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ORIGINAL PAPER

Heterosis for horticultural traits in Broccoli

Anna L. Hale · Mark W. Farnham · M. Ndambe Nzaramba · Collins A. Kimbeng

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Abstract Over the last three decades, broccoli (*Brassica oleracea* L., Italica Group) hybrids made by crossing two inbred lines replaced open-pollinated populations to become the predominant type of cultivar. The change to hybrids evolved with little or no understanding of heterosis or hybrid vigor in this crop. Therefore, the purpose of the present study was to determine levels of heterosis expressed by a set of hybrids derived by crossing relatively elite, modern inbreds ($n = 9$). An additional objective was to determine if PCR-based marker derived genetic similarities among the parents can be useful to predict heterosis in this crop. Thirty-six hybrids derived from a diallel mating design involving nine parents were evaluated for five horticultural characters including the head characteristics of head weight, head stem diameter, and maturity (e.g., days from transplant to harvest), and the plant vigor characteristics of plant height, and plant width in four environments. A total of 409 polymorphic markers were generated by 24 AFLP, 23 SRAP and 17 SSR primer combinations. Euclidean distances between parents were determined based on phenotypic traits. About half of the hybrids exhibited highparent heterosis for head weight (1–30 g) and stem diameter (0.2–3.5 cm) when averaged across environments. Almost all hybrids showed highparent heterosis for plant height (1–10 cm) and width (2–13 cm). Unlike other traits, there was negative heterosis for maturity, indicating that heterosis for this character in hybrids is expressed as earliness. Genetic similarity estimates among the nine parental lines ranged from 0.43 to 0.71 and were significantly and negatively correlated with highparent heterosis for all traits except for stem diameter and days from transplant to harvest. Euclidean distances were not correlated with heterosis. With modern broccoli inbreds, less heterosis was observed for head characteristics than for traits that measured plant vigor. In addition, genetic similarity based on molecular markers was more highly correlated with plant vigor characteristics than head traits. Unlike with molecular marker-based estimates of genetic similarity, euclidean distance determined using phenotypic trait data was not predictive of heterosis. In conclusion, this study has documented heterosis in *Brassica oleracea* L., and the ability to predict heterosis in this crop using molecular marker-based estimates of genetic similarity among parents used in producing the hybrid.

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A. L. Hale (✉) · M. W. Farnham
US Vegetable Laboratory, USDA/ARS,
2700 Savannah Hwy, Charleston, SC 29414, USA
e-mail: annajohnsonhale@gmail.com

M. N. Nzaramba
Department of Horticultural Sciences,
Texas A&M University, College Station, TX 77843, USA

C. A. Kimbeng
Louisiana State University Agricultural Center,
Baton Rouge, LA 70803, USA

Introduction

Broccoli (*Brassica oleracea* L., Italica Group) is an important horticultural crop in the US and is produced

commercially from hybrid seed. In the early 1960s, broccoli was a strictly open pollinated crop, but by the end of the decade and into the 1970s, some seed companies began to sell hybrid cultivars. By the 1990s virtually all commercial broccoli cultivars were hybrids; however, this conversion occurred with little knowledge of heterosis in broccoli. Hulbert and Orton (1984) observed significant heterosis for earliness of maturity in broccoli, but since then, to the knowledge of the authors, nothing has been published regarding heterosis in this crop.

Despite the lack of literature reporting hybrid vigor in broccoli, there is ample evidence supporting this phenomenon in closely related species. Riaz et al. (2001) analyzed plant height, days to maturity, and seed yield in *Brassica napus* L. and observed midparent heterosis for seed yield ranging from 26 to 169%. Shen et al. (2005) also reported heterosis in seed yield as well as seed oil content in *B. napus;* however, seed yield per plant was much more heterotic than oil content. Heterosis was reported in *Brassica carinata* A. Braun for days to maturity, plant height, seed yield, and numerous other agronomic traits (Teklewold and Becker 2005). The aforementioned studies on related species indicate that the amount of heterosis observed depends on the trait.

Hybrid development is an expensive and time-consuming endeavor. The key to, and one of the most costly aspects of developing good hybrids, is identifying parental combinations which produce superior progeny. Increasing evidence suggests that, for some traits, there is a strong correlation between genetic distance between the parents and F_1 performance or heterosis (Moll et al 1965; Teklewold and Becker 2006). Falconer and Mackay (1996) discussed the theory behind this relationship, explaining that differences in allelic frequency between two parental lines, among other things, affect the amount of heterosis observed in the F_1. Through algebraic proof, they theorize that heterosis is dependant on dominance, and loci with no dominance cause no heterosis. In other words, if the parental inbred lines do not differ in allelic frequency, no heterosis will be observed. Furthermore, heterosis will increase with the number of alleles that are differentially fixed in the two parents. This model assumes that, in theory, each inbred line is homozygous at all loci, and the more distantly related two parents are from one another, the less likely they will be fixed for the same alleles across all loci, and the more likely they are to exhibit heterosis.

Falconer and Mackay's (1996) theory has been tested numerous times on a wide range of species with results varying depending on the trait and species. In one of the earliest studies, Moll et al. (1965) investigated the relationship between heterosis and the degree of divergence in maize (inferred by probable ancestry and geographic separation). Results of the study indicated that heterosis for yield increased with increasing divergence of the parent populations within a certain range of diversities, while heterosis based on ear number and days to tassel showed no association with divergence. When parental populations were extremely diverse, as is the case with wide crosses, a decrease in yield heterosis was observed. Teklewold and Becker (2006) compared the ability of phenotypic and molecular distance (based on RAPD markers) to predict heterosis in 14 phenotypic traits in Ethiopian mustard (*B. carinata* A. Braun). They reported a significant correlation of phenotypic and molecular distance with hybrid vigor for plant height, seeds per plant, seed yield and number of pods per plant, and a significant correlation of molecular distance with number of secondary branches. The authors concluded, however, that distances estimated from phenotypic traits predicted heterosis better than those estimated from RAPD markers. When comparing heterosis and genetic similarity based on sequence related amplified polymorphism (SRAP) markers in *B. napus*, Riaz et al. (2001) reported similar findings. In their study, correlation coefficients indicated there was a significant relationship between genetic distance and midparent and highparent heterosis for seed yield, but a relatively weak relationship was reported for plant height, maturity and oil content. Many other studies report correlations between genetic similarity and heterosis (Morgan 1998; Diers et al. 1996; Knaak and Ecke 1995; Becker and Engqvist 1995; Ehiobu et al. 1990).

Currently, broccoli is produced as a hybrid crop; however, studies reporting hybrid vigor in the species are limited. Development of new hybrids is costly due to the need to hand pollinate or to facilitate crossing in cages, and then conduct extensive field evaluations of various parental combinations. Making this process more efficient could greatly reduce the production cost for variety development. For example, if genetic distance is correlated with heterosis for important broccoli traits, parents could be selected based on distance rather than trial and error. The objective of this study was to establish if select broccoli traits exhibit heterosis, and to determine if genetic distance based on molecular markers can be used as a predictor of heterosis among hybrids.

Materials and methods

Plant materials

A half-diallel population of broccoli was generated by crossing nine parental inbreds (doubled haploids) in all possible combinations to produce 36 F_1s. The parents were selected from relatively elite sources to represent diverse

genotypes and phenotypes. Entries in the study included USVL105-AR derived from 'Arcadia' (Sakata Inc., Salinas CA), USVL066-VI derived from 'Viking' (originally Peto Seed Co. Saticoy, CA), USVL032-GV derived from 'Green Valiant' (Sakata, Inc), USVL039-HS derived from 'High Sierra' (originally Asgrow, San Juan Bautista, CA), USVL024-MA and USVL048-MA derived from 'Marathon' (Sakata Inc.), USVL012-EV and USVL089-EV derived from 'Everest' (Syngenta Seed Co. Gilroy, CA), and USVL070-FU (derived from 'Futura' originally from Asgrow).

Plant culture, and scoring of phenotypic traits

Four field trials were planted into randomized complete block designs with three replications. In the first week of August in 2001 and 2003, all 45 entries (9 parents plus the 36 F_1s) were seeded into a commercial potting mix into trays in a greenhouse, and seedlings were transplanted into the field on 19 September. In 2002 and 2003, entries were seeded in a greenhouse the first week of February. In 2002, seedlings were transplanted to the field on 5 March and in 2003 they were transplanted on 27 February. Individual plots consisted of a single row of 8–12 plants of an entry. Previously described cultural practices were followed for all four trials (Farnham et al. 2000).

As plots approached maturity, they were observed every 2–3 days, and those heads which had reached 10–12 cm in diameter were evaluated and harvested. Each plot was evaluated for five phenotypic traits. Head weight and stem diameter were recorded for three heads per plot and the average number of days from transplant to harvest (DTH) was determined on a whole-plot basis. In addition, plant height and width were measured on six plants per plot. In order to estimate overall plant performance, a combined trait index (CTI) was calculated by dividing each of the trait by its standard deviation and adding them together.

Determination of heterosis

Heterosis was determined for each of the five phenotypic traits measured as well as the CTI. Absolute midparent heterosis (MPH) was expressed as the difference between the F_1 and the average of the parental varieties (MPV), and absolute highparent heterosis was (HPH) expressed as the difference between the F_1 and the high parent variety (HPV). Percentage of crosses exhibiting HPH was determined by the following formula: [number of F_1s exhibiting HPH/36) $\times$ 100]. Similarly, the percentage of crosses exhibiting MPH was determined by counting the number of F_1s that exhibited a plant trait that was higher than the MPV, dividing by 36 and multiplying by 100. Since Hulbert and Orton (1984) found that the direction for maturity heterosis is toward earliness in broccoli, a genotype was considered to have HPH if it was earlier than the earliest parent, and it was considered to exhibit MPH if it was earlier than the midparent average. Relative midparent heterosis (RMPH) was calculated by the following formula: $[(F_1 - MPV)/MPV] \times 100$. Similarly, relative HPH (RHPH) was calculated as follows: $[(F_1 - HPV)/HPV] \times 100$.

DNA extraction and marker development

Genomic DNA was isolated from leaves using DNeasy plant mini-kits (Qiagen Inc., Valencia, CA). Isolated DNA was quantified using a TKO 100 fluoriometer (Hoefer Scientific Instruments, San Francisco, CA) in conjunction with a Hoechst dye-based protocol.

Three types of markers, AFLPs, SRAPs, and SSRs, were used to determine genetic distance because it was previously determined that a mixture of these marker types gives a more accurate estimate of genetic distance than a single marker (Hale et al. 2006). Twenty-four AFLP, 24 SRAP, and 43 SSRs were used to determine genetic distance. PCR conditions and primers used were identical to Hale et al. (2006).

All AFLP and SRAP markers were scored as present (1) or absent (0), and percent polymorphism of the markers were determined. For SSRs, differences in band size were scored as different allelic forms of a given locus, and the data were transformed to binary presence (1) versus absence (0). The genetic similarity between each pair of lines was calculated according to the Dice similarity coefficient (Nei and Li 1979) using the appropriate routines in NTSYS-pc version 2.0 (Exeter Software, Setauket, NY). Values of genetic similarity ranged from 0 (no peaks in common) to 1 (identical peak patterns for all markers). Monomorphic peaks were not included in the analysis.

Clustering and statistical analysis

A cluster analysis was performed on the similarity matrix using NTSYS-pc to generate a dendrograph of the parental lines. Following the creation of the dendrograph, a bootstrap analysis was performed using the WinBoot software (Yap and Nelson 1996) to estimate the confidence limits for each of the clusters. Five thousand bootstrap resamplings were used.

Each of the nine parents fell into one of three major clusters (clusters 1, 2, and 3), and each of the F_1s was given a group designation depending on the parental cluster. For example, a cross made with two parents from cluster one would be given a group designation of 1×1, and an F_1 produced from a cross between a parent from cluster one and a parent from cluster two would be assigned to group

1 × 2. Thus, the F_1s fell into one of six groups (1 × 1, 2 × 2, 3 × 3, 1 × 2, 1 × 3, or 2 × 3). An analysis of variance and LSD mean separation analysis were performed on HPH and MPH with groups as the dependent variable using the GLM procedure of SAS.

In order to determine relationships between genetic distance and heterosis the five measured horticultural traits and the CTI along with their respective midparent and highparent heterosis measurements were regressed on genetic distance between the parents as determined by DNA markers.

Combined analysis of variance across all four environments (location-year combinations) was performed for each of the five phenotypic traits. Whenever a significant interaction effect was observed with environment (e.g. genotype × environment), the mean square of the interaction effects was used to test the significance of that variance component (e.g. genotype effect).

Genetic parameters were estimated following Gardner and Eberhart's analysis II linear model (Gardner and Eberhart 1966). This model is useful for the evaluation of parents (n) used in diallel crosses and their F1 progeny [$n(n-1)/2$]. The variation among genotypes was partitioned into varieties (parents) and midparent heterosis. Heterosis effects were further partitioned into the average variation across hybrids, variation attributed to a specific parent (variety), and variation associated with a specific (specific heterosis) (Hallauer and Miranda 1981; Murray et al. 2003). The single mean square for heterosis and its three partitions—average heterosis, parental heterosis, and specific heterosis—are all due to dominance and differences in allelic frequencies between populations, assuming a restricted genetic model composed of only additive and dominance effects (Gardner and Eberhart 1966; Ouendeba et al. 1996). The model below was used to calculate the ANOVA;

$$Y_{ij} = \mu_v + {}^1\!/_2(v_i + v_j) + \lambda(h + h_i + h_j + s_{ij})$$

where Y_{ij} is the mean of the cross between the ith and jth parents; μ_v is the mean of all parental varieties; v_i and v_j are variety effects of parents i and j; h is average heterosis contributed by all parental varieties used in crosses; h_i and h_j are the average heterosis contributed by variety i and j, respectively, in their crosses measured as a deviation from average heterosis; s_{ij} is the specific heterosis that occurs when parent i is crossed to parent j. Statistical analysis was done using the DIALLEL.SAS05 Program (Zhang et al. 2005).

Principal component analysis (PCA) was conducted using a combination of the five measured traits. Euclidean distances between pair-wise combinations of parents was used as the genetic diversity measure.

In the PCA plot, each of the nine parents grouped in one of five obvious clusters (clusters 1–5), and each of the F_1s was assigned a group designation as was done for the molecular clusters. Thus, for the phenotypic analysis, each of the F_1s fell into one of thirteen groups. Since two of the clusters only contained a single parent, for the phenotypic analysis, there were three intracluster groups and ten intercluster groups. An analysis of variance and LSD mean separation analysis were performed on HPH and MPH with groups as the dependent variable using the GLM procedure of SAS.

Results

Molecular marker and cluster analysis

Twenty-four AFLP, 23 SRAP, and 17 SSR primers rendered polymorphic banding patterns and were used in the final analysis for determination of genetic distance. The one SRAP and 24 SSR primers that did not produce informative markers either produced identical banding patterns, or no bands for all nine genotypes. The average number of polymorphic bands produced per primer for AFLP, SRAP and SSRs was eight, seven, and two, respectively.

A total of 409 polymorphisms were produced from the three marker types. Based on the polymorphic bands scored, the calculated genetic similarities ranged from 0.43 (between USVL070-FU and USVL105-AR) to 0.71 (between USVL039-HS and USVL032-GV; USVL012-EV and USVL089-EV; and USVL012-EV and USVL070-FU). Cluster analysis placed the nine parents into three major groups. Relatively high bootstrap confidence limits were found for all of the clusters (Fig. 1).

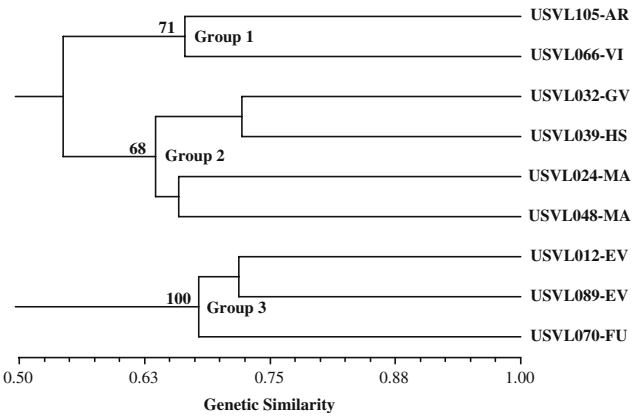

Fig. 1 Dendrograph showing genetic similarities between the 9 parents and bootstrap confidence limits based on 409 SSR, SRAP, and AFLP polymorphisms

Table 1 Mean squares from the analysis of variance and heterotic effects for 9 inbred parents and their 36 F_1 hybrids grown in four environments based on the Gardner-Eberhart analysis II method, for head weight (HW), stem diameter (SD), days from transplant to harves t (DTH), height, width, and the combined trait index (CTI)

Source	*df*	CTI	HW	SD	DTH	Plant width	Plant height
Environment	3	551.2**	42,145.6**	324.0**	5,709.2**	9,301.2**	4,352.8**
Reps/env	8	10.9**	1,672.2	22.9**	243.0**	295.9**	119.9**
Genotypes	44	27.5**	7,314.9**	111.1**	657.4**	508.5**	141.4**
Parents	8	83.5**	25,513.0**	490.6**	2,730.8**	1,069.6**	184.9**
Heterosis	36	15.2**	3,035.6**	26.5**	195.4**	386.8**	134.1**
Average het	1	206.5**	24,358.3	324.7*	3,806.9**	9,330.8**	3,257.5**
Variety het (GCA)	8	11.4	2,256.5	11.6	137.2*	95.5	55.0*
Specific het (SCA)	27	9.1**	2,405.5*	19.8**	79.9**	140.3**	2.5**
Genotype x env	132	4.8**	1,515.2	11.7**	59.7**	46.6*	24.8**
Parents × env	24	9.4**	2,095.6*	16.9**	194.0**	81.5**	47.2**
Heterosis × env	108	3.8	1,402.7	10.2	30.0*	38.2	18.8
Average het × env	3	2.0	2,902.7	25.8*	94.9**	75.0	63.1**
Variety het × env	24	6.0*	1,108.7	12.0	48.6**	46.9	21.0
Specific het × env	81	3.2	1,439.4	9.0	21.8	33.5	16.4
Error	350	3.3	122.8	8.4	22.8	34.1	16.2

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

Parental performance and heterosis

Combined analysis of variance results for the five phenotypic traits are presented in Table 1. Results show that stem diameter (SD), days from transplant to harvest (DTH), plant width, plant height, and CTI among genotypes (parents and F_1 crosses) differed as well as their relative yield in the different environments. This is indicated by significant genotype and genotype × environment interaction effects. Significant genotype and environment effects were observed for head weight among the parents and F_1 crosses as well, but there was no significant overall genotype × environment interaction. A significant interaction was observed between the parental genotypes and the environments for all traits analyzed, but little interaction was indicated between heterosis and the environment. Significant interactions between average heterosis and the environment were observed for stem diameter, DTH, and height, and a significant interaction between variety heterosis and the environment was observed for the CTI and DTH. All other interactions between the environment and heterosis were insignificant.

Partitioning of genotype effects into parental and heterosis effects resulted in significance for both components for all five traits. This implies that there were significant differences between parental genotypes for all of the analyzed traits. When heterosis effects were partitioned into average heterosis, variety heterosis, and specific heterosis, differences in significance were seen between the traits. Average heterosis was significant for stem diameter, DTH, width, height, and the CTI which indicated that F_1s were superior to midparent values for all traits except for head weight. Significant variety heterosis was observed only for DTH and height, implying that, for these traits, the heterotic pattern of at least one of the varieties differed from the others when crossed with the remaining varieties. For all of the traits, at least one of the specific crosses differed from the others due to nonadditive effects and differences in gene frequency with other varieties as indicated by the significant specific heterosis effects (Hallauer and Miranda 1981).

For the plant vigor characteristics of height and width as well as the CTI, almost all of the F_1s exhibited MPH and HPH. This trend was not observed for the head traits of stem diameter, head weight, and DTH, where less than half of the hybrids exhibited HPH. In fact, on average, the F_1s performed worse than their best parent for stem diameter and head weight. A higher degree of heterosis was observed in the plant characteristics than in the head characteristics (Table 2).

Genetic similarity and heterosis

Analysis of variance indicated a significant molecular marker group effect on MPH for DTH, head weight, height, width, and CTI but not for stem diameter. Identical results were seen for HPH. While not always significant, mean separation analysis showed a majority of intracluster crosses ranked below intercluster crosses (Table 3). The group effect was very distinct for plant characteristics such as height and width ($P < 0.0001$). Head characteristics, while significant, were not as definitive. There was no significant effect of group on heterosis for stem diameter.

The tendency for intracluster groups to rank below intercluster ones for mean separation of heterosis indicated a possible relationship between genetic similarity and hybrid performance. Therefore, MPH and HPH values of the F_1s for the five analyzed horticultural traits were regressed on

Table 2 Percentage of F_1s plants exhibiting relative midparent heterosis (RMPH), relative highparent heterosis (RHPH), absolute midparent heterosis (MPH) and absoulute highparent heterosis (HPH) for plant width, plant height, stem diameter (SD), days from transplant to harvest (DTH) head weight (HW), and the combined trait index (CTI). Also shown is the range and average of RMPH, RHPH, MPH, and HPH for the five traits of interest plus the combined trait index

Trait	RMPH	RHPH	MPH	HPH
Plant width				
Percentage	100	89	100	92
Range	5.2–24.5	–4.3 to 17.6	2.8–16.5	–4.3 to 12.6
Average	14.9	7.6	10.5	5.3
Plant height				
Percentage	100	100	100	100
Range	4.5–24.4	0.4–17.3	2.2–11.5	–0.1 to 8.3
Average	12.5	7.0	6.1	3.6
SD				
Percentage	92	44	92	36
Range	–3.2 to 15.8	–9.6 to 7.8	–1 to 5.5	–4 to 2.8
Average	6.5	–1.6	1.9	–0.9
DTH				
Percentage	97	42	97	42
Range	–14.2 to 3.7	–10.6 to 15.1	–13.4 to 2.1	–9.1 to 9.3
Average	–7.9	1.5	–6.5	0.6
HW				
Percentage	89	47	89	42
Range	–6.4 to 27.9	–14.2 to 18	–17 to 41	–36 to 27
Average	9.3	–1.0	15.5	–5.6
CTI				
Percentage	97	83	97	89
Range	–1.3 to 5.3	–2.1 to 5.0	–0.8 to 4.9	–1.6 to 4.1
Average	2.4	1.2	2.2	1.2

Table 3 Least significant difference (LSD) analysis for a group effect on head weight (HW), stem diameter (SD), days from transplant to harvest (DTH), height, width, and combined trait index (CTI)

Group	HW		SD		DTH		Plant height		Plant width		CTI	
	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH
1 × 3	25.91a	6.26a	2.40a	–1.07a	–5.85bc	6.06a	7.58ab	5.05ab	13.63a	9.25a	3.15a	2.37a
1 × 2	18.82ab	–1.82a	2.62a	0.31a	–8.18Cc	–1.20b	8.05a	5.29a	12.02a	6.68a	2.67ab	1.56a
2 × 3	16.31abc	–5.46ab	4.24a	0.99a	–6.13bc	1.69ab	5.38bc	3.07abc	11.20a	6.28a	2.33abc	1.33a
1 × 1	2.88cd	–24.17c	–0.292a	–2.17a	–2.04ab	6.92a	4.67c	0.58c	7.04b	0.92b	1.51bc	0.10b
2 × 2	9.30bcd	–11.33abc	1.17a	–0.71a	–7.50c	–2.32b	4.92c	2.68bc	6.85b	0.88b	1.19c	0.10b
3 × 3	–0.40d	–21.06bc	0.99a	–3.06a	–0.83a	1.71ab	3.21c	0.83c	5.63b	0.25b	1.22c	–0.34b
LSD	15.14	17.77	6.13	6.3	4.10	5.66	2.5	2.54	3.45	3.77	1.17	1.12

genetic similarity of the inbred parents used to make an F_1 to assess any linear relationship between them. There was a statistically significant relationship ($\alpha = 0.05$) between genetic similarity and the plant characteristics of height and width but not the head characteristics of head weight, stem diameter, DTH or CTI. The derived MPH values showed a significant relationship with genetic similarity for all traits except DTH, and HPH a significant relationship with genetic similarity all traits except stem diameter and DTH (Table 4). The R^2 values indicate that 19% of the plant width variation observed can be explained by genetic similarity, as can 17% of the variation in plant height. R^2 values for derived MPH and HPH were somewhat higher than for the traits themselves. The trait with the highest correlation between genetic similarity and heterosis was plant width, where 56% of the variation in MPH and 49% of the variability in HPH could be explained by genetic similarity ($r = –0.75$ and –0.7, respectively). Other traits had correlation coefficients ranging from –0.44 to –0.6 for MPH and from –0.42 to –0.66 for HPH. This meant that

Table 4 Correlation coefficients between genetic similarity (based on individual markers as well as a combination of all three marker types) and absolute midparent heterosis (MPH), absolute highparent heterosis (HPH), and themselves for head weight (HW), stem diameter (SD), days from transplant to harvest (DTH), height, width, and the combined trait index (CTI)

Trait	Marker type	MPH *r*	HPH *r*	Trait values *R*
HW	All markers	–0.49	–0.42	NS
	AFLP	–0.38	–0.37	–0.33
	SRAP	–0.35	–0.33	NS
	SSR	NS	NS	NS
SD	All markers	–0.44	NS	NS
	AFLP	–0.36	NS	NS
	SRAP	NS	NS	NS
	SSR	NS	NS	NS
DTH	All Markers	NS	NS	NS
	AFLP	NS	NS	NS
	SRAP	NS	–0.35	NS
	SSR	NS	NS	NS
Height	All markers	–0.5	–0.54	–0.41
	AFLP	NS	–0.36	NS
	SRAP	–0.44	–0.47	NS
	SSR	NS	–0.33	NS
Width	All markers	–0.75	–0.7	–0.43
	AFLP	–0.66	–0.69	–0.45
	SRAP	–0.54	–0.5	NS
	SSR	–0.33	NS	NS
CTI	All markers	–0.6	–0.66	NS
	AFLP	–0.46	–0.55	NS
	SRAP	–0.5	–0.53	NS
	SSR	NS	–0.44	NS

between 19 and 36% of the variation in MPH and between 18 and 44% of the variation in HPH could be explained by genetic similarity. For all traits analyzed, there was a negative association between genetic similarity and heterosis, indicating that as genetic similarity increased, heterosis decreased.

When individual marker types were used to estimate genetic similarity, the AFLP and SRAP based similarity were always more highly correlated with heterosis than SSR based genetic similarity. Regardless of the trait, genetic similarities based on a combination of all three types of markers had a higher correlation with heterosis than similarities based on any single type of marker (Table 4).

Phenotypic similarity and heterosis

Phenotypic similarity was assessed using PCA. In the PCA plot, the first and second principal components (factors) explained 54 and 17%, respectively, of the total variation

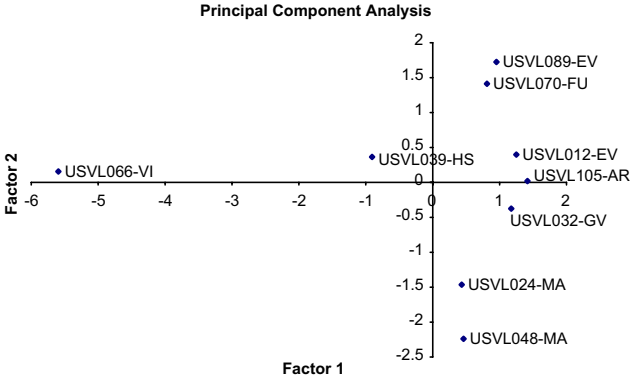

Fig. 2 Principal component analysis of 9 parental inbreds based on phenotypic measurments of five traits. The first and second principal components (Factor) explained 54 and 17% of the variation, respectively

partitioned. Head weight and stem diameter were the most influential traits in discriminating among the parents in the first principal component whereas the second principal component was largely influenced by DTH.

Some associations among parents in the PCA plot were similar to those observed in the dendrograph generated from molecular marker data; however, there were a few key differences (Fig. 2). Parental genotypes in group three of the marker-derived dendrograph also grouped together in the same quadrant of the PCA plot. Group two from the dendrograph also grouped together in the PCA plot, with the exception of USVL039-HS. This deviation appears to be primarily due to differences in factor 1 (head weight) since little difference was observed between USVL032-GV and USVL039-HS for factor 2. The primary difference between the dendrograph and the principal component analysis are seen in group 1. A very small difference was seen between the genotypes in group 1 for factor 2, so the difference between molecular and phenotypic groupings is attributed primarily to factor 1. The correlation between the two genetic distance measures was low and non significant ($r = –0.02$; $P > 0.05$). With the exception of head weight and high parent heterosis, no correlation was observed between Euclidean distance based on phenotypic traits and heterosis.

A significant phenotypic group effect (based on the principal component analysis) was observed for MPH and HPH for several of the traits, however, the trend seen in the molecular groupings of intracluster crosses ranking below intercluster crosses was conspicuously absent with the phenotypic groupings (Table or data not shown). There was no obvious advantage of crossing parents from different phenotypic clusters over parents within the same cluster. This observation was supported by the lack of a significant correlation between Euclidean distance based on phenotypic traits and heterosis. It was evident that the few

discrepancies between the phenotypic and molecular clustering made a significant difference in the results of the group analysis.

Discussion

The number of hybrids exhibiting heterosis and the amount of heterosis exhibited varied from trait to trait, with the plant characteristics of width and height showing more heterosis than head characteristics such as head weight and stem diameter. In general, parents and hybrids performed the same with respect to genotypic and environmental effects, but the hybrids showed less genotype by environment interactions than the inbred lines.

The polymorphisms generated from SRAP, AFLP, and SSR markers were able to distinguish among the nine inbred lines used in this study. Since these inbreds were derived from hybrids whose parentage is a trade secret, the true relationship among the lines is not known. However, some of the lines were derived from the same hybrid, and cluster analysis based on molecular marker data placed these related inbreds in the same major clusters. Overall, the relatively high values of the bootstrap analysis indicate a high confidence level for the three major clusters.

In general, the highest MPH and HPH for head weight, height and width were obtained by crossing parents from different molecular marker based clusters (i.e. more genetically distant). Similar results were reported by Riaz et al. (2001) who observed intercluster heterosis in *B. carinata*. Genetic similarity was significantly and negatively correlated with all characters for MPH except for DTH, and all characters for HPH except for DTH and stem diameter. This is in agreement with the theory presented by Falconer and Mackay (1996) as well as supporting evidence in *B. carinata* (Raiz et al. 2001), wheat (Morgan 1998), and maize (Betran et al. 2003). Contradictory results have been reported in a number of species including *B. napus* (Yu et al. 2005) and *Zea mays* (Shieh and Thseng 2002) which indicate that genetic distance estimated based on molecular markers is not a reliable predictor of heterosis. Conflicting results could be a reflection of the types of markers that were used, the genome coverage, or the specific set of genotypes evaluated. Yu et al. (2005) pointed out that many DNA fragments are located in non-expressed regions or have little or no association with horticulturally important traits and heterosis. Markers such as SRAPs which were used by Raiz et al. (2001) are generally more tightly linked to expressed regions of the genome than markers such as RAPDs, thus they could presumably more accurately represent areas contributing to heterosis. In this study, when genetic similarities were based on individual marker types, their association with heterosis was less pronounced than when using a combination of marker types. Genetic similarities based on SSRs were not as highly correlated with heterosis as AFLP and SRAP-based similarities. This is probably due to the low number of SSR markers used in this study and the lack of adequate genome coverage by these markers.

As Ehiobu et al. (1990) suggest, the results of inbreeding at individual loci are subject to large chance effects which could explain the discrepancies between studies and among traits. In this study, the genetic distance was calculated based on three different types of marker data to ensure good coverage representative of the entire genome. Therefore, if a small number of loci are responsible for the observed heterosis, the correlation between genetic distance and heterosis would not be large. Keeping this in mind, it is logical to assume that more complex traits may have a higher correlation between genetic similarity and heterosis. Thus, differences in correlation coefficients between the traits and genetic similarities could reflect the number of alleles and/or the degree of dominance involved in the expression of the trait.

Unlike genetic distance based on molecular markers, Euclidean distance based on phenotypic traits was not correlated with heterosis. The discrepancy between the two types of distance measures could be due to the fact that the molecular markers were random and not associated with any particular trait. The traits which were used to calculate distances between the parents are conditioned by a limited number of genes and subject to large chance effects. Furthermore, the traits which contributed the most in discriminating among the parents in the first principal components were head weight and stem diameter and these traits exhibited little heterosis. Thus, the lack of correlation of the phenotypic traits with heterosis was not unexpected.

It is important to recognize that there is a difference between heterosis and the absolute trait values, and that the degree of heterosis for a given trait does not necessarily reflect the trait values themselves. For example, in this study, when two low head weight parents were crossed, a high degree of heterosis was observed, meaning that the F_1 exceeded the parents in head weight. The head weight of the resulting F_1, however, even though it exceeded its two parents, was still relatively low when compared to other genotypes. Conversely, when two high head weight parents were crossed, little heterosis was observed, but the resulting F_1 had a relatively high head weight. This seems to indicate that favorable alleles can and, in some cases, probably are fixed, in desirable inbreds.

The highest average relative highparent heterosis was observed in the plant vigor traits of height and width, while the plant head characteristics of head weight and stem diameter had negative average HPH. This is likely due to the fact that, when selecting desirable inbreds, most

broccoli breeders focus on market triats (such as head characteristics), and the selection criteria for these traits tends to be the same regardless of the breeding program. Unlike selection for head traits, breeders have the freedom to select plant characteristics based on what is best adapted to the local environment and their own preferred phenotype. Therefore, it is likely that the same beneficial alleles for head characteristics are becoming fixed in most broccoli breeding programs while plant characteristics remain more diverse. While the data seem to indicate that inbred broccoli has the potential to perform as well as hybrids for market traits such as head weight, it is important not to ignore the importance of plant vigor. Plant vigor may be important for crop establishment as well as buffering against harsh environments. The CTI showed a positive, yet small RHPH of 1.2% indicating that even when plant vigor traits are taken into account, the average degree of heterosis observed in broccoli is still not exceptionally large. Although the average RHPH was negative for head quality traits, there were F_1s which exhibited a high degree of heterosis. These F_1s, however, tended to be produced by crossing two low head weight parents, and even though the heterosis was high, the relative head weight was not. The lack of high heterosis in broccoli compared to other *Brassica* species could be rooted in its narrow genetic base. With a narrow genetic base, there is less likelihood for allelic diversity between parents, which ultimately results in alleles becoming fixed in the modern gene pool through breeding for market traits.

In conclusion, average heterosis in broccoli was relatively low when compared to other *Brassica* species. Evidence does exist that inbreds can be fixed for head quality traits making the creation of hybrids an expensive and possibly unnecessary endeavor if high trait values are the goal. In order for seed companies to profit, variety protection is of utmost importance, and vigorous inbreds with high trait values do not contribute to this important goal. As a result, heterosis can be a major consideration for new commercial varieties. If high heterosis, as opposed to high trait values is the goal, certain combinations of parents will result in heterotic F_1s, and for some traits, selecting genetically distant parents could increase the heterosis in the F_1s. Based on the results of this study, the selection of parents to obtain highly heterotic offspring can be accomplished more efficiently by considering molecular marker based distances as opposed to phenotypic ones.

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