |
| BYG15-23 | 0      | 0      | 1      | 0      | 0      |
| MSU611B: Company 1 | 0  | 0      | 1      | 1      | 0      |
| Company 2 | 1  | 1      | 1      | 0      | 0      |
| Company 3 | 0  | 0      | 1      | 1      | 0      |
| Company 4 | 0  | 0      | 0      | 1      | 0      |
| USDA   | 0      | 0      | 1      | 1      | 0      |
| MSU826B: Company 1 | 1  | 1      | 1      | 1      | 1      |
| Company 2 | 1  | 1      | 1      | 0      | 0      |
| Company 3 | 1  | 1      | 0      | 1      | 0      |
| Company 4 | 1  | 1      | 1      | 1      | 1      |
| USDA   | 1      | 1      | 1      | 1      | 1      |
| MSU4535B: Company 1 | 1  | 1      | 0      | 1      | 0      |
| Company 2 | 1  | 1      | 0      | 1      | 0      |
| Company 3 | 1  | 1      | 0      | 1      | 0      |
| Company 4 | 1  | 1      | 1      | 1      | 0      |
| USDA   | 1      | 1      | 0      | 1      | 0      |
| MSU5718B: Company 1 | 1  | 1      | 0      | 1      | 0      |
| Company 2 | 1  | 1      | 1      | 1      | 0      |
| Company 3 | 1  | 0      | 0      | 1      | 0      |
| Company 4 | 1  | 1      | 0      | 1      | 0      |
| USDA   | 1      | 0      | 0      | 0      | 0      |

C = genetically characterized; segregations established.
U = genetically uncharacterized; segregations not demonstrated. For AE09 and AF12, the uncharacterized RAPD was polymorphic between AC43 and BYG15-23 but did not segregate.
Primers refer to Operon kit by letter and number, see Materials and Methods.
Sizes in base pairs estimated according to Schaffer and Sederoff (1981) using equal mixture of bacteriophage λ singly digested with HindIII and doubly digested with AvaI and BamHI.

Fig. 4. UPGMA cluster analysis of independently maintained, publicly released inbreds using the simple matching (SM) matrix from genetically characterized randomly amplified polymorphic DNA markers. Scale shows SM coefficients.

Fig. 5. Plot of the first three principal components generated from genetically characterized randomly amplified polymorphic DNA markers among independently maintained, publicly released inbreds. (■) MSU611B; (▲) MSU826B; (♦) MSU4535B; (●) MSU5718B; (▼) AC43; and (∇) BYG15-23. Numbers in parentheses under axis labels refer to the percent variation explained by the corresponding principal component.
among inbreds is a limited number, and the matrix correlation may have been greater if more data points were available. The literature is divided on the use of genetically uncharacterized RAPD markers in population studies. Demeke et al. (1992), Fukuoka et al. (1992), Heusden and Bachmann (1992), and Santos et al. (1994) support their use, while Hu and Quiros (1991) used segregation data and Thormann et al. (1994) completed Southern (1975) analyses to verify that polymorphic bands of similar size were homologous before using them for diversity studies. Because of the potential risk of misinterpreting relationships using the genetically uncharacterized RAPD markers, we omitted these data from our analyses. All inferences to population changes and relationships among inbreds were made solely on genetically characterized polymorphisms.

If a genetically characterized RAPD was present in all but one accession of an inbred, its loss could be due to drift or selection [MSU826B from Company 2 (Fig. 3)]. However, the appearance of a genetically characterized polymorphism in one accession and its absence in all other accessions could be evidence of contamination. This scenario occurred with MSU611B from Company 2; primer AG19 failed to amplify the genetically characterized polymorphic band for all other accessions of MSU611B (Fig. 3).

Other examples of potential contamination were observed for inbreds MSU611B from Company 1, MSU826B from Company 2, and MSU4535B from Company 4 with primer AB20 (Table 3).

As more RAPD markers become available and more genotypes are evaluated, we will be able to assess more confidently changes in independently maintained inbreds. Nevertheless, it is clear from this study that genetically characterized RAPD markers were able to reveal differences within a narrow genetic background.

We tried to use the genetically characterized RAPD markers to distinguish among the four inbreds. UPGMA cluster analysis of the SM matrix failed to confidently separate the inbreds (Fig. 4). PCA explained 62.4% of the observed variation with the first three principal components (Fig. 5). There was a tendency for different sources of the same inbred line to cluster, e.g., accessions of MSU611B formed a loose group (Figs. 4 and 5). However, MSU826B x Company 2 was weakly placed in the MSU611B cluster (SM coefficient of 0.697). For UPGMA cluster analysis and PCA, AC43 and BYG15-23 were distant from each other (Figs. 4 and 5) as expected because we selected RAPD markers segregating in this cross. The inability of our 11 genetically characterized RAPD markers to separate the four groups of inbreds clearly may be due to the limited data set, close relationships among the inbred lines, contamination as evidenced by the presence of S cytoplasm (Fig. 2) or the appearance of genetically characterized fragments in one inbred (e.g., AB20 in Table 3), selection or drift [loss of genetically characterized band in MSU826B (Fig. 3)], or the inefficiency of dominant markers in a highly heterozygous crop.

Because of the poor agreement between the genetically characterized and uncharacterized data sets, we chose to omit the uncharacterized polymorphisms from our analyses. As the numbers of genetically characterized RAPD markers increase, it will be possible to answer more sophisticated questions. Eventually, enough codominant RAPD markers or other PCR-based markers may be available to allow the seed industry to characterize inbreds quickly and easily and document changes in their constitution. However, there are serious caveats associated with the use of RAPD markers. Before any application, it is crucial that RAPD markers be characterized, either by segregation analysis or band sequence homology.

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