Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes

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**Abstract.** Genetic similarity among 45 *Brassica oleracea* genotypes was compared using two molecular markers, random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphisms (RFLPs). The genotypes included 37 broccolis (var. *italica*), five cauliflowers (var. *botrytis*) and three cabbages (var. *capitata*) which represented a wide range of commercially-available germplasm, and included open-pollinated cultivars, commercial hybrids, and inbred parents of hybrid cultivars. Fifty-six polymorphic RFLP bands and 181 polymorphic RAPD bands were generated using 15 random cDNA probes and 62 10-mer primers, respectively. The objectives were to compare RFLP and RAPD markers with regard to their (1) sampling variance, (2) rank correlations of genetic distance among sub-samples, and (3) inheritance. A bootstrap procedure was used to generate 200 random samples of size n (n = 2, 3, 5, ..., 55) independently from the RAPD and RFLP data sets. The coefficient of variance (CV) was estimated for each sample. Pooled regressions of the coefficient of variance on bootstrap sample size indicated that the rate of decrease in CV with increasing sample size was the same for RFLPs and RAPDs. The rank correlation between the Nei-Li genetic similarity values for all pairs of genotypes (990) was 0.745. Differences were observed between the RFLP and RAPD dendrograms of the 45 genotypes. Overlap in the distributions of rank correlations between independent sub-samples from the RAPD data set, compared to correlations between RFLP and RAPD sub-samples, suggest that observed differences in estimation of genetic similarity between RAPDs and RFLPs is largely due to sampling error rather than due to DNA-based differences in how RAPDs and RFLPs reveal polymorphisms. A crossing algorithm was used to generate hypothetical banding patterns of hybrids based on the genotypes of the parents. The results of this study indicate that RAPDs provide a level of resolution equivalent to RFLPs for determination of the genetic relationships among genotypes.

**Key words:** *Brassica oleracea* var. *italica* – *B. oleracea* var. *botrytis* – *B. oleracea* var. *capitata* – Genetic distance – Genetic similarity – Crossing algorithm – Cluster analysis

**Introduction**

Knowledge of genetic similarity (distance) between genotypes is useful in a breeding program because it facilitates efficient sampling and utilization of germplasm resources. The breeder can use genetic similarity information to make informed decisions regarding the choice of genotypes to cross for the development of populations, or to facilitate the identification of diverse parents to cross in hybrid combinations in order to maximize the expression of heterosis (Smith et al. 1990; Nienhuis and Sills 1992).

Estimates of genetic similarity based on restriction fragment length polymorphisms (RFLPs) have been shown to be consistent with expectations based on known breeding behavior and pedigrees in numerous crops, including maize (Lee et al. 1989; Melchinger et al. 1990a, b; Smith et al. 1990; Messmer et al. 1992) and among *Brassica oleracea* genotypes (Nienhuis et al. 1992).
In 1990 two groups of scientists independently described a new technique for detecting polymorphism at the DNA level using PCR with single random oligonucleotide primers of arbitrary sequence (Welsh and McClelland 1990; Williams et al. 1990). Such polymorphisms can occur either as a result of base pair or positional changes in the restriction sites (RFLP) or primer sites (RAPD) which flank a chromosomal location. RFLPs can be detected by hybridization of labeled DNA clones containing sequences homologous to a portion of the chromosomal fragment; whereas, RAPDs are detected by differential amplification of DNA fragments. The principal advantage of RAPDs compared to RFLPs is the technical simplicity of the methodology (Williams et al. 1990; Caetano-Anolles et al. 1991; Paran et al. 1991; Welsh et al. 1991). The principal disadvantage of RAPDs compared to RFLPs is that they are usually dominant rather than codominant markers. In addition, the reproducibility of RAPD banding patterns can be affected by different concentrations of reaction components and cycle conditions (Weeden et al. 1992).

In order for plant breeders to make informed decisions regarding the choice of molecular marker technology, comparisons of the inheritance and reliability of RAPDs and RFLPs are needed. The objectives of this study were to: (1) compare the sampling variance of RFLP vs RAPD markers, (2) compare estimates of genetic similarity based on RFLP and RAPD data, and (3) compare the inheritance of RAPD and RFLP banding patterns within and among three cultivated subspecies of Brassica oleracea, var. botrytis (cauliflower), var. capitata (cabbage) and var. italica (broccoli).

Materials and methods

Germplasm

Forty-five B. oleracea L. genotypes, previously described and characterized in a companion publication by Nienhuis et al. (1992) using RFLPs, were employed in this study. The choice of genotypes was influenced by the desire to focus on broccoli genotypes and also by the quantity and quality of remnant DNA samples. The genotypes included 37 broccolis (var. italica), five cauliflowers (var. botrytis) and three cabbages (var. capitata), which represented a wide range of commercially-available germplasm, and included open-pollinated cultivars, vintage cultivars, commerical hybrids, and inbred parents of hybrid cultivars. In addition, by identifying “off-type” plants in spaced plantings of commerical hybrid plants, the possible female parents corresponding to several hybrid cultivars were identified (Nienhuis et al. 1992).

RAPD and RFLP procedures

Plant DNA was isolated from lyophilized leaf tissue and a restriction endonuclease (EcoR1) was used to digest the crude DNA samples. The procedures for DNA isolation, restriction endonuclease digestion, electrophoresis, Southern blotting, hybridization and autoradiography have all been previously described (Song et al. 1988; Slocum et al. 1993). A total of 16 random Brassica genomic clones containing low-copy- number inserts were individually hybridized to EcoRI-digested total genomic DNA samples. The set of random genomic clones utilized as probes in this study included EW1G03, EW1D02, EW2D03, EW2A06, EW2A07, EW1D03, EW1F08, EW4A05, EW3D07, EW5F07, EW4G11, EW2B12, EW4G08, EW3C10, EW2E07 and EW1E04 (Pioneer Hi-Bred International, Johnston, Iowa). Frequently the probes hybridized to multiple restriction fragments within individual samples, which resulted in complex banding patterns. From one to seven bands, which were polymorphic among this sample of genotypes, were scored for each of the 16 probes resulting in a total of 56 scored fragments.

The same DNA samples used in the previous RFLP analyses were used as templates for RAPD reactions. The DNA was recovered from lyophilized remnant samples that had been stored for 3 years in a -70 °C freezer at Agridyne Incorporated, Salt Lake City, Utah. RAPD reactions were mixed in volumes of 10 μl using the following reagents: 20 ng of genomic template, 100 μM of dNTP, 0.4 μM of primer, 0.6 units of Taq polymerase (Promega, Madison, WIS.), 2.0 mM of MgCl₂, 50 mM of Tris pH 8.5, 20 mM of KCl, 5 μg/ml of BSA, 2.5% of Ficoll 400, and 0.002% xylene cyanole. The reactions were performed in glass capillary tubes in a thermal cycler (Idaho Technology, Idaho Falls, Idaho) programmed to cycle 40 times under the following conditions: for the first two cycles, denaturation for 60 s at 91 °C, annealing for 7 s at 42 °C and elongation for 70 s at 72 °C; the subsequent 38 cycles were run with the denaturation time reduced to 1 s at 91 °C. After amplification the reaction products were separated by electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light with Polaroid 667 film.

The number of polymorphic primers used from sets A, B, C, D, E, F, G, L, M and N (Operon Technologies, Alameda, Calif.) were 8, 10, 8, 4, 3, 4, 8, 10, 4 and 3, respectively. Information on specific primers is available from the authors. Polymorphic bands were classified as intense, medium or faint, based on resolution and degree of amplification (Weeden et al. 1992). Only bands classified as intense or medium were included in the analysis. From one to six bands, which were polymorphic among this sample of genotypes, were scored for each of the 62 probes, resulting in a total of 181 scored bands.

Genetic similarity estimates

Polymorphic RFLP or RAPD bands across all genotypes were each assigned numbers (1, 2, 3,... n) according to decreasing molecular weights. For both RAPD and RFLP data, each band was treated as a unit character, and the genotype was scored for the presence or absence of band and coded as 1 or 0, respectively. Genetic similarities were calculated between pairs of genotypes based on the method similar to that reported by Nei and Li (1979), i.e.,

\[ GS(XY) = C(XY)/[N(XY)] \]

where (GS(XY)) is the measure of genetic similarity between a pair of lines, C(XY) is the number of concordant bands (both present or absent) between lines X and Y, and N(XY) is the total number of bands scored for lines X and Y, respectively. The rank correlation between the RFLP and RAPD genetic similarity matrices was calculated.

Sampling variance

To compare the variance of genetic similarity estimates based on RFLP and RAPD data, 200 random bootstrap samples each of