Genetic Variation among and within United States Collard Cultivars and Landraces as Determined by Randomly Amplified Polymorphic DNA Markers

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Abstract. A collection of collard (*Brassica oleracea* L., Acephala group) germplasm, including 13 cultivars or breeding lines and 5 landraces, was evaluated using randomly amplified polymorphic DNA (RAPD) markers and compared to representatives of kale (Acephala group), cabbage (Capitata group), broccoli (Italica group), Brussels sprouts (Gemifera group), and cauliflower (Botrytis group). Objectives were to assess genetic variation and relationships among collard and other crop entries, evaluate intrapopulation variation of open-pollinated (OP) collard lines, and determine the potential of collard landraces to provide new *B. oleracea* genes. Two hundred nine RAPD bands were scored from 18 oligonucleotide decamer primers when collard and other *B. oleracea* entries were compared. Of these, 147 (70%) were polymorphic and 29 were specific to collard. Similarity indices between collard entries were computed from RAPD data and these ranged from 0.75 to 0.99 with an average of 0.83. Collard entries were most closely related to cabbage (similarity index = 0.83) and Brussels sprouts entries (index = 0.80). Analysis of individuals of an OP cultivar and landrace indicated that intrapopulation genetic variance accounts for as much variation as that observed between populations. RAPD analysis identified collard landraces as unique genotypes and showed them to be sources of unique DNA markers. The systematic collection of collard landraces should enhance diversity of the *B. oleracea* germplasm pool and provide genes for future crop improvement.

Collard (*Brassica oleracea* L.) is an important, leafy-green vegetable crop in the southeastern United States. Traditionally, collard is classified in the same botanical group, Acephala, as kale (Hortus Third, 1976). There are about ten cultivars, and five or more breeding lines readily available from commercial or public sources in the United States. Most of these are open-pollinated (OP) lines and the remainder are more recent F₁ hybrids. Little is known about the genetic makeup of the collard crop and its relationship to the other *B. oleracea* groups such as Italian broccoli or cabbage. There is an additional, generally unrecognized, *B. oleracea* germplasm pool of collard landraces that is being perpetuated by southeastern gardeners and farmers. It is unknown whether these landraces represent unique collard genotypes, and to the author's knowledge there has never been a systematic collection of collard germplasm undertaken in the southeastern United States.

Recent studies of *B. oleracea* using DNA-based markers including restriction fragment length polymorphisms (RFLPs) and randomly amplified polymorphic DNA (RAPD) markers have provided information regarding 1) the genetic relationships between *B. oleracea* and other related Brassica species (Song et al., 1988a; Song et al., 1988b; Demeke et al., 1992; Ren et al., 1995); 2) the use of DNA-based markers as cultivar or genotype fingerprinting tools (Hu and Quiros, 1991; Kresovich et al., 1992); and 3) the diversity existing within the *B. oleracea* germplasm pool (Neinhuis et al., 1993; dos Santos et al., 1994; Kresovich et al.,

1992). dos Santos et al. (1994) recently showed that the use of RAPD markers provide a level of resolution equivalent to RFLPs for determination of genetic relationships among *B. oleracea* genotypes. Likewise, Thorman et al. (1994) presented results that indicated RAPD markers give similar information as RFLP data when comparing genetic relationships of individuals within *B. oleracea* as well as within other Brassica species.

Recent molecular marker evaluations of *B. oleracea* germplasm have provided little information about the collard crop and its relationship to the other crop groups. Song et al. (1988b) included one collard cultivar, 'Georgia', in a study examining genetic relationships between crop groups of *B. oleracea*. In that study the collard entry was more closely related to cabbage representatives than to a kale. However, because Song et al. examined such a small portion of the *B. oleracea* germplasm pool, they did not draw broad conclusions about genetic relationships among the diverse groups. The present study was undertaken to 1) examine genetic variation among United States collard cultivars and landraces using RAPD markers and determine genetic relationships between different collard lines and also between collard lines and other *B. oleracea* crop representatives; 2) evaluate within line variation of an OP cultivar and an OP landrace; and 3) assess the potential of collard landraces in serving as new sources of collard or *B. oleracea* genes.

Materials and Methods

Eighteen entries of collard were obtained for genetic analysis in this study. These entries include 13 named cultivars or breeding lines of collard available from commercial or public sources and an additional five landraces obtained by the author from southeastern gardeners or farmers (Table 1). Although this a limited number of individual lines it is a thorough representation of the commercial collard crop in the United States. In addition to the collard entries, two broccoli, two cabbage, two kale, one cauliflower (Botrytis

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Table 1. Collard and other *Brassica oleracea* entries compared by RAPD genotyping in this study. Each entry is classified by crop, type of line, and source of seed.

Entry	Abbreviation	Crop ²	Type of line	Source
Vates	VA	Col	OP cultivar	Commercial
Morris Heading	MH	Col	OP cultivar	Commercial
Green Glaze	GG	Col	OP cultivar	Commercial
Champion	CH	Col	OP cultivar	Commercial
Georgia	GA	Col	OP cultivar	Commercial
Southern	SO	Col	OP cultivar	Commercial
SC Glaze	SG	Col	OP cultivar	Breeder
SC Header	SH	Col	OP cultivar	Breeder
Top Bunch	TB	Col	F ₁ hybrid	Commercial
Blue Max	BM	Col	F ₁ hybrid	Commercial
Flash	FL	Col	F ₁ hybrid	Commercial
Hicrop	HI	Col	F ₁ hybrid	Commercial
Mezic Zero	MZ	Col	OP landrace	N.C. gardener
J. Killough	JK	Col	OP landrace	Texas gardener
J. Hope	JH	Col	OP landrace	N.C. farmer
G. Simpson	SI	Col	OP landrace	N.C. farmer
G. Summersett	SU	Col	OP landrace	S.C. gardener
Vates kale	VK	Kal	OP cultivar	Commercial
Squire	SK	Kal	OP cultivar	Commercial
Chas. Wakefield	CW	Cab	OP cultivar	Commercial
Market Prize	MP	Cab	F ₁ hybrid	Commercial
Atlantic	AT	Bro	OP cultivar	Commercial
Packman	PA	Bro	F ₁ hybrid	Commercial
Valiant	VL	Bsp	F ₁ hybrid	Commercial
Snow Crown	SC	Cau	OP cultivar	Commercial
Heavicrop	HE	Col	F ₁ hybrid	Commercial

²Crop groups abbreviated as Col = collard, Kal = kale, Cab = cabbage, Bro = broccoli, BSP = Brussels sprouts, and Cau = cauliflower.

group), and one Brussels sprout (Gemmifera group) entries were used for comparison to collard (Table 1). Fifty seed of each entry were sown one seed per cell in a commercial peat mix (Metromix 360, Grace Sierra, Milpitas, Calif.) in 120-cell seedling trays with individual root cell volume of 40 ml. Seedlings were grown in a greenhouse 4 Sept. through 7 Oct. 1992 at which time leaves were sampled. Composite leaf samples of each entry were made by combining 0.15 g of leaf tissue from the youngest leaves from each of 45 seedlings. The composite sample was frozen in liquid N₂, ground and mixed in a mortar and pestle, and stored at -20C until the tissue was used for DNA extraction. In addition to composite sampling of all entries, leaf samples of 20 individual plants from the collard cultivar 'Morris Heading' and the landrace 'J. Hope' were collected and frozen as described above.

DNA was extracted from 2.0 g frozen leaf tissue using the CTAB protocol described by Doyle and Doyle (1987), and duplicate extractions were made for each entry. Single plant genomic DNA was similarly extracted from frozen leaves of the individual plants of the 'Morris Heading' and 'J. Hope' collard entries. Final DNA pellets were solubilized in 0.5 ml TE solution, and DNA content of all samples was determined using a DNA fluorometer (model TKO 100; Hoefer Scientific Instruments, San Francisco). Samples were diluted to a working concentration of 12.5 ng·ml⁻¹ with sterile distilled water.

About 30 decamer nucleotide primers, obtained from Operon DNA Technologies (Alameda, Calif.), were screened on a subset of six samples. Eighteen primers, including Operon-designated primers: A02, A03, A04, A05, A07, A17, A18, J05, J19, K12, K14, K18, T01, T08, T11, T15, T16, and T17; that showed consistent banding patterns and amplification were chosen for use in this

study. The polymerase chain reaction (PCR) DNA amplification protocol was a variation of that reported by Williams et al. (1990). Reactions were performed in a 25-μl volume containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 1.5 mM MgCl₂, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM dNTPs, 30 ng oligonucleotide decamer, 50 ng genomic DNA, and 1.0 unit Taq DNA polymerase (Promega, Madison, Wis.). Reactions were run on all duplicate samples. DNA amplification was performed using a thermal cycler (model 480; Perkin-Elmer-Cetus, Norwalk, Conn.). The thermal cycle used was 94C for 1 min; then 45 cycles of 94C (1 min), 35C (1 min), and 72C (2 min); and finally 72C for 3 min. A negative control PCR tube containing all components except genomic DNA was included in all thermal cycle runs. RAPD samples were electrophoresed on 1.5% agarose gels, stained with ethidium bromide and visualized under UV light (Fig. 1). Gels were photographed with black and white film (no. 55; Polaroid, Cambridge, Mass.).

Photographic negatives of duplicate reactions and gels were compared simultaneously. Amplification products of genotypes were scored as present or absent and identified by associate primer number and size in base pairs. Only amplification products that appeared consistently between runs were rated as present in composite scores of entries or individual plants and any bands that were classified as faint were not included in the data set as these were assumed to be unreliable markers. The size of amplification products were determined by comparison to a 100-bp DNA ladder (BRL, Bethesda, Md.) that was run alongside plant samples on every agarose gel.

Statistical analysis was conducted for the 18 collard entries combined with the eight other *B. oleracea* entries. Each RAPD band across all entries was assigned a number (1, 2, 3,n) and

each was treated as a unit character coded as 1 (present) or 0 (absent). Genetic similarities were calculated between all pairs of entries based on the following formula: $GS(ij) = S^N(i=j) / [S^N(i=j) + S^N(i \neq j)]$; which is a simple matching coefficient where $GS(ij)$ is the genetic similarity between entries i and j , $S^N(i=j)$ and $S^N(i \neq j)$ are the total number of concordant and discordant scores between accessions i and j , respectively (Sneath and Sokal, 1973). The complement of $GS(ij)$, the Genetic Distance [$GD(ij)$], was computed as $GD(ij) = 1 - GS(ij)$; between all entries. A dendrogram was constructed based on the genetic distance matrix data by applying unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using the SAS Proc Cluster program (SAS Institute, Cary, N.C.). An analysis as described above was also performed separately on each population of 20 individuals from each of the two collard entries 'Morris Heading' and 'J. Hope'.

Results and Discussion

Twenty-six *B. oleracea* entries, including 18 collard composites were analyzed as a group and a total of 209 RAPD bands were

scored. These bands ranged in size from 240 to 2300 bp. Between 7 to 16 bands were scored per primer and the average number was 11.5. The number of bands scored per primer for this set of *B. oleracea* entries is within the range observed by others evaluating Brassica germplasm. Hu and Quiros (1991) observed 10 bands per primer, Kresovich et al. (1992) about 8 per primer; and Thorman et al., (1994) 10.5 per primer. A relatively high average number of markers per primer for Brassica species was reported by Ren et al. (1995) who observed over 20 markers per primer. Of the total bands scored in this study, 147 or about 70% were polymorphic. This percentage of polymorphic bands is very similar to that observed in all of the above-cited studies.

In comparing the 26 *B. oleracea* entries, a total of 29 RAPD bands were specific to collard. Of that total, 21 bands were unique to at least one collard cultivar or breeding line. The remaining eight bands were specific to at least one of the collard landraces. Similarity indices among collard cultivars and lines ranged from 0.73 to 0.99 with a mean of 0.83 (Table 2). Two pairs of entries, 'Blue Max' and 'HiCrop', and 'Georgia' and 'Southern', had the highest similarity indices of 0.98 and 0.99, respectively. These two

pairs represent identical or nearly identical lines or genotypes, and phenotypic observations confirm this (data not shown). The differences observed between 'Blue Max' and 'HiCrop', both sold as F_1 hybrids, may represent the level of error in genotyping lines with RAPD markers in this study since all individuals of the hybrids would be expected to be genetically identical. Although that may be true with 'Georgia' and 'Southern' as well, it is also possible that the slight variations observed between these two latter, OP populations may be due to variation that exists between different lots or sources of these entries.

Similarity indices between collard cultivars and landraces ranged from 0.75 to 0.96, and the mean index was 0.82. Although comparison of collard landraces to cultivars indicates that the two groups are closely related, no landrace was exactly like any cultivar. Thus, each landrace represents a unique collard genotype. When collard entries were compared to other *B. oleracea* entries, collard were found to be most similar to cabbage (mean index = 0.83) and next to a Brussels sprouts variety (mean index = 0.80). In general, collard varieties had fewer bands in common with two broccoli (mean index = 0.72), one cauliflower (mean index = 0.74), and two kale (mean index = 0.73).

Relationships between collard and other *B. oleracea* entries, determined by UPGMA cluster

Fig. 1. RAPD products of collard and other *Brassica oleracea* entries generated by primer OPA-05. (A) Resulting gel electrophoresis and band visualization of composite samples of collard and other entries. The order of samples from left to right is identical to the order of entries listed in Table 1. Lanes 10 and 20 contain the 100-bp DNA ladder; darkest band near center of lane is 600bp. (B) Resulting profiles of 20 individuals (first 20 sample lanes) from the 'Morris Heading' population and also the composite sample of this cultivar (far right lane). Lanes 9 and 17 contain the 100-bp DNA ladder.

Table 2. Similarity index ($\times 100$) of 18 collard entries and 8 other *Brassica oleracea* lines. Entries are designated by abbreviations given in Table 1.

	Collard cultivars/lines													Collard landraces					Kale		Cabbage		Broccoli		BS	CA
	VA	MH	GG	CH	GA	SO	SG	SH	TB	BM	HI	FL	HE	MZ	JK	JH	SI	SU	VK	SK	CW	MP	AT	PA	VL	SC
VA	---	87	80	97	86	85	83	90	89	79	78	89	78	82	85	85	84	82	71	72	83	84	72	73	79	75
MH		---	80	88	86	87	85	85	83	79	78	81	79	79	85	89	83	83	70	72	83	82	69	71	78	72
GG			---	79	79	78	94	79	84	78	78	76	73	78	78	84	82	78	72	76	80	79	72	70	79	74
CH				---	85	85	83	91	89	80	79	89	87	83	83	85	86	82	73	71	86	85	72	74	82	76
GA					---	99	83	84	86	82	82	81	81	77	96	90	84	83	71	74	84	86	72	71	79	71
SO						---	83	83	85	83	83	81	80	76	96	90	84	83	71	73	84	86	71	70	79	71
SG							---	82	86	83	82	79	77	77	82	86	85	82	75	75	82	83	73	71	80	75
SH								---	88	80	80	86	83	81	83	83	82	80	71	75	83	85	74	74	80	77
TB									---	83	82	84	86	81	84	87	86	84	73	75	85	86	73	72	85	76
BM										---	98	78	78	72	81	84	80	80	72	71	81	83	73	74	80	75
HI											---	77	77	83	81	83	79	79	72	82	80	83	71	72	78	74
FL												---	82	81	82	79	78	79	74	73	85	84	73	73	79	77
HE													---	75	81	78	78	82	70	68	79	81	72	71	77	73
MZ														---	77	77	80	80	83	82	83	79	76	75	79	75
JK															---	89	82	83	72	75	84	85	72	71	79	72
JH																---	92	83	71	71	86	86	70	69	80	73
SI																	---	81	72	70	83	83	70	68	80	74
SU																		---	72	72	81	85	70	70	78	73
VK																			---	90	77	74	70	68	74	67
SK																				---	76	76	74	74	75	70
CW																					---	92	77	76	83	76
MP																						---	74	76	82	75
AT																							---	93	76	82
PA																								---	78	82
VL																									---	77
SC																										---

analysis, are presented in Fig. 2. In general, crop groups fell into distinct clusters. 'Snow Crown' cauliflower clustered independently, but closely, to the two broccoli entries, and the two kale entries clustered together. The one Brussels sprouts entry was also independently clustered as were the two cabbage entries, however, all three of these lines fell into a broad cluster that contained all collard entries. These results are in agreement with those of Song et al. (1988b) who found that one collard entry, among a group of *B. oleracea* representatives, had a relatively close genetic relationship to cabbage entries.

Within the overall collard cluster, several subclusters were identified (Fig. 2). One subgroup of entries contained a majority of collard cultivars. Two closely related open-pollinating cultivars in this group are 'Vates', one of the most widely grown collard cultivars, and 'Champion', a selection from 'Vates'. A similarity index of 0.97 for these two entries confirms their common genetic background. Three F_1 hybrids ('Heavycrop', 'Flash', and 'Top Bunch') as well as one breeding line ('SC Header') were also present in this cluster. It would not be unexpected if these entries had 'Vates' in their background, since that line was an industry standard for more than a decade.

A second subgroup of collard contained the open-pollinating line, 'Morris Heading' and two landraces, 'G. Simpson' and 'J. Hope'. The two latter entries were obtained from two different growers in Sampson Co., North Carolina. These two lines, like 'Morris Heading' tend to form a loose head at the main stem apex near the time of crop maturity. Also in this subgroup are the nearly identical entries, 'Georgia' and 'Southern', and also the landrace 'J. Killough'. This landrace had a similarity index of 0.96 between it and the former two cultivars, indicating that it could have been a selection out of those older open-pollinating cultivars.

Two glossy collard entries, 'Green Glaze' and 'SC Glaze' had a similarity index of 0.94, indicating a relatively similar genetic background, and they did not cluster with a particular group of collard entries. One landrace, 'G. Summersett', also appeared distinct from other collard entries. This line has a very large stature and also tends to form a loose head at maturity. The hybrids, 'Blue Max' and 'HiCrop', also clustered apart from other collard entries.

One collard landrace, 'Mesic Zero' was determined to be more closely related to two kale entries than to any collard. Although the person that saved 'Mesic Zero' called it a collard, it is indeed intermediate in appearance between collard and kale. This landrace exhibits significant plant-to-plant variation; however, most plants have kale-like leaves. It is possible that this line could represent a cross of collard by kale.

Genetic variation within the open-pollinating cultivar 'Morris Heading' and landrace 'J. Hope', as measured by RAPD analysis, was as great as the variation measured among collard composite entries (Table 3). The number of reproducible RAPD markers among 'Morris Heading' individuals was 168 and among 'J. Hope' individuals was 175. In both populations, about 55% of the bands were polymorphic. Mean similarity index between individuals of 'Morris Heading' was 0.82, and between 'J. Hope' individuals was 0.81. Computation of a similarity index between individuals and the composite sample of each respective population indicated that the magnitude of variation for such comparisons is equal to comparisons between individuals and that no single individual was exactly like the composite. In analyzing individuals for RAPD fingerprints, 13 bands were identified in at least one 'Morris Heading' individual that were not observed in the composite sample or any other collard entry. Likewise, 11 bands were found among 'J. Hope' individuals that were not previously identified.

Fig. 2. Dendrogram for 26 collard and other *Brassica oleracea* entries resulting from UPGMA cluster analysis of genetic distances derived from RAPD marker scores. Relationships between entries are quantified using the complementary similarity indices.

These bands were always polymorphic and they usually occurred at low frequencies in the population, at least partially explaining why they were not visible in composite samples. The intrapopulation genetic diversity observed in this study likely contributes a level of diversity to the collard germplasm pool that is of equal significance as the intercultural or population variation. The author is unaware of other studies of *B. oleracea* where RAPD markers have been used to measure intrapopulation genetic variation. If equal levels of within line variance are found in open-pollinating lines of other crop groups such as cabbage or broccoli, this would

represent a greater level of genetic variation previously unrecognized in studies examining only interpopulation genetic differences.

Although collard is officially classified as *B. oleracea* Acephala group, placing it in the same category as the kale crop, observations from this study indicate that collard is more closely related to cabbage (*Capitata* group). This conclusion is in agreement with that made by Song et al. (1988b) who found a collard individual to be more closely related to cabbage entries than to those of any other *B. oleracea* crop group. Indeed, this conclusion is more intuitively expected since collard has been commonly grown simultaneously with cabbage in the southeastern United States for two to three centuries. On the contrary, kale is a relative newcomer to the Southeast, as little was grown in this region until more recent times.

Table 3. Genetic similarity indices among individuals of the open-pollinating cultivar ‘Morris Heading’ and the landrace ‘J. Hope’.

Index comparison	Population	
	Morris heading	J. Hope
	Similarity index	
Mean similarity between individuals	0.82	0.81
Range of similarity between individuals	0.75–0.89	0.74–0.89
Mean similarity between an individual and the composite	0.80	0.81
Range of similarity between an individual and the composite	0.76–0.85	0.75–0.85

Several authors (Song et al., 1988b; Williams, 1985) have used the separate crop group label, “Sabellica”, to describe the collard group. Although this terminology is not officially recognized (Hortus Third, 1976) or used widely, it may be a reasonable approach to designate the collard as a separate group and to distinguish it from kale. Another approach would be to include collard germplasm as part of the Capitata group. However, a greater number of comparisons between collard and cabbage would be required before concluding that collard germplasm should be classed with the much larger Capitata group.

Collard landraces evaluated in this research represent unique genotypes compared to collard cultivars and breeding lines. RAPD markers specific to the landraces were identified when composite samples of collard cultivars and landraces, as well as cultivars of other cole crops, were compared. Additional unique markers were also uncovered when individuals of OP cultivar and landrace populations were examined. Thus, collard landraces are likely a pool of new genes that might be useful specifically in a collard breeding program, and in general for *B. oleracea* crop improvement. A systematic collection of collard landraces from sources in the southeastern United States should prove a worthwhile endeavor and help to increase the store of *B. oleracea* germplasm adapted to this region.

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