African Journal of Biotechnology Vol. x(xx), pp. xxx-xxx, xx xxxxx, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1107
ISSN 1684–5315 ©2011 Academic Journals

Full Length Research Paper

Inheritance of leaf color and sequence-related amplified polymorphic (SRAP) molecular markers linked to the leaf color gene in *Brassica juncea*

Y. X. Luo[1,2], D. Z. Du[1*], G. Fu[2], L. Xu[2], X. P. Li[2], X. R. Xing[2], Y. M Yao[2], X. M. Zhang[2], Z. Zhao[2] and H. D. Liu[2]

[1]College of Agriculture, Northwest A and F University, Shaanxi 712100, P. R. China.
2Qinghai Academy of Agriculture and Forestry, Qinghai University, Qinghai 810016, P. R. China.

Accepted 15 July, 2011

Leaf color inheritance in *Brassica juncea* was studied in F_1, F_2 and BC_1 populations. Leaf color was found to be under the control of gene, and the purple leaf trait was dominant over the green leaf trait. Segregation analysis reveal that one pair of gene controlled the leaf color. To develop markers linked to the leaf color gene, SRAP (sequence-related amplified polymorphism) combined with BSA (bulked segregant analysis) technology was used to screen the parents and bulks were selected randomly from an F_2 population (Ziye × Lvye) consisting of 192 individuals. From a survey of 170 SRAP primer combinations, 3 SRAP markers linked to leaf color gene were identified, and the average distance between markers was 3.3 cM. The closet markers (ME7EM9 and ME2EM2) were at a distance of 1.2 and 5.5 cM from the target gene, respectively. The leaf color trait controlled by a single dominant gene together with the available molecular markers will greatly facilitate the future breeding of purple leaf varieties. The markers found in this study could accelerate the step of map-based cloning of the target gene.

Key words: *Brassica juncea*, leaf color, inheritance, sequence-related amplified polymorphism (SRAP).

INTRODUCTION

The *Brassica* genus is remarkable for containing more important agricultural and horticultural crops than any other genus. The major crop types include three diploid species, *Brassica rapa*, *Brassica nigra* and *Brassica oleracea*, and also three amphidiploid species, *Brassica napus, Brassica juncea* and *Brassica carinata* (UN, 1935). *B. napus* (AACC, 2n = 38) is widely cultivated as an oilseed crop in China, Canada, Australia and Europe. *B. napus* has limited variability (Prakash et al., 1980). Intensive breeding has also exhausted this variability to a considerable extent. *B. juncea* (AABB, 2n = 36) has a number of potential advantages over *B. napus*, including enhanced seedling vigor, blackleg resistance and shatter resistance, plus higher tolerance to drought and high temperature stresses (Mahmood et al., 2007; Feng et al., 2009; Srivastava et al., 2010). Both *B. juncea* and *B. napus* possess a common A genome and a few homologous chromosomes between the genomes B and C, which provide the foundation for the transfer of valuable agronomic traits from *B. juncea* into *B. napus* by the inter-specific crossing (Roy, 1984; Woods et al., 1991; Turi et al., 2010). *B. juncea* is not only an important species for planting in drought areas but also the valuable resource for the genetic improvement of *B. napus* (Fariduddin et al., 2009).

China is one of the original centers of *B. juncea* with abundant genetic resources, such as yellow-seeded mustard, tetralocular siliqua mustard and purple leaf mustard. However, research and utilization of *B. juncea* are just beginning. Some local varieties, especially Ziye mustard, are main and unique ecological types in *B. juncea* (Qi et al., 2007). Purple leaf coloration is due to anthocyanins in foliar tissues. Anthocyanins are water soluble pigments derived from flavonoids via the shikimic acid pathway. While foliar anthocyanins are develop-

*Corresponding author. E-mail: dezhidu@yahoo.cn. Tel: 86-971-13709769842. Fax: 86-971-5366520.

mentally or environmentally transient in some species, they are permanent leaf components in Ziye mustard.

Purple leaf trait is desirable in any oilseed *Brassica* species. Anthocyanins serve to depress the freezing point of water in tissues and inhibit water migration into extracellular spaces. Purple leaf color trait is an indicative character of freezing resistance. Huang et al. (2009) reported that purple leaf varieties have high anthocyanins content than green leaf varieties, which has a significant positive correlation with freezing resistance of *B. juncea*. Purple gene could be used as a visible selectable marker for plant transformation as an alternative to chemically selectable markers, such as kanamycin resistance. It is simple, visual, rapid and has less pollution in comparison with other reporter genes (kortstee et al., 2011). Purple leaf trait gene could be used in hybridization to improve hybrids purity and yield because the trait is easily scored and expressed at all developmental stages (Irani and Grotewold, 2005). Additionally, Ziye mustard could be used for ornamental crops.

Few results about the inheritance of plant color in *Brassica* have been reported. In *B. napus*, Tu et al. (1999) indicated that purple stem was controlled by a dominant gene. But Lv et al. (2008) reported that leaf color was under the control of nuclear gene, the purple leaf trait was partially dominant over the green trait and a single gene locus controlled the partial dominance of purple leaf color. In *B. juncea* and *B. rapa*, the purple leaf has rarely been genetically studied. For developing purple leaf varieties, a detailed understanding of the genetics of leaf color and an easy way of identifying the character are required.

Efficient selection of desirable traits is of great concern for crop improvement. Traditionally, phenotypic analysis is used for the selection of desirable plants. Molecular markers are complementary tools to traditional selection (Karuppanapandian et al., 2006; Sorkheh et al., 2009; Kalia et al., 2011; Ali et al., 2011; Khan et al., 2011), which may modify the breeding objectives and reduce the cost of producing breeding lines and accelerate the breeding program dramatically. Many molecular marker technologies have been applied in plant breeding, such as restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and sequence related amplified polymorphism (SRAP). So far, few molecular markers linked to the leaf color gene have been identified in *Brassica*. In *B. napus*, five AFLP markers linked to purple leaf color gene (Lv et al., 2008) have been identified, and four of which have been converted into sequence characterized amplified region (SCAR) markers. In *B. rapa*, Burdzinski et al. (2007) developed three AFLP markers linked to non-purple stem and assigned the white locus to *B. rapa* linkage group R9. But no marker associated with purple leaf color gene has been identified in *B. juncea* yet.

In order to make good use of and clone this gene, it is imperative to construct a fine map. In this study, we analyzed the inheritance of leaf color of the purple leaf mustard, identified three SRAP markers tightly linked to the leaf color gene and constructed a map around the target gene, which would be helpful for future breeding and gene cloning.

MATERIALS AND METHODS

B. juncea local species, Ziye mustard (P_1) grown in the Qinghai-Tibet Plateau with purple leaf and brown-seeded and local species, Lvye mustard (P_2) grown in the Central Plains with green leaf and yellow-seeded were analyzed for association with leaf color. P_1 was crossed reciprocally with the pure local species P_2, and their F_1 populations were backcrossed with both parents. These F_1, F_2, B_1 (P_1) and B_2 (P_2) were grown in the field at the Qinghai University (36° 38′N, 101° 45′E) and Yuanmou farm (25° 25′N, 101° 35′E) in Yunnan province. Segregation of leaf color was studied in F_2 and backcross populations. Plants of the segregated populations were grouped into purple and green color leaf classes. A χ^2 test was performed on the grouped data to check the goodness-of-fit of the segregating populations to the expected Mendelian phenotypic segregation ratio. Meanwhile, 192 F_2 plants derived from $P_1 \times P_2$ were used as the mapping population to identify SRAP markers linked to the leaf color gene.

DNA extraction and preparation of bulks

Genomic DNA was isolated from fresh leaves by CTAB (cetyl trimethyl ammonium bromide) method (Li et al., 2001; Sharma et al., 2003). Equal quantities of DNA from 8 purple F_2 plants and 8 green F_2 plants were pooled to form the purple bulk (BP) and green bulk (BG), respectively. DNA concentration was adjusted to 50 ng/µl.

SRAP analyses

SRAP methodology was according to Li et al. (2001) with minor modifications. Genomic DNA was isolated from the parents and the F_2 populations as described previously. Primers were synthesized by Shanghai Sangon Company. SRAP primers sequence information was from Huazhong Agricultural University. The total volume was 10 µl containing 1× reaction buffer, 200 µmol/L dNTPs, 0.5 U Taq, 50 ng template, 50 ng of each forward and reverse primers. The parameters for PCR were 94℃ pre-denaturing for 2 min, followed by 5 cycles of 94℃ for 1 min, 35℃ for 1 min, 72℃ for 1 min and 35 cycles of 94℃ for 1 min, 50℃ for 1 min and 72℃ for 1 min and a final extension of 7 min at 72℃. PCR products were stored at 4℃. Amplification products were separated on 6% polyacrylamide denaturing sequencing gel, fixed in 10% acetic acid for 45 min, rinsed with H_2O for 20 min, then visualized by silver staining system and dried on glass plates.

Gel electrophoresis

Amplification products were run through electrophoresis on 6% denaturing polyacrylamide gels for about 45 min with constant power of 80 W. The gel was composed of 57 g/L acrylamide (19 Ac r: 1 Bis), 3 g/L bisacrybmide, 420 g/L urea, 200 ml/L 5×TBE, 0.03 g/L ammonium persulfate solution, 30 µl/L TEMED (N, N, N0, N0, -tetramethyene diamine).

Figure 1. Plant materials. (a): lvyejie (P_2, left) and ziyejie (P_1, right); (b): F_2 individuals.

Table 1. Expected ratio of F_2, B_2 (P_1) and B_2 (P_2) plants from $P_1 \times P_2$. χ^2 Tests are for a single gene hypothesis.

Cross	Generation	Total number	Purple-leaf number	Green-leaf number	Expectant ratio	χ^2	P
	$B_1(P_1)$	86	86	0	1:0	0	1
$(P_1 \times P_2)$	$B_2(P_2)$	221	105	116	1:1	0.55	0.4-0.5
	F_2	590	433	157	3:1	0.81	0.3-0.4

Silver staining

Gel was fixed in a solution of 10% (v/v) acetic acid for 40 min, washed two times with deionized water, 10 min each and soaked in staining solution (0.15% w/v silver nitrate) without the formaldehyde for 60 min. After rinsing for about 5 s with de-ionized water, the gel was soaked in pre-cooled developing solution containing 6% (w/v) sodium carbonate, 0.15% formaldehyde and 0.003% sodium thiosulfate. When the image was sufficiently developed, the process was stopped by placing the gel in 10% (v/v) acetic acid for 5 min. Gels were then washed for 30 min with de-ionized water before storage.

Linkage analysis

The F_2 population consisting of 192 individuals was utilized to analyze the SRAP markers. The specific SRAP fragments that showed reproducible polymorphism between purple and green individuals were regarded as dominant or co-dominant markers, respectively. These marker data and individual phenotypes were analyzed with the MAPMAKER/ EXP 3.0 program. A minimum LOD score of 3.0 was used for map construction. Map distances were calculated using Kosambi's mapping function.

RESULTS

Leaf color inheritance in *B. juncea*

To elucidate the inheritance of purple leaf color in *B. juncea*, Chinese Ziye mustard inbred was crossed with the Lvye mustard inbred, and their F_1, F_2 and BC_1 progenies were phenotyped for leaf color (Figure 1). Analysis results of seedling color showed that purple leaf color in *B. juncea* was under the control of nuclear gene and that purple leaf trait was dominant over green character. Leaves of F_2 population segregated into purple and green leaf color groups. Of the 590 F_2 plants derived from $P_1 \times P_2$, 433 were purple color leaf, 157 were green color leaf. The chi-square test showed that leaf color segregated in a ratio of 3:1 (χ^2 = 0.81, P = 0.3 - 0.4), confirming a monogenic inheritance of the trait. One hundred and sixty four B_2 plants from the cross were also used for leaf color segregation analysis. The leaf color in B_2 also segregated into purple and green color groups. Of the 201 B_2 plants, 105 were purple and 116 were green. Chi-square tests showed that leaf color segregated in a ratio of 1:1 (χ^2 = 0.55, P = 0.4 - 0.5). This ratio is consistent with monogenic inheritance of this dominant trait. No green plant was observed in the B_1 progenies, which further confirmed the dominance (Table 1).

Microsatellite markers

For genotype, the population efficiency, bulked segregant analysis (BSA) was used to identify polymorphism of the markers. The DNA pools were used to test 170 pair of SRAP primers (Table 2). Most primers were from *B. napus*

Table 2. The SRAP primer combinations.

Forward primer		Reverse primer	
Name	**Sequence 5'–3'**	**Name**	**Sequence 5'–3'**
ME1	TGAGTCCAAACCGGAAA	EM1	GACTGCGTACGAATTAAA
ME2	TGAGTCCAAACCGGAAC	EM2	GACTGCGTACGAATTAAC
ME3	TAGGTCCAAACCGGAAG	EM3	GACTGCGTACGAATTAAG
ME4	TAGGTCCAAACCGGAAT	EM4	GACTGCGTACGAATTAAT
ME5	TGAGTCCAAACCGGACA	EM5	GACTGCGTACGAATTACA
ME6	TGAGTCCAAACCGGACG	EM6	GACTGCGTACGAATTACC
ME7	TAGGTCCAAACCGGACC	EM7	GACTGCGTACGAATTACG
ME8	TGAGTCCAAACCGGACT	EM8	GACTGCGTACGAATTACT
ME9	TGAGTCCAAACCGGAGA	EM9	GACTGCGTACGAATTAGA
ME10	TAGGTCCAAACCGGAGC	EM10	GACTGCGTACGAATTAGC
		EM11	GACTGCGTACGAATTAGG
		EM12	GACTGCGTACGAATTAGT
		EM13	GACTGCGTACGAATTATA
		EM14	GACTGCGTACGAATTATC
		EM15	GACTGCGTACGAATTATG
		EM16	GACTGCGTACGAATTATT
		EM17	GACTGCGTACGAATTCAA

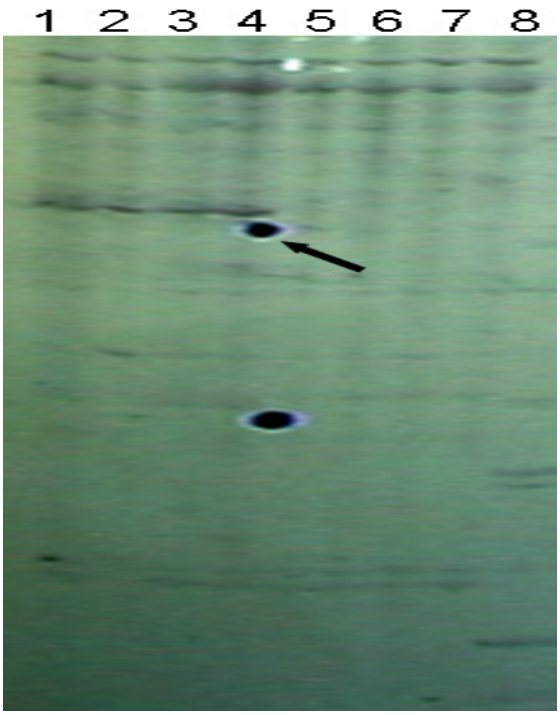

Figure 2. DNA products amplified by ME7EM9 on parents and bulks. 1 and 2: P_1; 3 to 4: BP; 5 to 6:P_2; 7 to 8: BG; arrow: specific band.

and *B. rapa*, but a few were from *B. nigra*, which were tested since the sequence homology between *Brassicas* in the U's Triangle that allowed analysis of markers was first identified in *B. napus*, *B. rapa, B. nigra* and *B. oleracea* (Ling et al., 2007; Lombard et al., 2001; Plieske and Struss 2001; Saal et al., 2001). Of the 170 SRAP primer pairs, six SRAP primer combinations showed polymorphism between the purple and green pools. These six pairs of candidate primers were then further tested in parents and F_1 plants. The PCR products of two SRAP primers in parents were not identical to DNA pools. PCR products of four SRAP primers in parents or F_1 were identical to DNA pools (Figure 2). However, difference in molecular weights of PCR products amplified by one SSR primers was not significant between two parents. Therefore, they were not used for the further analysis due to the difficulty in differentiating them.

Furthermore, the three SRAP primer pairs were used to test the 16 individuals (eight purple leaf individuals and eight green leaf individuals) comprising the bulks. All primers amplified the polymorphic products in the purple pool, purple parent, F_1 hybrid, and 8 plants established purple pool, but no target bands in green pool, green parent and 8 plants established green pool, indicating that they are dominant markers (Figure 3). The primer sequences that generated good candidate molecular markers are presented in Table 3.

Linkage analysis

To confirm the linkage relationship of the purple gene with these markers, an F_2 population including 192 plants was used. After we identified markers that were polymorphic in the parental generation and bulks, we evaluated each as a candidate for linkage to the purple leaf color locus, followed by more detailed linkage

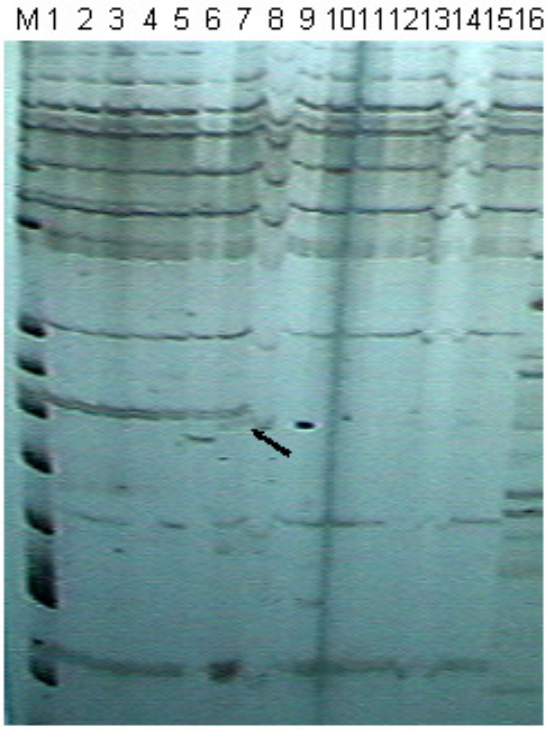

Figure 3. DNA products amplified by ME1EM7 on 16 plants used for gene pool construction M: marker; lanes 1 to 8: purple-leaf plants in F_2; lanes 9 to 16: green-leaf plants in F_2; arrow: specific band.

analysis of candidates. All primer pairs used for mapping produced bands that segregated from each other as alleles, thus verifying that the markers are single locus markers. We identified the linkage phase between purple leaf color and each marker in the F_1 generation and then F_2 progeny as either parental or recombinant. Three SRAP markers were candidates for linkage to leaf color due to significant deviation from a 1:1 ratio of parental to recombinant genotypes (Table 3). Finally, we used multi-point LOD score analysis with Mapmaker/exp, version 3.0 to assemble a linkage group containing markers ME1EM7, ME7EM9, ME2EM2 and the target gene PL. The markers covered a region of 13.1 cM around the purple leaf gene with an average interval of 3.3 cM. ME7EM9 and ME2EM2 were the closest markers linked to the purple leaf locus, at a genetic distance of 1.2 and 5.5 cM, respectively (Figure 4). The discovery of molecular markers flanking target gene is important in integrating the purple leaf color locus linkage group into known *B. juncea* linkage groups, and for comparative mapping studies.

DISCUSSION

Data from this study clearly indicate that purple leaf trait was under the control of a single dominant gene. This is the first report about the inheritance of the leaf color in Ziye mustard from Qinghai-Tibet plateau. This result is in conformity with the findings of Yan (2008) but in contrast with those reported by Zhang (2010) and LV (2008). The differences observed in the genetic control of leaf color in various studies could be attributed to the difference in the genotypic background of the species used.

In order to make use of this valuable genetic resource, it is necessary to study the molecular markers linked to this gene. We employed SRAP technologies along with BSA to identify markers linked to the leaf color gene. This technology has been popularized for gene mapping, especially for quality trait controlled by a single gene (Padmaja et al., 2005; Massand et al., 2010). We screened 170 SRAP primer combinations. Six SRAP combinations revealed polymorphism between the purple and green bulks. These six SRAP combinations were then further examined in parents, F_1 and the 16 individuals (eight purple leaf individuals and eight green leaf individuals) comprising of the bulks. The PCR products of three SRAP primers in parents, F_1 and 16 individuals were identical to DNA pools. The discrepancy between the results of bulk and parents analyses is not uncommon (Kim et al., 2010) and may be attributed to the composition of the bulk. After examined in individuals of F_2 population, PCR products of the three primers were identified as being tightly linked to the leaf color trait. All primers amplified a specific fragment only in purple individuals, which followed a dominants inheritance mode. We could not find any co-dominant marker linked to the purple leaf color gene, and therefore, we could not distinguish the homozygous from heterozygous genotypes in F_2 population. To our knowledge, this is the first report about the molecular markers being linked to purple leaf trait in *B. juncea.* The present markers, ME1EM7, ME7EM9 and ME2EM2, could be used to screen leaf color trait in marker assisted selection breeding programs. A molecular linkage map of three SRAP markers was constructed by using F_2 population. This linkage map could also be useful in screening genetically diverged populations for the leaf color trait.

The SRAP marker system is a simple and efficient marker system that can be adapted for a various purposes in crops, including map construction and map-based cloning. It has several advantages over other systems: simplicity, reasonable throughput rate, allows easy isolation of bands for sequencing and, most importantly, it targets ORFs (Li et al., 2001; Chen et al., 2007; Feng et al., 2009). However, few SRAP-based maps have been constructed, and the density of maps constructed by previous researchers is very low in *B. juncea* when compared with *B. napus* (Lombard et al., 2001; Pradhan et al., 2002; Lionneton et al., 2004; Ramchiary et al., 2007). It was difficult to compare the two maps, because the SRAP markers they used are different from the ones we used. Thus, we suggested that developing common markers adapted for mapping in

Table 3. Primers, polymorphism and map distances of markers linked to the purple leaf color gene.

Name	Polymorphism	Distance from purple locus (cM)
EM2ME2	Dominant	5.5
EM7ME9	Dominant	1.2
EM7ME1	Dominant	7.6

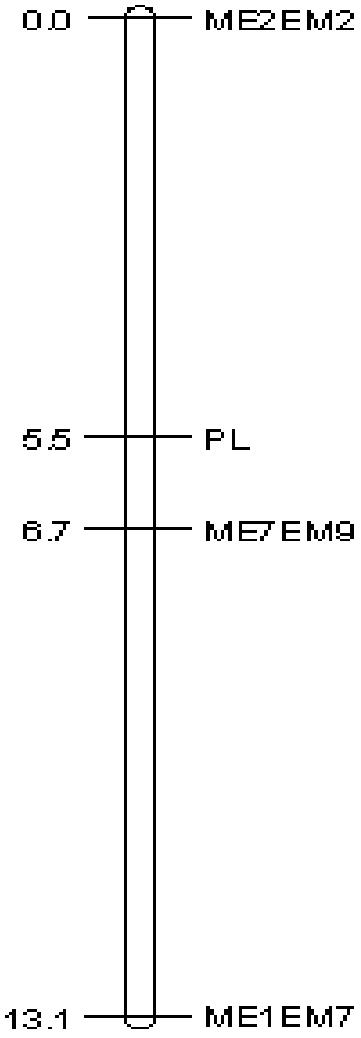

Figure 4. Linkage map shows SRAP markers flanking the purple leaf color gene in *B. juncea*. Map distance in the left is reported in Kosambi centiMorgan (cM) units, the markers are listed in the right. PL means the purple leaf color gene.

Brassica seems to be very urgent (Lowe et al., 2004; Rahman et al., 2007; Cheng et al., 2009). This will be helpful for comparative mapping, as well as chromosome location of agronomic traits in *Brassica*. Within a species of *Brassica*, we can analyze linkage relationship between agronomic traits which have been identified by comparison of common markers without consideration of difference of parents and mapping populations. Between species of *Brassica*, we can analyze the same agronomic characters linked to common markers in order to accelerate progress of map integration in *Brassica*. Of course, specific markers for different *Brassica* crops are absolutely necessary for gene mapping.

Conclusion

A dominant gene was responsible for the leaf color trait *in B. juncea*. We have identified three molecular markers linked to it. This linkage map of leaf color gene in *B. juncea* represents the first localization of a purple leaf gene in the *Brassica*, and may be used for *B. juncea* map enhancement and comparative mapping of related species.

ACKNOWLEDGEMENTS

This study was supported by National Key Technology Research and Development Program (2007BAD64B03) and Program from Science and Technology Department of Qinghai Province (2009-Z-702). The authors thank Prof. Fu Zhong, in Qinghai Academy of Agriculture and Forestry, for kindly providing the mapping population.

REFERENCES

Ali S, Munir I, Arif M, Inmullah, Farhatullah, Ali I, Iqbal A, Ahmad M, Khan MW, Abbad J, Swati ZA (2011). Characterization of *Brassica napus* germplasm based on molecular marker. Ari. J. Biotech. 10(16):3035-3039.

Burdzinski C, Wendell DL (2007). Mapping the Anthocyaninless (anl) Locus in Rapid-Cycling *Brassica rapa* (RBr) to Linkage Group R9. BMC Genet. 8:64-70.

Chen C, Yu Q, Hou S, Li Y, Eustice M, Skelton RL, Veatch O, Herdes RE, Diebold L, Saw J (2007). Construction of a sequence-tagged high-density genetic map of papaya for comparative structural and evolutionary genomics in *Brassicales*. Genetics 177:2481–2491.

Cheng X, Xu J, Xia S, Gu J, Yang Y (2009). Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor. Appl. Genet. 118:1121-1131.

Fariduddin Q, Khanam S, Ali B, Hayat S (2009). Effect of 28-homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of *Brassica juncea* L.. Acta Physiol. Plant. 31:889-897.

Feng FJ, Chen MM, Zhang DD, Sui X, Han SJ (2009). Application of SRAP in the genetic diversity of *Pinus koraiensis* of different provenances. Afr. J. Biotech. 8(6):1000-1008.

Huang C, Guo T, Zhen SC (2009). Increased cold tolerance in

Arabidopsis thaliana transformed with Choristoneura fumiferana glutathione S-transferase gene. Biol. Plantarum 53: 183-187.
Irani NG, Grotewold H (2005). Light-induced morphological alteration in anthocyanin-accumulating vacuoles of maize cells. BMC Plant Biol. 5: 1-15.
Kalia RK, Rai MK, Kalia SJ, Singh R, Dhawan AK (2011). Microsatellite markers: an overiew of the recent progess in plants. Euphytica 177: 309-334.
Karuppanapandian T, Karuppudurai T, Sinha PB, Kamarul Haniya A, and Manoharan K (2006). Genetic diversity in green gram [*Vigna radiata* (L.)] landraces analysed by using random amplified polymorphic DNA (RAPD). Afr. J. Biotechnol. pp. 1214-1219.
Khan WM, Munir I, Farhatullah, Artif M, Iqbal A, Ali I, Ahmad D, Ahmad M, Mian A, Bakht J, Inamullah, Swati ZA (2011). Assessment of genetic diversity of *Brassica juncea* germplsm using randomly amplified polymorphic DNA (RAPD) markers. Afr. J. Biotech. 10(19): 3654-3658.
Kim CK, Park SH, Kikuchi S, Kwon SJ, Park S (2010). Genetic analysis of gene expression for pigmentation in Chinese cabbage (*Brassica rapa*). Biochip J. 4: 123-128.
Kortstee AJ, Khan SA, Helderman C, Trindade LM (2011). Anthocyanin production as a potential visual selection marker during plant transformation. Transgenic Research, Online First™, 21 February 2011.10.1007/s10681-011-0351-z.
Li G, Quiros CF (2001). Sequence related amplified polymorphism (SRAP) a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103: 455-461.
Ling A, Kaur J, Burgess B, Hand M, Hopkins C, Li X (2007). Characterization of simple sequence repeat markers derived in silico from *Brassica rapa* bacterial artificial chromosome sequences and their application in *Brassica napus*. Mol. Ecol. Notes, 7: 273-277.
Lionneton E, Aubert G, Ochatt S, Merah O (2004). Genetic analysis of agronomic and quality traits in mustard (*Brassica juncea*). Genetic analysis of agronomic and quality traits in mustard (*Brassica juncea*). Theor. Appl. Genet. 109: 792-799.
Lombard V, Delourme R (2001). A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations. Theor. Appl. Genet. 103:491-507.
Lowe AJ, Moule C, Trick M, Edwards KJ (2004). Efficient largescale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor. Appl. Genet. 108:1103-1112.
Lv JV (2008). Primary mapping of the purple leaf color gene in *Brassica napus*. Ph. D. dissertation (in Chinese) Huazhong Agricultural University.
Mahmood T, Rahman MH, Stringam GR, Yeh F (2007). Quantitative trait loci for early maturity and their potential in breeding for earliness in *Brassica juncea*. Euphytica, 154: 101-111.
Massand PP, Yadava KS, Sharma S (2010). Molecular mapping reveals two independent loci conferring resistance to Albugo candida in the east European germplasm of oilseed mustard *Brassica juncea*. Theor. Appl. Genet. 121: 137-145.
Padmaja KL, Arumugam N, Gupta V, Mukhopadhyay A, Sodhi YS, Pental D, Pradhan AK (2005). Mapping and tagging of seed coat colour and the identification of microsatellite markers for marker-assisted manipulation of the trait in *Brassica juncea*. Theor. Appl. Genet. 111: 8-14.
Plieske J, Struss D (2001). Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in *Brassica*ceae species. Theor. Appl. Genet. 102: 689-694.
Pradhan A, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi Y (2002). A high-density likage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor. Appl. Genet. 106: 607-617.
Prakash S, Hinata K (1980). Taxonomy, cytogenetics and origin of crop *Brassicas*, a review. Opera Bot. 55: 1-57.
Qi XH, Zhang MF, Yang JH (2007) Molecular phylogeny of Chinese vegetable mustard (*Brassica juncea*) based on the internal transcribed spacers (ITS) of nuclear ribosomal DNA. Genet. Resour. Crop Eviron. 54: 1709-1716.
Rahman M, Mcvetty PBE, Li G (2007). Development of SRAP, SNP, and Multiplexed SCAR molecular markers for the major seed coat color gene in *Brassica rapa* L. Theor. Appl. Genet. 115:1101-1107.
Ramchiary N, Padmaja KL, Sharma S, Gupta V, Sodhi YS (2007). A high-density linkage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor. Appl. Genet. 106: 607-617.
Roy NN (1984) Interspecific transfer of *Brassica juncea*-type high blackleg resistance to *Brassica napus*. Euphytica, 33:295-303.
Saal B, Plieske J, Hu J, Quiros CF, Struss D (2001). Microsatellite markers for genome analysis in *Brassica*. II. Assignment of rapeseed microsatellites to the A and C genomes and genetic mapping in *Brassica oleracea*. Theor. Appl. Genet. 102: 695-699.
Sharma R, Mahla RH, Mohapatra T, Bhargava CS, Shama MM (2003). Isolating plant genomic DNA without liguid nitrogen. Plant Mol. Biol. Rep. 21:43-50.
Sorkheh K, Shiran B, Kiani S, Amirbakhtiar N, Mousavi S (2009). Discriminating ability of molecular markers and morphological characterization in the establishment of genetic relationships in cultivates genotypes of almond and related weld species. J. Forest. Res. 20: 183-194.
Srivastava AK, Lokhande VH, Patade VY, Suprasanna P, Sjahril R (2010). Comparative evaluation of hydro-, chemo- , and hormonal-priming methods for imparting salt and PEG stress tolerance in Indian mustard (*Brassica juncea* L.). Acta Physiol. Plant. 32: 1135-1144.
Tu JX, Fu TD, Zheng YL, Yang GS (1999). Studies on the recessive genetic male sterility and its genetic markers in rapeseed (*Brassica napus*). Ⅱ. DNA molecular confirmation for p6-9 purple stem gene linked to male fertility gene of GMS. Acta Agron. Sci. (in Chinese). 25: 669-673.
Turi NA, Raziuddin, Farhatullah, Khan NU, Munir I, Shah AH, Khan S (2010). Combining ability analysis in *Brassica juncea* L. for oil quality traits. Afr. J. Bioteh. 9(26): 3998-4002.
UN (1935). Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn. J. Bot. 7: 389-452.
Woods DL, Capcara JJ, Downey RK (1991) The potential of mustard (*Brassica juncea*) as an edible crop on the Canadian prairies. Can. J. Plant Sci. 71: 195-198.