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Comparison of RSAP, SRAP and SSR markers for genetic analysis in hot pepper

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ABSTRACT

The restriction site amplified polymorphism (RSAP), combining simplicity, reliability and moderate throughput ratio was used to assess the genetic divergence between different hot pepper (*Capsicum annuum* L.) inbred lines. The utility of RSAP markers was compared to that of sequence-related amplified polymorphism (SRAP) and simple sequence repeat (SSR) markers. The result suggested that RSAP was significantly informative among the three genetic marker systems studied on hot pepper genetic divergence. The highest number of polymorphic bands per assay ratio and the highest marker index was obtained using it. The clusters of RSAP and SSR were found to have of pepper inbred lines representing similar geographic origins, as well as reflecting important role of fruit characteristics in pepper classification. The correlations of genetic relationships were significantly high between RSAP datasets and SSR markers, but very low between SRAPs and SSRs, which corresponded to the polymorphic regions detected by different marker systems.

Key words: RSAP, SRAP, SSR, *Capsicum annuum* L., genetic distance, pepper.

INTRODUCTION

Hot pepper (*Capsicum annuum* L.) a native of tropical America was domesticated in different parts of South and Central America for thousands of years. After European contact, pepper was rapidly disseminated around the globe (Eric, 5). Generation in different areas of the world, pepper evolved many types and varieties through long-term artificial and natural selection. Now pepper is an economically important crop plants grown on a large scale as fresh and processed vegetable crops, also being a leading condiment. One of the primary needs of the crop industry is the estimation of genetic divergence between cultivated accessions for identification and breeding purposes. To this end, various DNA marker techniques have been successfully applied to study the genetic divergence of various plant species.

Studies on the genetic diversity of pepper have involved the use of morphological markers and molecular markers such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPDs) (Lefebvre, 9), simple sequence repeats (SSRs), sequence-specific amplification polymorphisms (SSAPs) (Tam *et al.*, 24) and sequence-related amplified polymorphisms (SRAP) (Ren *et al.*, 20). Various markers differ in different aspects, such as number of detected bands per assay (multiplex ratio), levels of detected polymorphism, the distribution of target sequences throughout the genome, assay reproducibility, and the cost associated with the marker system. Therefore, choosing the most appropriate marker system for any given investigation is important and valuable.

Restriction site amplification polymorphism (RSAP) developed by us in recent years is a multiplex marker system aiming at detecting restriction sites polymorphism in a simple PCR. The main principle of RSAP is that it uses two primers of 18 bases, in which the 12-14 bases starting at the 5' end are sequences of no specific constitution ('filler' sequences), followed by 4-6 bases of restriction site sequences at the 3' end. The difference of two primers lies on the restriction site recognizing sequences and filler sequences. Moreover, RSAP employs the two step PCR procedure. The first 5 PCR cycles with the low annealing temperature (35°C) ensures the primers binding to the restriction sites by partial match with the target DNA template for the successful PCR amplification, and the resulting PCR products can reach to a rational level enough to be the template for further stringent PCR amplification. Then, the annealing temperature is increased to 46^{0}C for the subsequent 35 cycles, ensuring to amplify efficiently and consistently. The amplicons of RSAP could be detected on 6% denaturing polyacrylamide gel. Therefore, it combines the simplicity with reliability and moderate throughput ratio (Du *et al.*, 3). RSAP has been successful used to study genetic divergence for various plant species, such as *Loropetalum chinese* var. *rubrum* (Li *et al.*, 12), *Porphyra* (Qiao *et al.*, 19), Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*) and

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purple-caitai (*Brassica compestris* L. var. *purpurea* Bailey) (Zhang *et al.*, 32). The primary objective of the study reported here was to assess the usefulness of RSAP as a marker system for analysing the genetic divergence of pepper collections and also to compare the effectiveness of the RSAP marker system with that of other dominant and co-dominant marker systems, namely SRAP and SSR.

MATERIALS AND METHODS

Ten inbred lines (designated 401 to 410) of pepper (*Capsicum annuum* var. *longum*) provided by Vegetable Institute, Guangdong Academy of Agriculture Science were used in the study (Table 1). Genomic DNA was extracted from fresh young leaves using the SDS method (Wang *et al.*, 28). Ten primers (Table 2) of RSAP were designed according to the principle of RSAP marker system (Du *et al.*, 3). The PCR was conducted in a final reaction volume of 25 µl, which contained 30 ng DNA, 1×PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.6 mM each primers, 1.5 U *Taq* polymerase (Sino-American Biotech. Co., Luoyang, China). The amplifications were carried out by initially denaturing template DNA at 94°C for 5 min., then performing 5 cycles at 94°C for 1 min., 35°C for 1 min., and 72°C for 2 min., followed by 35 cycles at 94°C for 1 min., 46°C for 1 min., and 72°C for 1 min. The final extension step was at 72°C for 10 min. The PCR products were separated by 6% denaturing polyacrylamide gel, followed by the fast silver staining protocols (Xu *et al.*, 30) with minor modification. After electrophoresis, the glass plate with gel was placed into plastic bin containing 1% $AgNO_3$ solution and gently shaken for 10-15 min. Then the gel was rinsed with deionized water once. Thereafter, the glass plate with gel was put into a new plastic bin with 2l solution (containing 40 g NaOH, 0.8 g Na_2CO_3, and 8 ml formaldehyde) and gently shaken until bands appeared distinctly and background turned into relatively uniform yellow (approx. 2-3 min.). Subsequently, the gels were rinsed with water for 3-4 min. and dried before scoring bands. The primers of SRAP and the PCR procedure used in the study were the same as those in the original protocol (Li and Quiros, 11). The primers were commercially synthesized (Jikang Biotechnology Ltd. Co. Shanghai, China). The gel analysis was same as described in the RSAP method.

For SSR analysis 23 primers were adopted, which has been located on the different pepper chromosomes (Anon, 1) and their primers with PCR conditions were publishing (Lee *et al.*, 8). The SSR markers adopted in our study were as follows: *Hpms* 1-139, *Hpms* 1-148, *Hpms* 1-214 and AF039662, which are located on chromosome 1, *Hpms* 1-106 and *Hpms* 1-143 located on chromosome 2, *Hpms* 1-173 and AA840692 located on chromosome 3 and CAN130829 located on chromosome 4, *Hpms* 2-18 located on chromosome 4a, *Hpms* 2-45 located on chromosome 5, *Hpms* 1-172 located on chromosome 7, *Hpms* 1-216 located on chromosome 7a, *Hpms* 1-41, *Hpms* 1-43, *Hpms* 1-62, *Hpms* 1-155, *Hpms* 1-281, *Hpmshp*MADS and AA840739 located on chromosome 8, *Hpms* 1-3, *Hpms* 1-117 and *Hpms* 2-41 located on chromosome 9. In 25 µl final volume of PCR reaction, 20 ng DNA, 1×PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 mM each primers and 1U *Taq* polymerase were mixed. The PCR procedures were performed according to Lee *et al.* (8) and the gel analysis was as same as described in the RSAP method.

All distinct bands were scored as present (1) or absent (0). Genetic variation of ten inbred lines were studied using POPGENE ver.1.32, which allowed the analyses of dominant (SRAP /RSAP) and codominant (SSR) diploid data. The data obtained include the observed (n_a) and expected (n_e) number of alleles, percentage polymorphism, the polymorphic index content (PIC) or expected heterozygosity. The PIC was determined over all loci as PIC = $1-\sum P_I^2$ where P_I is the frequency of the Ith allele. A product of PIC/expected heterozygosity, the effective multiplex ratio (the proportion of polymorphic loci per primer set), the total number of loci detected per primer set, and the marker index (the product of the effective multiplex ratio and the average expected heterozygosity for the polymorphic markers; MI) (Powell *et al.*, 18) were calculated and used to compare the usefulness of RSAP in relation to the other marker systems. To estimate the genetic similarities among genotypes, the simple matching similarity coefficient for dominant marker data (SRAP and RSAP) and the Nei and Li coefficient for co-dominant marker data (SSR) were adopted (Powell *et al.*, 18). Cluster analysis based on similarity matrices was performed to construct dendrograms using the unweighted pair-group method with arithmetic averages (UPGMA) on the MVSP 3.1(Kovach Computing Services, 7). The Mantel's test was used to assess the strength of correlation between different distance matrices produced with the three markers system and the test statistic tested for significance against 1,000 random permutations using the MXCOMP module in the NTSYSpc 2.10e software (Rohlf, 21).

RESULTS AND DISCUSSION

For RSAP, forty-one primer sets yielded 2,121 bands in the pepper collections studied, of which 538 (25.4%) were polymorphic. The average number of bands generated per primer set was 51.7 (range: 28-100), with an average of 13.1 polymorphic bands per primer set (range: 3-32) (Table 3). The most frequent RSAP fragments ranged from 100 bp to 1 kb in size

Table 1. Pedigree, origin and main traits of ten inbred lines in hot pepper.

Code	Name of inbred line	Pedigree	Origin	Fruit length (cm)	Fruit width (cm)	Fruit shape (length/ width)	Pericarp thickness (cm)	Fruit wt. (g)	Fruit stalk (cm)
401	4591	Unknown	Thailand	11.84	1.83	6.46	0.21	14.67	4.63
402	5904	Derived from Hunan local variety ‘Guangpi Jiao’	Hunan, China	13.39	2.54	5.26	0.30	32.51	5.47
403	Tian Niu Jiao Jiao 8	Unknown	USA	16.18	4.43	3.65	0.37	65.58	5.6
404	388	Unknown	Anhui, China	20.35	2.01	10.11	0.25	23.79	3.6
405	LW101	Derived from Hunan local variety ‘Fudijian’	Hunan, China	16.21	1.91	8.50	0.19	15.94	4.27
406	8216	Hunan local variety ‘Hexi Niujiao Jiao’	Hunan, China	18.83	3.13	6.01	0.29	40.01	6.17
407	Male parent of ‘Luo Jiao 98A’	derived from Henan local variety ‘Yunyang Jiao’	Henan, China	18.12	3.37	5.38	0.31	48.53	4.73
408	8802	Derived from a cross between Anhui local variety and ‘Beifang Niujiao Jiao’	Anhui, China	18.21	2.07	8.81	0.22	19.97	4.2
409	Chifeng Niu jiao jiao	Derived from a cross between local variety in Northern China and Bulgaria	Inner Mongolia, China	16.49	4.46	3.70	0.32	55.37	4.33
410	410	Derived from ‘HenanYangjiao jiao’	Henan, China	18.55	2.48	7.49	0.29	34.86	5.93

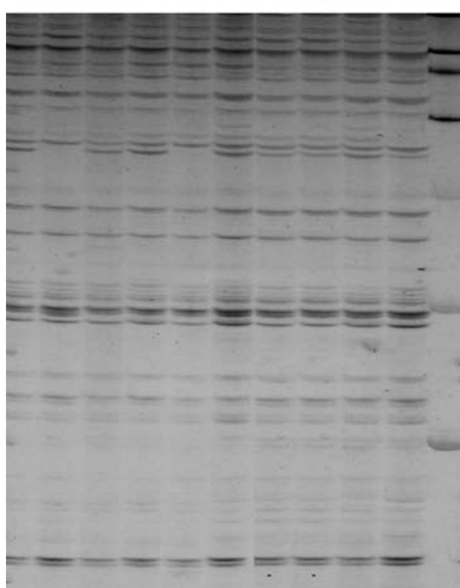

Fig. 1. RSAP amplification patterns of 10 pepper inbred lines with R6-R10 primer set.

represented by percentage polymorphism and PIC/H_e was the highest when calculated from the SSR dataset and the lowest when calculated from the RSAP dataset and from the SRAP dataset respectively. Therefore, there was no association between percentage of polymorphic loci and total number of scored loci. The highest ratios of number of polymorphic loci per primer set were observed for RSAP, being 1.3-fold higher than for SRAP and 14.4-fold higher than for SSR (Table 3). Correlation between pairwise genetic distance matrices detected by RSAP and SSR was highest (r = 0.5782) and highly significant (1,000 permutation, $P<0.01$)using Mantel's test, followed by that detected by RSAP and SRAP(r = 0.3237), however the correlation between the distance matrices detected by the SRAP and SSR were very low (r = -0.033).

All of the clusters show well-resolved relationships among the pepper lines (Fig. 2), with the average genetic distances between ten inbred lines detected by RSAP, SSR and SRAP being 0.4115, 0.4551 and 0.3591 respectively, in which the SSR cluster show highest range of genetic distances (0.171-0.789) (Table 3). The clusters of ten pepper lines inferred

Table 2. Details of ten RSAP primers used in the study.

Primer	Sequence 5' to 3'	Restriction site	Restriction enzyme
R1	ATTACAACGAGTGGATCC	GGATCC	*BamH* I
R2	CACAGCACCCACTTTAAA	TTTAAA	*Dra* III
R3	GACTGCGTACATGAATTC	GAATTC	*EcoR* III
R4	TATCTGGTGAGGGATATC	GATATC	*EcoR* III
R5	TTGGGATATCGGAAGCTT	AAGCTT	*Hind* III
R6	ATTTCAGCACCCACGATC	GATC	*Mbo* III
R7	ATAGTCCTGAGCGGTTAA	TTAA	*Mse* I
R8	ATAACTGTGTACCTGCAG	CTGCAG	*Pst* I
R9	GTACATGCATTACTGCGA	TGCGA	*Taq* I
R10	ATTGGACTGGTCTCTAGA	TCTAGA	*Xba* I

(Fig.1). For SRAP, 776 bands were scored from the twenty-three primer sets, of which 230 (29.6%) were polymorphic. The average number of bands generated per primer combination was 34 (range: 21-83), with an average of 10 polymorphic bands per primer combination (range: 1-58) (Table 3). For SSR, twenty-three primer sets were used to analyse the pepper collections and yield 41 microsatellite loci, of which 21(51.2%) were polymorphic. The average number of loci generated per primer set was 1.78 (range: 1-4), with an average of 0.91 polymorphic loci per primer set (range: 0-4) (Table 3).

A wide variation was observed for the average number of loci recovered per primer set for each marker system. However, SSR recovered the lowest ratio and RSAP the highest ratio of loci. Genetic variation

from RSAP and SSR marker systems were generally agreed with each other and with their origins. The RSAP cluster separates ten inbred lines into four groups at similarity coefficients 0.61 (Fig. 2). Except for 406 coming from China alone forming one group, one group includes lines from USA (403) excluding 409 from China, one group consists of line from Thailand (401), another group consists of lines all come from China comprising three subgroup, the first subgroup consisting lines from Henan province of China (407 and 410), the second subgroup including lines from Anhui province of China (404 and 408), the third subgroup consists of lines from Hunan province of China (402 and 405) (Fig. 2). The SSR cluster separated ten lines into five groups at similarity

Table 3. Comparison of information and genetics distance obtained with RSAP, SRAP and SSR marker systems.

Particulars	RSAP	SRAP	SSR
No. of primer sets	41	23	23
No. of bands scored	2121	776	41
No. of polymorphic bands scored	538	230	21
Polymorphism (%)	25.4	29.64	51.2
No. of loci per primer set	51.7	34	1.78
No. of polymorphis loci per primer set	13.1	10	0.91
Observed No. of alleles per locus (n_a)	1.2324	1.2964	1.878
Effective No. of alleles per locus (n_e)	1.1599	1.1445	1.3815
PIC(RSAP, SRAP) or H_e(SSR)	0.0919	0.0871	0.1983
Marker index (MI)	1.204	0.871	0.353
Mean genetic distance	0.4115	0.3591	0.4551
Minimum genetic distance	0.323	0.235	0.171
Maximum genetic distance	0.491	0.53	0.789
Genetic distance among Chinese lines	0.4006	0.3741	0.3968
Genetic distance among Thailand and Chinese lines	0.4163	0.319	0.5401
Genetic distances among USA and Chinese lines	0.4453	0.3581	0.5498

coefficient of 0.63. Except for lines from Anhui province of China comprising 404 alone forming a group and 408 interspersing among the first subgroup of another group from Henan province of China, the others grouping of SSR cluster are as same as that of the RSAP (Fig. 2).The average genetic distances between lines originated from different countries detected by SSR and RSAP are also correspond to their geographical origins, with the highest average distance among the American line (403) and eight Chinese lines (0.4453 for RSAP, 0.5498 for SSR), followed by the Thailand line (401) and eight Chinese lines (0.4163 for RSAP, 0.5401 for SSR) , the lowest among eight Chinese lines(0.4006 for RSAP, 0.3968 for SSR). However, the cluster and the average genetic distances inferred from SRAP show no correspondence to geographical origin. The clusters of RSAP and SSR are matched with pepper fruit characteristics as well. For instance, one group includes line 403 and 409 both having the biggest fruit width(>4 cm) and fruit weight, the thickest pericarp thickness (>0.3 cm) and the smallest fruit shape index (fruit length/width<4). One group consist of 401 having the smallest fruit width (<2 cm) and fruit weight .One group consist of 406 having the second biggest fruit weight, the longest fruit stalk (6 cm) and the worst fruit surface smoothness. However, the SRAP cluster is mismatched with pepper fruit characteristics as a whole, except for clustering line 403 and 409.

On the whole, we found that SSAP was an efficient marker system for studying genetic variation in pepper. RSAP, SRAP and SSR adopted in the article are all PCR-based molecular markers and very convenient to operate compared with other molecular markers, just need a simple PCR for accomplishment. Regarding marker establishment, SSR application needs genomic sequence information which development was costly. However, the establishment of RSAP and SRAP are easier without any other information known in advance (the known restricted sites for RSAP, the common features of open reading frame (ORF) for SRAP) (Li and Quiros, 11). As far as the comprehensiveness of polymorphism detection in genome is concerned, SRAP mainly amplify for ORF belong to the single-copy regions, SSR just for simple repeat sequences regions (Tautz and Renz, 25). But RSAP detects restricted sites which dispersed on the whole genome including single-copy and multi-copies zones (Vos *et al.*, 27). Therefore, RSAP may be more comprehensive for reflecting genetic divergence between lines than SSR and SRAP. Although the restriction sites act as the basis of many marker systems including RFLP(Botstein *et al.*, 2), AFLP (Vos *et al.*, 27), and various modified AFLP (Roy *et al.*, 22; Wurff *et al.*, 2000; Zhang *et al.*, 32), those marker systems rely on restriction digestion and subsequent complex procedures to detect polymorphism. Whereas, RSAP is more convenient in operation without complex steps for amplification polymorphism of restriction sites, just a simple PCR and obtain the electrophoresis profiles (Fig. 1) similar to AFLP. The comparison of marker efficiency between RSAP and AFLP was not carried out in the article. But that could be indirectly inferred from the studies between diversity analysis by AFLP previously and by RSAP in this paper. In the former

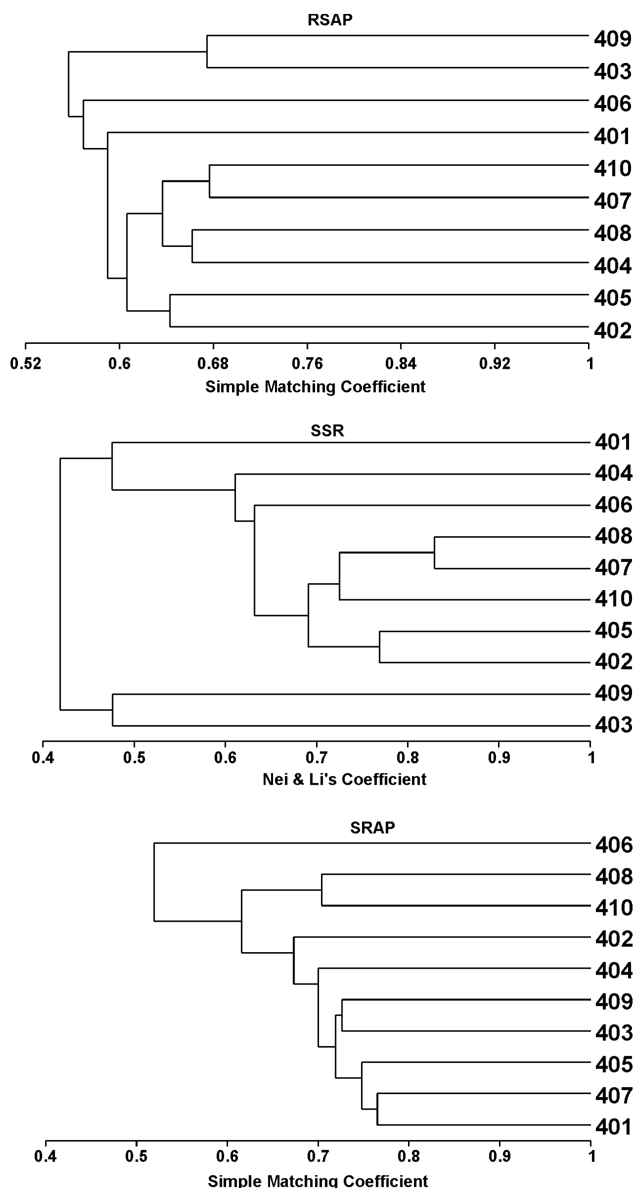

Fig. 2. Dendrograms of ten pepper inbred lines based on RSAPs, SSRs and SRAPs data.

AFLP presented 13.1 polymorphism bands per primer set in 35 pepper lines including bell pepper (*C. annuum* var*. grossum*) and conical pepper (*C. annuum* var*. longum*) (Tam *et al*., 24). In the later RSAP produced 12.78 polymorphic bands per primer set in 10 pepper inbred lines (*C. annuum* var*. longum*), suggesting RSAP can produce polymorphic bands similar as or more than AFLP in *C. annuum*.

The difference of geography and climate is the important cause for plant genetic divergence. Breeders have deduced the genetic relationship within collection according to the geographical origins of these cultivars for combination of breeding parents and germplasm preserved. However, the deduction has become more and more difficult due to the complexities of genetic context with the wide germplasm communication and the rapid development of breeding. Fortunately, the development of DNA molecular markers provides a powerful tool for the genetic distances estimated. Molecular genetic distances estimated were affected by the variance of experimental error and the repartition of the markers on the genetic map of the species (Lombard *et al*., 13). Lefbvre *et al*. (9) suggested using evenly spaced markers with known positions on a genetic map to compute the genetic distances avoiding redundancy was advisable. The genetic relationships between ten inbred lines inferred from the SSR markers spreading in different chromosome was generally agree with their geographical origins. However, the SSR cluster may be less precise due to its least number of polymorphism loci in the analysis. Meanwhile, the RSAP cluster was highly correlated with the SSR cluster and in accordance with the geographical origins better with its most number of polymorphism sites in the analysis, and reflected the genetic relationship more accurately. Minor inconformity between the RSAP/SSR clusters and the geographical origins involve in the line 409 from China grouping with line 403 from America, which can be explained by two lines have many common fruit characteristics indicting fruit characteristics being an important reflection of relationship within pepper collection as well as geography. The fruit characteristics being determinant in pepper classification was testified by some previous studies (Lefebve *et al*., 9,10; Paran *et al*.,16 ; Tam *et al*., 24). Differences among marker techniques with respect to grouping lines on the basis of genetic distance have also been shown in earlier investigations. Some investigators reported a good correlation between datasets (Thormann *et al*., 26; Lu *et al*., 14; Nagaoka and Ogihara, 15), while others reported lower correlations (Powell *et al*., 18; Pejic *et al*., 17; Giancola *et al*., 6). Lefebvre *et al*. (9) reported that RAPDs correlated well with AFLPs (r = 0.95) in pepper. Tam *et al*. (24) reported that the correlation were low among SSRs, AFLPs and SSAPs in pepper. Russell *et al*. (23) suggested that higher band sharing would make a technique more suitable for genetic relatedness studies. The correlations between SRAP and SSR were lower than the correlation between RSAP with SRAP and RSAP with SSR, which would be attribute to different marker systems aiming at various regions in genome respectively. That is, SRAP detecting polymorphism of ORF and SSR aiming at simple sequence repeates making the sharing bands of SRAPs and SSRs were lowest. However, RSAP detect the polymorphic variation on restriction sites

which dispersed and ubiquitous in organism genome including coding sequences and simple repeats sequences, which making the more sharing bands were between RSAPs and SSRs, as well as RSAPs and SRAPs.

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REFERENCES

1. Anonymous 2007. United States Department of Agriculture, National Institute of Food and Agriculture. 2005. Sol genomics network. 10 March 2006. <http://www.sgn.cornell.edu/oldhighlights.pl>.

2. Botstein, D., White, R.L., Skolnick, M.H. and Davies, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Amer. J. Human Genet.* **3**: 314-31.

3. Du, X. 2006. Development of RSAP- a novel DNA marker technique and its application in pepper genetics and breeding. Ph.D. thesis, Northwest A&F University, Yangling, Shaanxi, P.R. China.

4. Du, X., Wang, D. and Gong Z. 2006. Development and refinement of a new marker technique--Restriction site amplified polymorphism (RSAP). *J. Northwest A&F Uni. (Nat. Sci. Ed.)* **34**: 45-49, 54(*in Chinese*).

5. Eric, J.V., Jit, B.B. and Paul, W.B. 2005. Genetic diversity of chili (*Capsicum annuum* var. *annuum* L.) landraces from northern New Mexico Colorado and Mexico. *Econ. Bot.* **59**: 8-17.

6. Giancola, S., Marcucci Poltri, S., Lacaze, P. and Hopp, H.E. 2002. Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean. *Euphytica*, **127**: 95-113.

7. Kovach, W.L. 2007. MVSP - a multivariate statistical package for windows, ver. 3.1. Kovach Computing Services, Pentraeth, Wales, U.K.

8. Lee, J.M., Nahm, S.H., Kim, Y.M. and Kim, B.D. 2004. Characterization and molecular genetic mapping of microsatellite loci in pepper. *Theor. Appl. Genet.* **108**: 619-27.

9. Lefebvre, V., Goffinet, B., Chauvet, J.C., Caromel, B., Signoret, P., Brand, R. and Palloix, A. 2001. Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RAPD and phenotypic data. *Theor. Appl. Genet*. **102**: 741-50.

10. Lefebvre, V., Palloix, A. and Rives, M. 1993. Nuclear RFLP between pepper cultivars. *Euphytica*, 7**1**: 189-99.

11. Li, G. and Quiros, C.F. 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**: 455-61.

12. Li, Y., Xiong, X., Yu, X., Chen, H., Li, Y. and Li, D. 2009. Establishment and optimization of RSAP-PCR reaction for *Loropetalum chinese* var. *rubrum*. *J. Hunan Agric. Univ. (Nat. Sci.)* **35**: 65-75.

13. Lombard, V., Baril, C.P. and Zhang, D. 2000. Improvement of the precision of genetic distance estimates between rapeseed cultivars based on AFLPs by using information from their position on a consensus linkage map. **In**: Heller, S.R. (Ed.) *Int. Conf. Status Plant Animal Genome Res.*, VIII (abstracts). San Diego, California, USA.

14. Lu, J., Knox, M.R., Ambrose, M.J., Brown, J.K.M. and Ellis, T.H.N. 1996. Comparative analysis of genetic diversity in pea accessed by RFLP- and PCR-based methods. *Theor. Appl. Genet*. **93**: 1103-11.

15. Nagaoka, T. and Ogihara, Y. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet*. **94**: 597-602.

16. Paran, I., Aftergoot, E. and Shifriss, C. 1998. Variation in *Capsicum annuum* revealed by RAPD and AFLP markers. *Euphytica*, **99**: 167-73.

17. Pejic, I., Ajmone-Marsan, P., Morgante, M., Kozumplick, V., Castiglioni, P., Taramino, G. and Motto, M. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. *Theor. Appl. Genet*. **97**: 1248-55.

18. Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. 1996. The utility of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.* **2**: 225-38.

19. Qiao, L., Weng, M., Kong, F., Dai, J. and Wang, B. 2007. The application of RSAP marker technique in diversity detection and germplasm Identification of *Porphyra*. *Period. Ocean Univ. China,* **37**: 951-56. (*in Chinese*).

20. Ren, Y., Zhang, Y., Yin, J. and Wang, D. 2008. *Parent grouping of 31 elite inbred lines in hot pepper* (*Capsicum annuum* L.). *Hereditas,* **30**: 237-45. (in *Chinese*).

21. Rohlf, F.J. 2000. NTSYSpc, numerical taxonomy and multivariate analysis system version 2.10e. Exeter Software, Setauket.

22. Roy, J.K., Balyan, H.S., Prasad, M. and Gupta, P.K. 2002. Use of SAMPL for a study of DNA polymorphism, genetic diversity and possible gene tagging in bread wheat. *Theor. Appl. Genet*. **104**: 465-72.

23. Russell, J.R., Fuller, J.D., Macaulay, M., Hatz, B.G., Jahoor, A., Powell, W. and Waugh, R. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. *Theor. Appl. Genet.* **95**: 714-22.

24. Tam, S.M., Mhiri, C., Vogelaar, A., Kerkveld, M., Pearce, S.R. and Grandbastien, M.A. 2005. Comparative analyses of genetic diversities within tomato and pepper collections detected by retro-transposon-based SSAP, AFLP and SSR. *Theor. Appl. Genet*. **110**: 819-31.

25. Tautz, D. and Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Res*. **12**: 4127-38.

26. Thormann, C.E., Ferreira, M.E., Camargo, L.E.A., Tivang, J.G. and Osborn, T.C. 1994. Comparison of RFLP and RAPD markers to estimate genetic relationships within and among cruciferous species. *Theor. Appl. Genet*. **85**: 976-84.

27. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*. **23**: 4407-14.

28. Wang, D., Peng, S., Liu, Z. and Zheng, X. 1998. Genomic DNA extraction and RAPD analysis of hot pepper. *Acta Agric. Univ. Jiangxiensis*, **20**: 180-83. (*in Chinese*).

29. Wurff, A.W.G. van der, Chan, Y.L., Straalen, N,M, van and Schouten, J. 2000. TE-AFLP: combining rapidity and robustness in DNA fingerprinting. *Nucleic Acids Res*. **28**:105.

30. Xu, S., Tao, Y., Yang, Z. and Chu, J.Y. 2002. A simple and rapid methods used for silver staining and gel preservation. *Hereditas,* **24**: 335-36. (*in Chinese*).

31. Zhang, J., Lu, Y. and Yu, S. 2005. Cleaved AFLP (cAFLP), a modified amplified fragment length polymorphism analysis for cotton. *Theor. Appl. Genet*. **111**: 1385-95.

32. Zhang, M., Zhang, L., Gong, Z. and Hui M. 2009. New primers design and reaction system optimization of restriction site amplified polymorphism (RSAP) marker technique. *J. Northwest A&F Uni. (Nat. Sci. Ed.)* **37**: 148-54. (*in Chinese*).

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