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Inheritance Analysis and Quantitative Trait Loci Detection of Head Splitting Resistance in Cabbage (*Brassica oleracea* L. var. *capitata*)

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***Abstract*. Head splitting resistance (HSR) in cabbage is an important trait closely related to appearance, yield, storability, and mechanical harvestability. In this study, a doubled haploid (DH) population derived from a cross between head splitting-susceptible inbred cabbage line 79-156 and resistant line 96-100 was used to analyze inheritance and detect quantitative trait loci (QTLs) for HSR during 2011–12 in Beijing, China. The analysis was performed using a mixed major gene/polygene inheritance method and QTL mapping. This approach, which uncovered no cytoplasmic effect, indicated that HSR can be attributed to additive-epistatic effects of three major gene pairs combined with those of polygenes. Major gene and polygene heritabilities were estimated to be 88.03% to 88.22% and 5.65% to 7.60%, respectively. Using the DH population, a genetic map was constructed with simple sequence repeat (SSR) markers anchored on nine linkage groups spanning 906.62 cM. Eight QTLs for HSR were located on chromosomes C4, C5, C7, and C9 based on 2 years of phenotypic data using both multiple-QTL mapping and inclusive composite interval mapping. The identified QTLs collectively explained 37.6% to 46.7% of phenotypic variation. Three or four major QTLs (Hsr 4.2, 7.2, 9.3, and/or 9.1) showing a relatively larger effect were robustly detected in different years or with different mapping methods. The HSR trait was shown to have a complex genetic basis. Results from QTL mapping and classical genetic analysis were consistent. Our results provide a foundation for further research on HSR genetic regulation and molecular marker-assisted selection (MAS) for HSR in cabbage.**

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Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the world's most widely cultivated vegetables. In addition to being a significant year-round component of the global vegetable supply, cabbage is of immense importance for human nutrition, providing dietary fiber, vitamins, and cancer-preventing substances (Fahey and Talalay, 1995; Masarirambi et al., 2011). During later stages of vegetative growth, cabbage heads are vulnerable to cracking. This phenomenon seriously affects appearance, yield, storability, and mechanical harvestability. In addition, susceptibility to head splitting hinders the prolongation of harvest time and thus the ability of growers to select harvest times for optimal selling price. Head splitting resistance is thus a very desirable property in cabbage (Holt and Schoorl, 1983). Previous studies (Liu et al., 2009; Zeng et al., 2009) about this trait mainly concentrated on cultivating conditions, physiological, cytological characters, and types of the head. It is proved that many factors, such as irrigation, fertilizer, planting density, endogenous hormones content, leaf surface microconfiguration, and tissue character could affect the cracking head. To alleviate late-stage head splitting, farmers generally reduce irrigation times during cultivation. Such practices affect normal cabbage head growth, however, and cannot completely prevent head splitting. Under water-sufficient conditions, head splitting in cabbage is mainly caused by genetic factors (Qin et al., 1994). Improvement of HSR in newly developed varieties has therefore become a priority in cabbage breeding programs. The complex genetic nature of the HSR trait has only been explored in a few studies. After performing a cross between early- and late-splitting cabbage lines and obtaining their F_1, F_2, and backcross progenies, Chiang (1972) concluded that HSR is controlled by at least three pairs of genes within an average of six generations. Gene action was found to be mostly additive. Partial dominance for early splitting was detected, and narrow sense heritability was estimated as 47%. Zhuang et al. (2009) further analyzed combining ability and heritability according to the Griffing IV method. Using 15 cross combinations involving six backbone parents, they revealed that HSR is controlled by both additive and nonadditive effects. Additive effects were found to be the most important, with the percentage contribution of additive effects increasing with prolongation of field duration. These classical genetic methods revealed that HSR is complex and controlled by many genes, and also estimated the total gene effect. Nevertheless, major gene effects and polygene effects were not clearly distinguished and the gene relationships were not identified.

Major gene/polygene genetic segregation analysis and QTL mapping are the main approaches used to clarify the genetic basis of quantitative traits (Gai et al., 2007; Zhang et al., 2003). These methods have been successfully applied to uncover inheritance patterns and QTLs of important traits in many crops, vegetables, and flowers (Anbessa et al., 2006; Cheng et al., 2011; Hao et al., 2008; Liang et al., 2014; Wang et al., 2012b; Zhang et al., 2010, 2013).

For QTL mapping, the use of DH populations is a powerful tool. Because each line is homozygous, they can be replicated between test sites, and trialed over multiple years. So the standard error of QTL genotype means is thus decreased, thereby allowing a better estimate of trait heritability and increasing QTL detection ability (Pink et al.,

2008; Simon et al., 2008). At the same time, multiple mapping methods for detecting QTLs are needed to effectively identify and verify important QTLs (Su et al., 2010; Xing et al., 2012).

Recent progress in genetic mapping and molecular marker development in *B. oleracea* have laid an important foundation for genetic studies and QTL analysis of important *B. oleracea* traits (Brown et al., 2014; Gao et al., 2007; Walley et al., 2012; Wang et al., 2012a). QTL mapping studies in cabbage have mainly concentrated on plant size (Lan and Paterson, 2001), flowering time (Okazaki et al., 2007), and disease resistance (Kifuji et al., 2012; Nagaoka et al., 2010; Mei et al., 2013). No QTL mapping studies associated with cabbage HSR have been reported.

To thoroughly dissect the genetic architecture of HSR in cabbage, we first developed an intracrop 'immortal' DH mapping population derived from a cabbage x cabbage cross and used it in a segregation analysis over multiple generations (P_1, P_2, and DHs) to explain HSR inheritance. We then constructed a framework linkage map based on this DH population with SSR markers. Finally, we located the first-known cabbage QTLs for HSR on the generated linkage map and analyzed QTL stability across years with different mapping programs.

Materials and Methods

Plant materials. Two inbred cabbage lines (Fig. 1), 96-100 and 79-156, were used as parents to develop a DH population. The male parental line, 96-100 (P_2, about 50 d form transplantation to maturity in autumn), is resistant to head splitting and was self-developed from germplasm of Indian origin introduced by the Bejo Sheetal Company; the female parental line, 79-156 (P_1, about 50 d form transplantation to maturity in autumn), is susceptible to head splitting and self-developed from a germplasm introduced from Denmark. Both of the parents are early maturing inbred lines and were provided by the Cabbage and Broccoli Laboratory, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (CAAS). The DH population was derived from the F_1 (79-156 × 96-100) by microspore culture (Yuan et al., 2011, 2012) during 2009–12.

Fig. 1. The difference between parents in head-splitting resistance trait.

Seeds were obtained from 157 DH individuals in the summer of 2011 and from 181 individuals in 2012. All DH lines obtained in the 2 years were used for genetic segregation analysis. For linkage map construction and mapping, data from the first 157 lines were used.

Field experiments, resistance evaluation, and statistical analysis. Parents, F_1 (79-156 × 96-100), RF_1 (96-100 × 79-156), and DH lines were planted in the field at the experimental farm of the Institute of Vegetables and Flowers, CAAS (Changping, Beijing, China) in 2011 and 2012. Seeds of each generation were sown in a greenhouse in mid-July, and the resulting seedlings were transplanted into 10-cm diameter plastic pots after 3 weeks. The plants were then transplanted into the field at ≈5 weeks of age (19 Aug. 2011 and 22 Aug. 2012). Parental, F_1, and RF_1 lines were planted in a randomized complete block design with three replicates. In each replicate, parents and F_1 lines were planted in three rows; each row comprised 16 plants, spaced 40 cm apart within a row and 50 cm between rows. For the DH population, a block in replication design was adopted with three replicates (Gai, 2000; Supplemental Table 1). Each replicate consisted of single rows of 16 plants. The experimental plots were surrounded by two additional rows planted to serve as a protective buffer. Irrigation and insecticide application were consistent with commercial production conditions. In each replicate, 15 plants at the same growth level of each parental, F_1, and RF_1 line and 10 plants of each DH line were marked for phenotypic measurements.

Because of differences in maturation rates due to genotypic variation, maturation dates were recorded for each DH line. At maturity, the height and the circumference of each marked cabbage head at its widest point were measured with a flexible plastic ruler. The arc length and width of the largest split were also measured with a flexible plastic ruler 15 d after maturity. Splitting was assessed on a 6-point scale, assigned according to the number of split layers and the ratio of the split size to the entire surface area (calculated as S [%] = $S_1/S_2 \times 100\%$, where S_1 = arc length × width of the largest split and S_2 = height × half of the largest circumference of the head) (Fig. 2). Assigned splitting scores were as follows: 0 = no split; 1 = 1 split layer; 2 = 2 split layers and $S < 50$; 3 = 3–5 split layers and $S < 50$, or 2 split layers and $S \geq 50$; 4 = 6–10 split layers and $S < 50$, or 3–5 split layers and $S \geq 50$; 5 = more than 10 split layers, or 6–10 split layers and $S \geq 50$. The head splitting index was calculated as: $\sum$ (splitting score × number of plants with that score)/(total number of plants × highest possible splitting score) × 100%. The data were analyzed with Excel 2003 and SAS 8.1 software.

Joint segregation analysis. To determine an appropriate genetic model for the HSR trait, the cabbage DH population dataset was analyzed by major gene/polygene mixed inheritance analysis (Gai, 2006; Gai et al., 2003). Joint segregation analysis was performed following Hao et al. (2008) and Zhang et al. (2010) under the basic assumptions described by those authors. To select the genetic model best explaining the quantitative trait variation, 38 genetic models of 7 different types were considered (Supplemental Table 2). Maximum likelihood estimates of component parameters in each genetic model were generated using the iterated expectation and conditional maximization algorithm (Zhang et al., 2003). The proportion, mean, and variance of each component distribution in the likelihood function were included in the estimates. Best-fit models were chosen according to the Akaike information criterion (AIC) (Akaike, 1977) and a suite of goodness-of-fit tests. Finally, genetic parameters of major genes and polygenes were estimated based on the least squares principle.

DNA extraction. Total DNA was isolated from expanding leaves of 3-week-old plants using the modified cetyltrimethylammonium bromide method (Saghai-Maroof et al., 1984). The genomic DNA samples were diluted to 100 ng/µl with Tris-EDTA (pH 8.0) and stored at –20 °C for use as polymerase chain reaction (PCR) templates. In addition, leaf tissue was lyophilized for use in future DNA extractions.

Genetic linkage map construction and QTL mapping. A set of 2,170 SSR markers developed from cabbage sequence scaffolds (Wang et al., 2012a) and 1,013 expressed sequence tag (EST)-SSR markers (Chen et al., 2010a) were used to scan for polymorphisms between the two parents. DNA amplification of SSR markers was carried out in 20-µl volumes containing 1 unit of *Taq* polymerase, 0.1 µM of each primer, 200 µM dNTPs, 2 µl of 10 × buffer (25 mM Mg^{2+}), and 100 ng genomic DNA template. The PCR thermal profile was as follows: initial denaturation for 5 min at 94 °C, followed by 35 cycles of DNA denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and extension for 45 s at 72 °C, and a final extension for 7 min at 72 °C. PCR amplifications were carried out in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). The resulting products were subjected to 8% polyacrylamide gel electrophoresis at 160 V for 1.5 h followed by silver staining (Brant et al., 1991).

Framework map construction and QTL analysis. For map construction, the DH population was genotyped for all SSR markers that showed polymorphisms between the parental 79-156 and 96-100 lines. The genotyping data were coded as type "a" or "b", corresponding to parental lines 79-156 and 96-100, respectively, with ambiguous and missing data indicated by "-". A linkage map was constructed using JoinMap 4.0 software (Van Ooijen, 2006). For map distance calculations, recombination frequencies were converted to centiMorgans (cM) using Kosambi's method, and linkage groups were assigned to chromosomes C1–C9 of *B. oleracea* based on markers in common with the reference (Lv et al., 2014; Wang et al., 2012a).

QTLs were estimated by multiple-QTL modeling (MQM) with MapQTL 4.0 (Van Ooijen et al., 2002) and by inclusive composite interval mapping (ICIM) using QTL IciMapping v3.0 software (Li et al., 2007). In MQM, a 1,000-permutation run was first performed to estimate the significance threshold of the test statistic for a QTL based upon a 5% experiment-wise error rate. Interval mapping at 1-cM intervals along the chromosomes was then used to scan for QTLs based on a logarithm of odds (LOD) threshold of 2.5. Markers closely linked to positions with the highest LOD score were taken as cofactors for MQM analysis. To select significant markers during the first step of ICIM stepwise regression, *P* values for entering and removing variables were set respectively at 0.001 and 0.002; in the second step, a minimum LOD threshold of 2.5 was used to declare a QTL significant.

Results

Phenotypic assessment of parental, F_1, RF_1, and DH lines. Parents 79-156 and 96-100 differed significantly with respect to HSR (Fig. 1; Table 1). Line 96-100 was resistant to head splitting, with a head splitting index of 9.33 and 9.36 in 2011 and 2012, respectively; in contrast, 79-156 was highly susceptible to head splitting, with corresponding head splitting index values of 86.87 and 85.32. Head splitting indexes of F_1 and RF_1 were not significantly different from one another; the values were intermediate to those of the parents, indicating that there is no cytoplasmic effect on HSR inheritance.

Analysis of variance revealed significant differences ($P < 0.01$) in HSR among DHs in both 2011 and 2012 (Table 2), indicating the existence of heritable variation and is suitable for genetic analysis. Significant differences between years and among lines were observed at $P < 0.05$ and $P < 0.01$ levels, respectively; this result demonstrates that the HSR trait is mainly under genetic control, with climate also playing an important role.

In the DH population, the head splitting index showed continuous variation, suggesting that comprehensive HSR in cabbage is a typical quantitative genetic character (Fig. 3). Nevertheless, the frequency distribution of phenotypes deviated from a normal distribution, with skewness and kurtosis values of 0.76 and –0.73 in 2011 and 0.97 and –0.29 in 2012, respectively. These skewed and multipeak phenomena indicate the possible existence of major genes for HSR. The head splitting index of DHs ranged from 0.00 to 98.52 in 2011 and 0.00 to 100.00 in 2012, with maximum values of the index much greater than that in the parents. The observed transgressive segregation indicates that genes controlling HSR are scattered throughout the genome. Extreme phenotypes can produce in both positive and negative directions through gene recombination. In 2011 and 2012, the corresponding average head splitting index values were 35.16 and 30.46, with the distribution of the data in the DH population skewed toward the resistant parental type.

Inheritance analysis for HSR. Models F-1, F-2, G-0, and G-1 in 2011 and B-1-2, F-1, G-0, and G-1 in 2012 were chosen as candidates according to the smaller Akaike's Information Criterion (AIC) (Table 3). Fitness evaluation including the uniform test (U_1^2, U_2^2, U_3^2), Smirnov test (nW^2) and Kolmogorov test (D_n) ruled out the applicability of F-2, F-1, and G-1 models because 5, 4, and 4 of the 15 parameters were observed to be significant in the 3 respective tests, while no statistic was significant under the G-0 model in 2011. The B-1-2 model could be eliminated in 2012 because five of the 15 parameters were found to be significant. Although only one parameter was significant under the other three models, the G-0 model was regarded as the best fit because it was associated with the lowest AIC score (Supplemental Table 3). We therefore deduced that HSR in cabbage can be described by a model corresponding to three pairs of additive-epistatic major genes plus additive-epistatic polygenes.

Maximum likelihood frequency values of eight component distributions were estimated under the G-0 model, with first- and second-order genetic parameters then calculated from the results (Table 4) using the least squares method. The calculated parameters showed similar tendencies in both years; additive effects (*d*) of the three major genes were estimated as 20.4, 8.59, and 15.89. All the additive effects were positive, indicating that the susceptible parental line 79-156 had important effects on total variability. The additive epistatic effect of the first two major genes, i_{ab}, was 3.64, while that between the first and third major genes, i_{ac}, was 10.94. The additive epistatic effect of the second and third major genes, i_{bc}, was only –0.86, while that among the three major genes, i_{abc}, was –5.81. Similar results were observed in 2012. Heritabilities of major genes and polygenes in 2011 were 88.03 and 7.60%, respectively, with corresponding values of 88.22 and 5.65% in 2012. The heritability values of the major genes were much larger than those of the polygenes. These observations demonstrate that HSR is mainly controlled by major genes, and that selection for this trait should be carried out in early generations.

Linkage map construction and analysis. A preliminary screening of 79-156 and 96-100 parental genotypes using SSR markers developed from cabbage sequence scaffolds and *B. oleracea* ESTs identified 252 polymorphic markers between the two parents. Of these polymorphic markers, 149 were used to genotype 157 members of the DH population. In addition to 12 EST-SSR markers, 123 scaffold-SSR markers were incorporated into the DH linkage map. The linkage analysis uncovered nine linkage groups. Based on the presence of reference SSR markers, the linkage groups were designated as C1–C9 in accordance with the nomenclature used by Wang et al. (2012a) and Lv et al. (2014).

The framework linkage map (Fig. 4) was 906.62 cM in length, with an average between-marker distance of 6.72 cM, a minimum

Fig. 2. The grade 0–5 of head-splitting resistance in DH population. 0 = no split; 1 = 1 split layer; 2 = 2 split layers and S < 50; 3 = 3–5 split layers and S < 50, or 2 split layers and S ≥ 50; 4 = 6–10 split layers and S < 50, or 3–5 split layers and S ≥ 50; 5 = more than 10 split layers, or 6–10 split layers and S ≥ 50. a = arc length and b = width of the largest split; c = height of the head and d= the largest circumference of the head. S [%] = $S_1/S_2 \times 100\%$, where $S_1 = a \times b$ and $S_2 = c \times 1/2$ d.

Table 1. Statistical summary of head splitting resistance in parents, F_1, RF_1, and doubled haploid (DH) populations.

	Parents, F_1 and RF_1				DH populations				
Head-splitting index	79–156	96–100	F_1	RF_1	Mean	SD	Variation range	Skewness	Kurtosis
2011	86.87 c[z]	9.33 a	30.50 b	31.10 b	35.16	28.27	0.00–98.52	0.76	–0.73
2012	85.32 c	9.36 a	33.12 b	32.28 b	30.45	28.55	0.00–100	0.97	–0.29

[z]Values within a given row followed by the same lowercase letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

between-marker distance of 0.46 cM, and a maximum distance of 34.90 cM. The largest linkage group, encompassing 189.46 cM, was C3; the smallest, C2, spanned 32.01 cM (Table 5). The maximum average distance between markers (10.67 cM) was that of C2, which featured the lowest number of markers (3); the minimum average distance (3.34 cM) was found on C7, which had the highest number of markers (24). The cabbage genome has been variously estimated to comprise 603 (Uzunova et al., 1995) or 630 Mbp (Liu et al., 2014). Using the latest estimate of Liu et al. (2014), the average physical distance between mapped markers was calculated to be 4.67 Mbp.

Segregation distortion in the DH population. Segregation distortion is a feature common to *Brassica* DH populations, indicating possible preferential selection of genotypes responsive to microspore culture and/or the ability to produce seed during regeneration and seed-bulking phases (Sebastian et al., 2000; Wang et al., 2012a). Based on a χ^2 test for goodness of fit to the expected 1:1 Mendelian segregation ratio, 90 of 135 loci displayed varying degrees of distortion ($P \leq 0.01$) (Table 5). Although slightly more, 79-156 alleles (63.3%), were present among the 90 distorted loci compared with 96-100 (36.7%) alleles (Table 5), there was not a significant departure from a 1:1 Mendelian ratio. Linkage groups C1, C4, and C9 had clusters of markers that were distorted toward 96-100 alleles, whereas linkage groups C3, C5, C6, and C7 contained clusters of markers distorted in favor of 79-156. Linkage group C8 included small clusters of both parental genotypes. No heterozygous loci were scored during genotyping of the molecular markers.

QTL mapping for HSR in cabbage. As shown in Table 6 and Fig. 4, eight QTLs for HSR during 2 years were detected using MQM and ICIM methods with Map QTL 4.0 and IciMapping 3.0 software. The QTLs were located on cabbage chromosomes C4, C6, C7, and C9 and individually explained 5.50% to 13.94% of observed phenotypic variation. Using the MQM program, six QTLs were detected on chromosomes C4, C7, and C9; these QTLs collectively accounted for 38.4% and 38.0% of the phenotypic variation in 2011 and 2012, respectively, with the effect of each QTL ranging from 7.1 to 12.1% over the 2 years. *Hsr 4.2* and *Hsr 7.2*, which were robust QTLs showing a relatively large effect, could be detected in both years and were distributed between markers Scaffold12597a/Scaffold55516 and Scaffold195/Scaffold46873. In both years, the 96-100 allele at the *Hsr 4.2* locus increased the tendency toward HSR, whereas this allele at the *Hsr 7.2* locus decreased it. Using ICIM, five of the same QTLs were detected on chromosomes C4, C6, and C9 and together explained 37.6% and 46.7% of observed phenotypic variation in 2011 and 2012, respectively. Their individual effects ranged from 5.5% to 13.9%—the same trend observed using MQM. Three of the QTLs (*Hsr 4.2, Hsr 9.2*, and *Hsr 9.3*) either overlapped or were adjacent to the corresponding regions detected using MQM. *Hsr 9.3* was located in the interval between BOE344–BOE975 on chromosome C9; it explained 10.1% to 13.9% of the phenotypic variation and showed the largest effect of the three QTLs. The locus *Hsr 9.1*, detected only by ICIM, was a major QTL explaining 9.2% to 10.5% of the phenotypic variation in both years. The 96-100 allele increased HSR at the *Hsr 9.3* locus and decreased it at *Hsr 9.1* in both years.

Table 2. Analysis of variance of head splitting resistance (HSR) in the doubled haploid population.

Yr	Source	*DF*	*SS*	*MS*	*F*
2011	Block	13	44,468.71	3,420.67	152.81
	Line	141	346,887.36	2,460.19	109.91**
	Replication	2	89.02	44.51	1.99
	Block × rep	26	557.36	21.44	0.96
	Error	282	6,312.42	22.38	
	Corrected total	464	398,314.87		
2012	Block	10	41,866.24	4,186.62	117.53
	Line	169	402,077.30	2,379.16	66.79**
	Replication	2	95.09	47.54	1.33
	Block × rep	20	664.32	33.22	0.93
	Error	338	12,040.46	35.62	
	Corrected total	539	456,743.42		
2011 and 2012	Year	1	784.70	784.70	5.78*
	Line	154	236,825.86	1,537.83	11.34**
	Error	154	20,891.50	135.66	
	Corrected total	309	258,502.06		

* and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively. SS, sum of squares; DF, degree of freedom; MS, mean squares; F, Fisher.

Discussion

Genetic analysis. HSR is an important agronomic trait, being associated with appearance, yield, mechanical harvestability, marketability, and storability. Qin et al. (1994) have reported that head splitting in cabbage under water-sufficient conditions is mainly due to genetic factors. In the present study, head splitting is mainly controlled by genetic features, with environmental conditions having a lesser influence. Similar results have been recorded for chinese cabbage as well as watermelon, tomato, and various other fruit crops cracking (Budan, 1999; Cortés et al., 1983). The development of

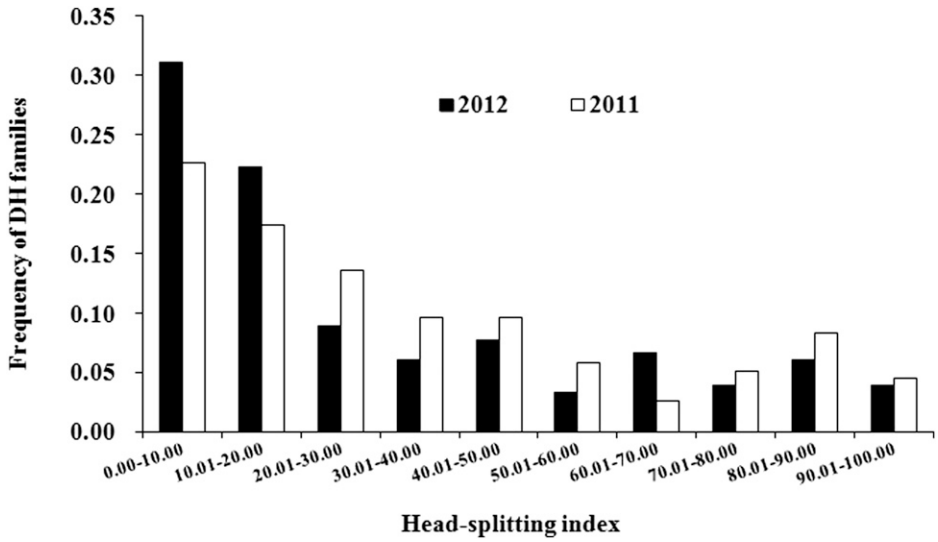

Fig. 3. Frequency distribution of head-splitting index in DH families in 2011 and 2012, arrows indicate the mean head-splitting index of the parental lines.

Table 3. Akaike information criterion (AIC) values estimated for different genetic models.

Model	AIC 2011	AIC 2012	Model	AIC 2011	AIC 2012
A-0	1576.176	1829.380	E-2-0	1442.933	1724.153
A-1	1477.031	1733.070	E-2-1	1442.181	1722.841
B-1-1	1448.086	1736.838	E-2-2	1467.829	1722.419
B-1-2	1439.054	1678.730	E-2-3	1446.232	1692.444
B-1-3	1464.138	1695.314	E-2-4	1440.953	1721.049
C-0	1533.019	1778.552	E-2-5	1440.953	1721.049
C-1	1541.363	1788.985	E-2-6	1465.257	1720.842
D-0	1464.339	1719.479	E-2-7	1465.042	1719.195
D-1	1463.836	1718.423	E-2-8	1465.042	1719.196
E-1-0	1440.924	1723.253	E-2-9	1534.623	1779.918
E-1-1	1440.172	1721.397	F-1	1438.600	1683.141
E-1-2	1465.832	1720.422	F-2	1426.862	1708.692
E-1-3	1453.709	1689.240	F-3	1444.692	1691.289
E-1-4	1438.942	1719.573	F-4	1558.399	1713.401
E-1-5	1438.942	1719.573	G-0	1434.154	1677.556
E-1-6	1463.393	1719.397	G-1	1433.911	1678.578
E-1-7	1463.114	1717.723	G-2	1465.797	1720.432
E-1-8	1463.114	1717.723	G-3	1461.501	1779.675
E-1-9	1532.740	1778.148	G-4	1455.627	1691.234

Underlined models were selected as candidate models because of their smaller AIC values.

Table 4. Estimates of genetic parameters under the G-0 model over 2 years.

Distribution parameter	Estimate 2011	Estimate 2012	First order parameter	Estimate 2011	Estimate 2012	Second order parameter	Estimate 2011	Estimate 2012
μ_1	90.57	90.62	m	37.78	32.93	σ_p^2	512.54	581.13
μ_2	79.43	65.17	d_a	20.4	20.13	σ_{mg}^2	451.22	512.70
μ_3	32.23	12.80	d_b	8.59	7.04	σ_{pg}^2	38.94	32.81
μ_4	12.44	12.80	d_c	15.89	12.42	σ^2	22.38	35.62
μ_5	50.25	43.65	i_{ab}	3.64	7.04	h_{mg}^2 (%)	88.03	88.22
μ_6	12.44	12.80	i_{ac}	10.94	12.42	h_{pg}^2 (%)	7.60	5.65
μ_7	12.42	12.80	i_{bc}	–0.86	–0.67			
μ_8	12.42	12.80	i_{abc}	–5.81	–0.68			

σ_p^2, phenotypic variation; σ_{mg}^2, major gene variation; σ_{pg}^2, polygenic variation; σ^2, environmental variation; h_{mg}^2, major gene heritability; h_{pg}^2, polygene heritability.

Table 5. Distribution of simple sequence repeat molecular markers on the genetic map.

Linkage group	Length (cM)	Number of markers	Between marker interval (cM) Min	Max	Avg	No. of distorted makers ($P \leq 0.05$)	No. of alleles 79-156	96-100
C1	64.15	16	1.32	12.19	4.01	11	0	11
C2	32.01	3	15.83	16.17	10.67	1	1	0
C3	189.46	19	0.46	19.08	9.97	16	16	0
C4	111.43	16	3.23	16.44	6.96	8	0	8
C5	93.88	13	3.32	18.73	7.22	9	9	0
C6	88.23	9	5.10	23.07	9.8	8	8	0
C7	80.07	24	0.97	7.94	3.34	16	15	1
C8	92.81	19	1.06	12.46	4.88	12	8	4
C9	154.57	16	3.67	34.90	9.66	9	0	9
Total	906.62	135	—	—	—	90	57	33

varieties having HSR is thus an essential strategy for breeders.

Few relevant genetic analyses have been conducted, however, and previous genetic studies (Chiang, 1972; Zhuang et al., 2009) of HSR inheritance have focused only on HSR as a polygenic system and ignored the effect of individual genes. The major gene/polygene genetic segregation analysis, used in the present study, has the ability of determining individual effects up to major genes and the collective epistatic effects of polygene (Wang et al., 2001). It is strongly recommended for plant breeders to apply it as a simple and useful technique to know the number of major genes, their kinds of genetic effects, heritability values and genetic information on all kinds of genetic effects, and heritability value of whole polygenes without any extra requirements on laboratory conditions except a precise experiment (Gai et al., 2007). It was used for head cracking in our previous study with six basic generations (Su et al., 2012). However, the present approach designed for the six basic populations was only capable to find the genetic mechanism up to two major genes plus polygenes (Gai et al., 2003) and the separation generations can't be repeated, which may lead to large errors. In this study, we performed a segregation analysis of the HSR trait using a DH population comprising more than 150 lines with three replications over 2 years. HSR is primarily controlled by hereditable factors with the additive-epistatic effects of three major genes as well as polygenes, and no cytoplasmic effect was observed. Additive effects predominated overall other types of genetic effects, and, as in previous studies, higher heritabilities of the trait were recorded with respect to major genes than polygenes (Chiang, 1972; Su et al., 2012; Zhuang et al., 2009).

Strategic considerations and QTL research. Compared with F_2, DH populations are ideal for the genetic study of quantitative traits, because they are composed of genetically fixed DH lines, which can be replicated between test sites and trialed over years, decreasing the standard error of QTL genotype means and allowing a better estimate of trait heritability and increased power to detect QTLs (Soller and Beckmann, 1990). Although the number of available polymorphic loci may be reduced using intracrop crosses, such a strategy—as pointed out by Walley et al. (2012)—allows a direct relationship to be established between trait and crop type; the genetic variation captured in this fashion reduces the time required to incorporate important agronomic traits into elite breeding material. Here, we report a new cabbage linkage map using SSR markers in a DH population derived from an intrasubspecies cross of cabbage. Our newly constructed map and the generated DH lines are therefore not only important for research on related characteristics of cabbage, but will also contribute to the exchange of materials between laboratories and successive research in the future (Walley et al., 2012; Wang et al., 2012a).

With the advent of molecular markers, QTL mapping has become increasingly important in molecular breeding. Marker-assisted selection and gene discovery are now widely used for the breeding of field crops and vegetables (Cheng et al., 2011; Sabouri, 2009; Walley et al., 2012). Although some QTLs have been identified in cabbage, research is still at a preliminary stage: QTL cloning has not yet been reported and functional analysis studies are rare (Lv et al., 2014). For cabbage breeders, HSR is an important characteristic that affects both cabbage yield and quality, but no QTL studies have previously been reported for this trait.

The use of different genetic models, algorithms, and mapping procedures can produce different mapping results, even for the same set of data (Su et al., 2010). The results of different mapping methods can potentially complement one another. Whole-genome scanning with multiple mapping methods has thus been recommended for QTL mapping (Xing et al., 2012). Using data obtained over multiple years increases environmental heterogeneity and also allows improved estimates of QTLs that may not reach the genome-wide significance threshold in just one environment (Chen et al., 2010b; Piepho, 2005; Van Eeuwijk et al., 2010). We used two

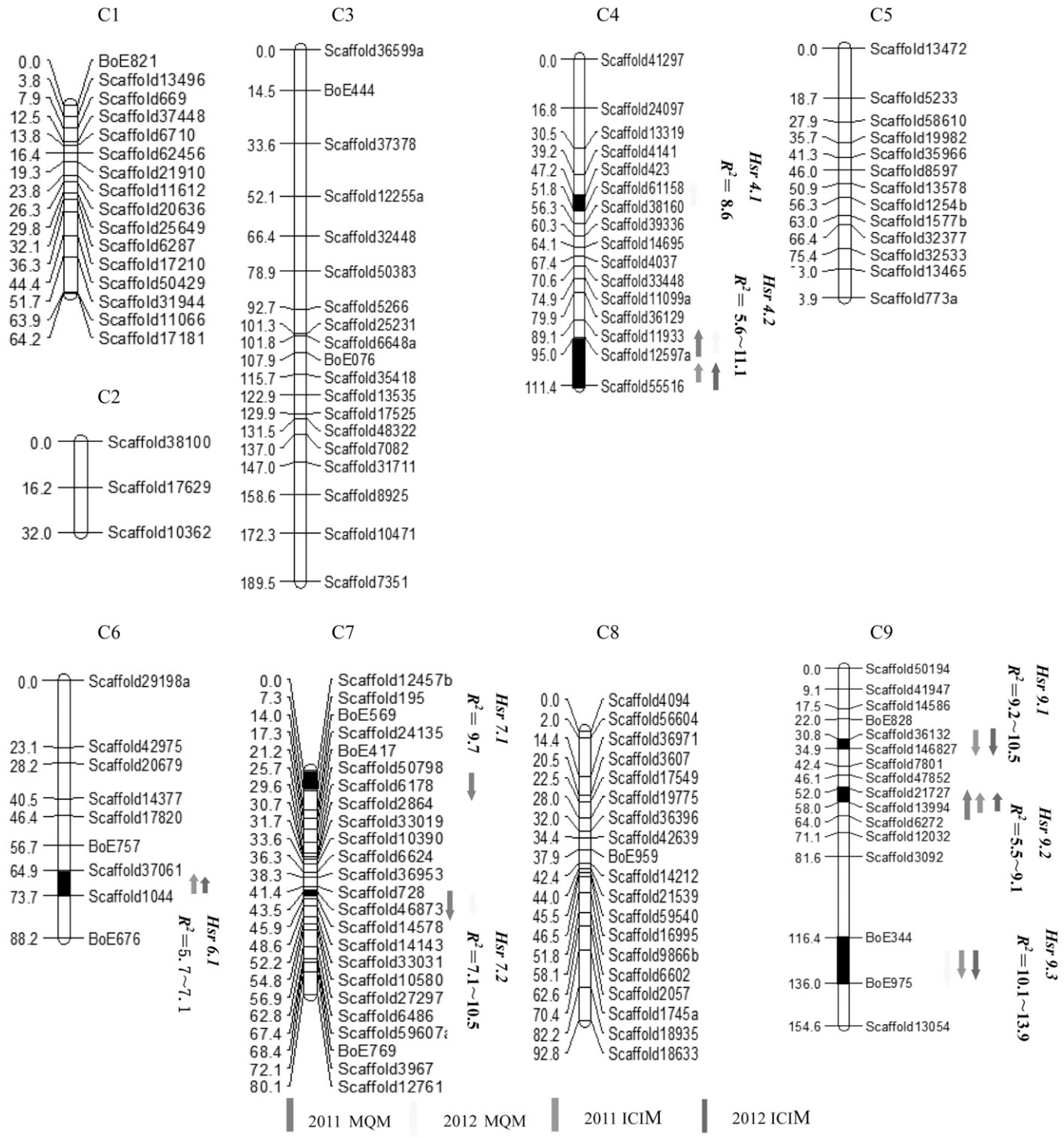

Fig. 4. Genetic linkage map and positions of quantitative trait loci (QTLs) associated with heading splitting resistance in a cabbage DH population. Marker locations are listed to the right and recombination distances (cM) to the left of each linkage group. Locations of QTLs are indicated by names and arrows to the right of the linkage groups. Arrows indicate the relative effect of the 96-100 allele with upward for increasing and downward for decreasing. Red, yellow, green, and blue represent MQM and ICIM method in 2011, 2012, respectively. R^2 means proportion of the phenotypic variation explained by each QTL.

mapping methods to analyze agronomic trait data collected during 2 years in a DH population, and our study results, the first to identify QTLs associated with the HSR trait in cabbage, provide support for the above-mentioned assertions. For example, QTLs distributed on C7 and C6 were only identified by one mapping method; in addition, *Hsr 9.2* and *Hsr 9.3* could both be detected in 2011 and 2012 by ICIM, but only one was identified using MQM. On chromosome C9, QTL *Hsr 9.1* with a larger effect is physically quite close to *Hsr 9.2*. Whether these QTLs are independent or instead both components of a larger QTL is an open question. In brief, three or four major QTLs (*Hsr 4.2, Hsr 7.2, Hsr 9.3*, and/or *Hsr 9.1*) and some minor QTLs were detected over 2 years using the two mapping methods. This result is consistent with the results of classical genetic analysis, both segregation analysis and QTL mapping with molecular markers can be used as a mutual control and supplement (Gai et al., 2007).

Because cabbage lines resistant to head splitting have traditionally been selected in the field during the mature stage, splitting severity depends on environmental factors and plant conditions. The use of DNA markers enables reliable selection of resistant plants,

Table 6. Analysis of quantitative trait loci (QTLs) for head splitting resistance in a cabbage doubled haploid (DH) population using multiple-QTL mapping (MQM) and inclusive composite interval mapping (ICIM) approaches.

Yr	QTL names[z]	Linkage group	Position (cM)	LOD	Marker interval	R^2 (%)[y]	Add[x]
	Hsr 4.2	C4	100.0	3.01	Scaffold12597a-Scaffold55516	9.1	9.19
	Hsr 7.1	C7	7.3	4.03	Scaffold195-BOE569	9.7	–9.80
	Hsr 7.2	C7	43.5	4.3	Scaffold195-Scaffold46873	10.5	–10.81
2011 (MQM)	*Hsr 9.2*	C9	51.1	3.38	Scaffold47852-Scaffold21727	9.1	10.84
	Hsr 4.1	C4	51.8	3.05	Scaffod61158-Scaffod38160	8.6	8.75
	Hsr 4.2	C4	95.0	3.61	Scaffold12597a-Scaffold55516	10.2	8.81
	Hsr 7.2	C7	41.4	2.48	Scafford728-Scaffold46873	7.1	–7.62
2012 (MQM)	*Hsr 9.3*	C9	126.4	3.06	BOE344-B0E975	12.1	–12.93
	Hsr 4.2	C4	95.0	2.55	Scaffold12597a- Scaffold55516	5.6	6.87
	Hsr 6.1	C6	68.0	2.73	Scaffold37061-Scaffold1044	7.1	8.08
	Hsr 9.1	C9	34.0	2.84	Scaffold36132-Scaffold146827	9.2	–9.08
	Hsr 9.2	C9	58.0	2.54	Scaffold21727-Scaffold13994	5.6	7.18
2011 (ICIM)	*Hsr 9.3*	C9	128.0	3.60	BoE344-BoE975	10.1	–9.38
	Hsr4.2	C4	95.0	5.41	Scaffold12597a- Scaffold55516	11.1	9.15
	Hsr 6.1	C6	66.0	2.67	Scaffold37061-Scaffold1044	5.7	6.86
	Hsr 9.1	C9	34.0	3.54	Scaffold36132-Scaffold146827	10.6	–9.14
	Hsr 9.2	C9	58.0	2.79	Scaffold21727-Scaffold13994	5.5	6.76
2012 (ICIM)	*Hsr 9.3*	C9	128.0	5.84	BoE344-BoE975	13.9	–10.40

[z]QTLs are named according to the trait (i.e., *Hsr*, head splitting resistance) followed by the chromosome number and position.
[y]Proportion of the phenotypic variation explained by each QTL.
[x]Additive effect: positive and negative values indicate that parental lines 96-100 and 79-156 respectively bear the HSR-enhancing allele.

even at the seedling stage. The makers link to QTLs identified in our study will be helpful for the identification of genes related to HSR and for MAS in cabbage breeding programs.

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Supplemental Table 1. Design of the field experiments.

Year		The order of parents, F_1, RF_1 and the block number in each repeat of DHs																	
2011	I	P_1[z]	P_2	F_1	RF_1	1[y]	6	3	9	2	14	10	11	4	12	8	5	13	7
	II	P_2	P_1	RF_1	F_1	2	9	14	7	1	10	8	4	6	11	5	13	3	12
	III	P_1	F_1	RF_1	P_2	14	5	12	4	8	1	10	6	9	13	3	7	2	11
2012	I	P_1	P_2	F_1	RF_1	1	5	6	11	9	7	2	3	10	4	8			
	II	F_1	P_1	RF_1	P_2	6	9	7	2	10	8	4	1	5	11	3			
	III	P_1	RF_1	F_1	P_2	3	5	11	4	8	10	6	9	1	7	2			

[z]Parents, F_1, and RF_1 plants were distributed according to a randomized complete block design with three replicates.
[y]Doubled haploid (DH) lines were first divided randomly into 14 blocks in 2011 and 11 blocks in 2012; the blocks were then distributed according to a randomized complete block design with three replicates.

Supplemental Table 2. Genetic models tested during joint segregation analysis of doubled haploid populations (adopted from Gai et al., 2003).

Pairs of major gene	Model	Composition distribution proportion	Composition distribution average	Model type[z]		Parameters estimated[y]	
				Only major gene	Major gene and polygene	Major gene	Polygene
0	-	-	$\mu_1=m$	A-0	C	-	[d]
1	Additive	1	$\mu_1=m+d$ $\mu_2=m-d$	A-1	D1	m, d	[d]
2	Additive-epistasis	Equal	$\mu_1=m+d_a+d_b+i$ $\mu_2=m+d_a-d_b-i$ $\mu_3=m-d_a+d_b-i$ $\mu_4=m-d_a-d_b+i$	B-1-1	E-1-1	m, d_a, d_b, i	[d]
	Additive	Equal	$\mu_1=m+d_a+d_b$ $\mu_2=m+d_a-d_b$ $\mu_3=m-d_a+d_b$ $\mu_4=m-d_a-d_b$	B-1-2	E-1-2	m, d_a, d_b	[d]
	Equal additive	1:2:1	$\mu_1=m+2d$ $\mu_2=m$ $\mu_3=m-2d$	B-1-3	E-1-3	m, $d=d_a=d_b$	[d]
	Dominant epistasis	1:2:1	$\mu_1=m+d_a$ $\mu_2=m- d_a+d_b$ $\mu_3= m- d_a-d_b$	B-1-4	E-1-4	m, d_a, d_b	[d]
	Recessive epistasis	1:1:2	$\mu_1=m+d_a+d_b$ $\mu_2=m+d_a- d_b$ $\mu_3= m-d_a$	B-1-5	E-1-5	m, d_a, d_b	[d]
	cumulative	1:2:1	$\mu_1=m+2d+i$ $\mu_2=m-i$ $\mu_3=m-2d+i$	B-1-6	E-1-6	m, $d=d_a=d_b$, i	[d]
	Complementary	1:3	$\mu_1=m+i^*$ $\mu_2=m-i^*$	B-1-7	E-1-7	m, i^*	[d]
	Duplicate	3:1	$\mu_1=m+i^*$ $\mu_2=m-i^*$	B-1-8	E-1-8	m, i^*	[d]
	Inhibitory	3:1	$\mu_1=m-i^*$ $\mu_2=m+i^*$	B-1-9	E-1-9	m, i^*	[d]
3	Additive-epistasis	Equal	$\mu_1\sim\mu_8$	F-1	G-1	m, d_a, d_b, d_c, i_{ab}, i_{ac}, i_{bc}, i_{abc}	[d]
	Additive	Equal	$\mu_1\sim\mu_8$	F-2	G-2	m, d_a, d_b, d_c	[d]
	Equai additive(1)	1:3: 3:1	$\mu_1\sim\mu_4$	F-3	G-3	m, $d=d_a=d_b=d_c$	[d]
	Equai additive(2)	1:1 :2 :2 :1:1	$\mu_1\sim\mu_6$	F-4	G-4	m,$d_1=d_a=d_b$,$d_2=d_c$	[d]

[z]B-1-X or E-1-X denote models without linkage, while B-2-X or E-2-X denote models with linkage.
[y]m, population mean; d, major gene additive effects for models A and D; d_a, d_b, and d_c, additive effects of the first, second, and third major genes, respectively, for models B, E, F, and G; i, additive × additive effect of the two major genes for models B and E; i_{ab}, i_{ac}, i_{bc}, and i_{abc}, interaction effect of the first and second major genes, the first and third major genes, the second and third major genes, and the three major genes, respectively, for models F and G; i^*, includes additive and additive × additive effects.

Supplemental Table 3. Tests for goodness of fit of alternative models.

Year	Model	Generation	U_1^{2z}	U_2^2	U_3^2	${}_nW^2$	D_n	No. of significant parameter(s)
2011	F-2	P1	1.65(0.20)	1.82(0.18)	0.18(0.68)	0.23(>0.05)	0.58 (>0.05)	5
		P2	0.35(0.55)	0.04(0.84)	2.20(0.14)	0.15(>0.05)	0.47(>0.05)	
		DH	16.30(0.00)[y]	10.47(0.00)	7.28(0.01)	2.16(<0.01)	0.21(<0.05)	
	G-0	P1	0.09(0.77)	0.34(0.56)	1.42(0.23)	0.10(>0.05)	0.41(>0.05)	0
		P2	0.00(0.97)	0.02(0.90)	0.42(0.52)	0.04(>0.05)	0.24(>0.05)	
		DH	0.09(0.76)	0.02(0.90)	0.42(0.52)	0.11(>0.05)	0.09(>0.05)	
	G-1	P1	0.00(0.96)	0.07(0.79)	3.27(0.07)	0.08(>0.05)	0.35(>0.05)	4
		P2	0.10(0.76)	0.22 (0.64)	0.48(0.49)	0.05(>0.05)	0.30 (>0.05)	
		DH	4.82(0.03)	6.64 (0.01)	3.27(0.07)	0.55(<0.05)	0.11 (<0.05)	
	F-1	P1	0.00(0.96)	0.17 (0.68)	2.15 (0.14)	0.10(>0.05)	0.38 (>0.05)	4
		P2	0.16(0.69)	0.65 (0.42)	2.92(0.09)	0.16(>0.05)	0.49(>0.05)	
		DH	4.94(0.03)	6.68 (0.01)	3.00 (0.08)	0.55(<0.05)	0.11 (<0.05)	
2012	G-0	P1	0.09(0.77)	0.34 (0.56)	1.42 (0.23)	0.10(>0.05)	0.41 (>0.05)	1
		P2	0.00(0.97)	0.02 (0.90)	0.42 (0.52)	0.04(>0.05)	0.24 (>0.05)	
		DH	2.88(0.09)	3.31 (0.07)	0.51(0.47)	0.43(>0.05)	0.10 (<0.05)	
	G-1	P1	0.12(0.73)	0.07 (0.79)	0.07(0.78)	0.07(>0.05)	0.31 (>0.05)	1
		P2	0.61(0.43)	0.84 (0.36)	0.42(0.52)	0.10(>0.05)	0.41(>0.05)	
		DH	3.42(0.06)	3.73 (0.05)	0.32(0.57)	0.45(>0.05)	0.10 (<0.05)	
	B-1-2	P1	0.51(0.47)	1.04 (0.31)	1.72(0.19)	0.16(>0.05)	0.52(>0.05)	5
		P2	0.14(0.70)	0.65(0.42)	3.06(0.08)	0.17 (>0.05)	0.50(>0.05)	
		DH	35.75(0.00)	24.59(0.00)	11.03(0.00)	3.98(<0.01)	0.27(<0.05)	
	F-1	P1	0.76(0.38)	1.29(0.26)	1.36(0.24)	0.18(>0.05)	0.55(>0.05)	1
		P2	0.13(0.72)	0.62(0.43)	3.09(0.08)	0.17(>0.05)	0.49 (>0.05)	
		DH	3.01(0.08)	3.51(0.06)	0.60(0.44)	0.45(>0.05)	0.11(<0.05)	

[z]U_1^2, U_2^2, and U_3^2, χ^2 statistics; nW^2, Smirnov's statistic; D_n, Kolmogorov's statistic. Values in parentheses after U_1^2, U_2^2, U_3^2, and D_n values are probabilities; values of nW^2 are 0.461 and 0.743 at $P < 0.05$ and $P < 0.01$ significance levels, respectively.
[y]Underlined values are significant.