African Journal of Biotechnology Vol. 6 (24), pp. 2784-2791, 17 December, 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

A genetic linkage map of cucumber (*Cucumis sativus L*) combining SRAP and ISSR markers

Martin A.Yeboah[1,3], Chen Xuehao[2]*, Chen Rong Feng[2], Guohua Liang[1] and Minghong Gu[1]

[1]The Key Laboratory of Plant Functional Genomics of The Ministry of Education, Yangzhou University, 12 East Wenhui Road, Yangzhou, Jiangsu 225009, P. R. China.
[2]School of Horticulture and Plant Protection, 12 East Wenhui Road, Yangzhou University, Jiangsu 225009, P. R. China.
[3]Savanna Agricultural Research Institute (SARI), P.O. Box 52, Tamale, Ghana.

Accepted 31 October, 2007

Sequence-related amplified polymorphism (SRAP) and Inter-simple sequence repeat (ISSR) markers are both simple and efficient maker systems adapted to many crops and for multiple purposes. In this study a genetic map based on SRAP and ISSR markers was constructed for cucumber (*Cucumis sativus* L.) based on the segregations of SRAP and ISSR markers in 112 plants of F_2 population derived from a cross between two cucumber inbred lines PW0832 and PW0801. In the investigation of polymorphisms with 50 ISSR primers and 132 SRAP primer combinations, 13 (26%) ISSR primers and 26 (20%) SRAP primer pairs were polymorphic generating a total of 109 polymorphic markers of which 48 were ISSR and 61 were SRAP. The average polymorphic bands were four for ISSR and two for SRAP. All the 109 polymorphic markers were scored for segregation of which 86 satisfied the Mandelian segregation ratio of 3:1. These data were used to construct an integrated linkage map for cucumber consisting of 62 loci, distributed in seven linkage groups (LGs) spanning a total of 992.2 cM, with an average distance of 16.0 cM between two adjacent loci. These markers would be very useful tool for marker assisted selection in cucumber breeding as well as for studies in quantitative traits.

Key words: SRAP, ISSR, linkage mapping, cucumber.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most widely cultivated fruit vegetables in many areas in the world and is the fourth most important vegetable crop after tomato, cabbage and onion (Tatiloglo, 1993) It is mostly consumed sliced and raw preferably as salad or prickles. Cucumber is a highly polymorphic species with variations in both vegetative and fruit characteristics (Staub et al., 1997). Despite its large morphological variability cucumber displays a low level of DNA polymorphism (Kennard et al., 1994). This low level of DNA polymorphism has limited the number of polymorphic DNA markers available for cucumber breeding and has been an obstacle in the construction of linkage maps, marker assisted selection and cultivar identification. The genome of cucumber (750 – 1000 cM) is estimated to have seven linkage groups (Staub and Melglic, 1993).

*Corresponding author. E-mail: xhchen@yzu.edu.cn.

However different linkage groups have been observed in several studies. In their studies to identify linkage groups in cucumber, Knerr and Staub (1992) and Meglic and Staub (1996) assigned 12 and 17 loci respectively to four linkage groups. Their linkages spanned 215 cM and 584 cM, respectively. In another studies using RFLP, RAPD, isozyme, morphological and disease resistance markers Kennard et al. (1994) constructed 50 and 70 points maps with wide and narrow crosses of cucumber respectively. They observed ten linkage groups in each map, spanning 766 and 480 cM for the narrow and the wide crosses respectively. In 2001, Bradeen et al. (2001) expanded and integrated the linkage maps constructed in the previous studies to produce a consensus ten linkage-group map in cucumber. But recent works have all supported the seven group accession. For example Fazio et al. (2003) constructed a genetic map of cucumber with both RIL and F_2 populations using 14 SSR, 24 SCAR, 27 AFLP, 62 RAPD and three morphological markers. They constructed seven linkage groups spanning 706 cM with mean

Table 1. The nucleotide sequences of Inter-simple sequence repeat (ISSR) use in the mapping.

Primer	Sequence[a]
UBC807	(**AG**)$_8$T
UBC834	(**AG**)$_8$YT
UBC835	(**AG**)$_8$YC
UBC840	(**GA**)$_8$YT
UBC842	(**GA**)$_8$YG
UBC858	(**TG**)$_8$ RT
UBC862	(**AGC**)$_6$
UBC859	(**TG**)$_8$RC
UBC887	DVD(**TC**)$_7$
UBC882	BVB(**AT**)$_7$
A34	GSGC(**GT**)6
A35	(**AG**)$_8$CTT
N92	(**GA**)$_8$CC

[a]Primer motif is bolded.
Y = (C or T), S = (G or C) , R = (A or G), D = (A, G or T), B = (C, G or T) and V = (A, C or G).

marker interval of 5.6 cM. Nonetheless, Pan et al. (2005) in their studies on first flower nodes in cucumber with Sequence-Related Amplified Polymorphism (SRAP) markers constructed nine linkage groups spanning 1,114.2 cM with mean marker interval of 14.5 cM.

Sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR) markers both reveal a much larger number of polymorphic fragments per primer and also do not require prior knowledge of DNA sequence for primer design (Kantey et al.,1995; Li and Quiros, 2001). They are effective methods for genomic DNA analysis and the simplicity of their techniques allows for the identification of genotypes and the construction of high saturated genetic maps (Tsumura et al., 1996; Li and Quiros, 2001). They are both PCR-based techniques allowing for the detection of more than one independent band in a single PCR reaction. However, while ISSR is dominant and targets simple sequence repeats (microsatellites) that are abundant throughout the eukaryotic genome (Nagaoka and Ogihara,1997), the SRAP markers, which is mainly dominant but with moderate number of co-dominant markers, is aimed at the amplifications of open reading frames (ORFs) which are coding sequences in the genome (Li and Quiros, 2001).

A genetic linkage map represents the relative order of genetic markers along a chromosome. Recombination frequencies are used to determine the relative distance between the markers. Genetic mapping also describes the arrangements of genes based on the relationship of the linkages. Construction of linkage map includes firstly grouping markers and secondly ordering the markers within each group. Linkage grouping is placing markers into linkage groups based on their linkage relationships. The criteria used for linkage grouping are established by testing the independence between two loci in segregating population by means of goodness of fit or log likelihood ratio. Recombination fraction, lod score (base-10 log likelihood ratio) and significant P-value are used as a criteria to infer whether each pair of loci belongs to the same linkage group (Liu, 1998; Larsen, 1979). High resolution genetic maps provide breeders with powerful tools to analyze the inheritance of genes of interest and also for map based cloning (Kumar, 1999). However, previous maps constructed with SRAP markers in cucumber (Pan et al., 2005; Gang et al., 2005) with low number of loci could not offer a high resolution and utility for cucumber breeders.

In our study, we have identified ISSR primers and primer combinations for SRAP that proved highly polymorphic between the two cucumbers inbred parents, PW0832 and PW0801. The paper also describes a genetic linkage map of cucumber using F_2 population of the parents.

MATERIALS AND METHODS

Plant material

F_2 population of 112 individuals created from F_1 seeds from a across between two inbred lines PW0832 (flood tolerant) and PW0801 (flood sensitive) parental lines was used as the mapping population in this study. The two lines were obtained from the Vegetable Department of School of Horticulture, Yangzhou University, China.

DNA extraction

DNA was isolated from young cucumber leaves as described in the protocol of Levi and Thomas (1999). The DNA concentration of samples was measured with visible spectrophotometer (Pharmacia Biotech) at $A_{260.}$ Fifty (50) ISSR and 132 SRAP primers were screened for polymorphism using the two parental lines.

SRAP and ISSR analysis

A set of 50 ISSR primers mostly from the University of British Columbia, Canada (UBC set #9) representing di, tri, tetra and penta repeats were used (Table 1). Different concentrations of template DNA and Taq polymerase were tested for optimal amplification products. The optimal amplification mixture of 25 ul contained 100 ng DNA, 1 uM ISSR primer (Sangong Inc.) 0.5 mM dNTPs, 1 mM $MgCl_2$, 1x PCR buffer and 1 U Tag DNA polymerase (Sangong Inc.). PCR amplifications were performed in a Peltier Thermal Cycler PTC-200 (MJ Research) with an initial step at 95^{o}C for 1 min, followed by 35 cycles of denaturation at 94^{o}C for 30 s, annealing temperature of 50^{o}C for 30 s and elongation at 72^{o}C for 2 min. Finally, an additional extension for 10 min at 72^{o}C was used. Amplified DNA products were denatured at 95^{o}C for 5min and separated by electrophoresis along with marker ladders (Sangon Inc.), for molecular weight determination, in a 6 % polyacrylamide sequencing gel containing 7 mol of urea. Each gel was run in 0.5 x TBE buffer (100 mM Tris, 100 mM boric acid, 2 mM EDTA, PH 8.0) at 50 W for 4 h and then stained with silver nitrate as described in the protocol by Yun-Tao et al. (2007). Gel images were visualized with a UVP white light transilluminator for band scoring and photographed with UVP Bioimaging System.

For the SRAP marker, two primers were used following the protocol of Ferriol et al. (2003). The two primer type comprised the

Table 2. The forward and reverse sequence-related amplified polymorphism (SRAP) primer information in the study.

Forward primers								Reverse primers						
Me1	TGA	GTC	CAA	ACC	GG	ATA		Em1	GAC	TGC	GTA	CGA	ATT	AAT
Me2	TGA	GTC	CAA	ACC	GG	AGC		Em2	GAC	TGC	GTA	CGA	ATT	TGC
Me3	TGA	GTC	CAA	ACC	GG	AAT		Em3	GAC	TGC	GTA	CGA	ATT	GAC
Me4	TGA	GTC	CAA	ACC	GG	ACC		Em4	GAC	TGC	GTA	CGA	ATT	TGA
Me5	TGA	GTC	CAA	ACC	GG	AAG		Em5	GAC	TGC	GTA	CGA	ATT	AAC
Me6	TGA	GTC	CAA	ACC	GG	ACA		Em6	GAC	TGC	GTA	CGA	ATT	GCA
Me7	TGA	GTC	CAA	ACC	GG	ACG		Em7	GAC	TGC	GTA	CGA	ATT	CAA
Me8	TGA	GTC	CAA	ACC	GG	ACT		Em8	GAC	TGC	GTA	CGA	ATT	CAC
Me9	TGA	GTC	CAA	ACC	GG	AGG		Em9	GAC	TGC	GTA	CGA	ATT	CAG
DC1	TAA	ACA	ATG	GCT	ACT	CAA	G	Em10	GAC	TGC	GTA	CGA	ATT	CAT
OD3	CCA	AAA	CCT	AAA	ACC	AGG	A	Em11	GAC	TGC	GTA	CGA	ATT	CTA
SA4	TTC	TTC	TTC	CTG	GAC	ACA	AA							

forward and reverse primers, the forward primer is 17-20 bp long made up of 14-17 nucleotides rich in C and G and three selective bases at the 3' end. The second primer, the reverse primer has 18 bp made up of 15 nucleotides rich in A and T with three selective bases at the 3' end (Table 2). The forward and the reverse primers amplify the exonic and intronic regions respectively. Each PCR contained a reaction mixture of 25 ul made up of 60 ng of genomic DNA, 200 uM of dNTPs, 1.5 mM of $MgCl_2$, 0.3 uM of each primer, 2.5 ul of PCR buffer, 1 unit of Tag polymerase (Sangong Inc.) and sterile doubled distilled water. Samples were also amplified in a Peltier Thermal Cycler PTC-200 (MJ Research) programmed at 5 min of initial denaturation at 94°C followed by 5 cycles of 1 min denaturing, 1 min annealing at 35°C and 2 min of elongation at 72°C, after these, 30 cycles of 1 min denaturing, 1 min annealing at 48°C ending with an elongation step of 5min at 72°C. The PCR products were fractionated on 6 % polyacrylamide gel at 50 W for 4 h and stained with $AgNO_3$ (Yun-Tao et al., 2007).

ISSR and SRAP amplifications were repeated at least twice and only bands reproduced were scored for analysis. Nomenclature for both ISSR and SRAP markers was based on the primer name, for the primer that amplified more than one polymorphic band, subscripts 1, 2, 3, etc (starting from the lowest to the highest molecular weight band) were assigned after the primer name.

Linkage map construction

One-hundred and twelve (112) F_2 plants were scored for 50 ISSR markers and 132 SRAP marker combinations. Individual plants were classified according to parents' band type. Those with same band type as the PW0832 were given a value=1 and those of the band type PW0801 were given a value=2, the unclear band types or those lacking data were given the value=0. Segregation data were tested for their deviation from the expected 3:1 Mendelian ratio using chi-square test and only markers that fitted the ratio were used to construct a linkage map. Eighty-eight (88) dominant markers out of the total 109 fitted the expected 3:1 Mendelian ratio ($P < 0.05$) and were used in the linkage analysis. Linkage software MAPMAKER/EXP3.0 (Lander et al., 1987; Lincoln et al., 1992) was used to construct a linkage map. Markers were first grouped using a minimum log of odds (LOD) score of 2.5 and maximum recombination value of 0.30. For each of the linkage groups, markers were ordered by the "order" command with a LOD score of 3.0 and recombination value of 0.25. Unmapped markers were placed by the "Try" command. The ordered marker sequences were confirmed by the "Ripple" command and finally the linkages maps were generated with the "Map" command by means of the "Kosambi" map function (Kosambi, 1944). The "Error-detection" command was employed to identify errors in marker scoring after which putative errors were retested. The map was drawn according to the program developed by Liu and Meng (2003).

RESULTS AND DISCUSSION

ISSR and SRAP markers analyses

A total number of 50 ISSR primers and 132 SRAP primer combinations were used for analysis. Five ISSR primers and 15 SRAP primer combinations failed to amplify products of sufficient quality for analysis. However, for the remaining 45 ISSR primers and 117 SRAP primers combinations, 17 (37.8%) and 32 (27.7%), respectively, showed polymorphisms of which 13 of the ISSR primers and 26 of the SRP primer combinations were reproducible enough for marker analysis (Tables 3 and 4). Each of these 13 ISSR and 26 SRAP polymorphic primers produced at least one scorable polymorphic DNA band which was visible enough for detection and scoring. In total there were 109 scorable polymorphic bands made up of 48 ISSR bands and 61 SRAP bands which were used as markers in this study. Figure 1 gives a representative gel photograph of the analyses of UBC834 (ISSR) and OD3EM5 (SRAP). These primers detected six and five polymorphism bands respectively which indicate their level of polymorphism in this study. Both ISSR and SRAP were considered as dominant markers in this study and therefore scored for their presence or absence.

There were 206 bands generated across all 13 ISSR primers with primer UBC807 yielding the highest number of products (27 bands) and primer A35 the least (10 band). However, among the SRAP markers ME1EM5 had the highest number of bands (20) and the least was recorded in SA4EM8 with seven bands.

For number of polymorphisms bands, primer UBC840 was the only primer that generated eight polymorphic bands while ISSR primers UBC834, UBC887 and SRAP

Table 3. Primers used for ISSR analysis and their polymorphism levels.

Primer	Primer[1] type	No. of amplified bands	No. of Polymorphic bands	% polymorphism	Mapped markers	Origin of amplicon	
						PW0832	PW0801
UBC807	ISSR	27	4	14.8	4	0	4
UBC834	ISSR	21	6	28.6	4	4	0
UBC835	ISSR	20	1	5.0	1	1	0
UBC840	ISSR	19	8	28.6	6	1	5
UBC842	ISSR	21	4	14.3	2	2	0
UBC858	ISSR	12	1	8.3	0	0	0
UBC862	ISSR	16	5	46.9	2	2	0
UBC859	ISSR	11	2	18.2	0	0	0
UBC887	ISSR	12	6	50.0	5	5	0
UBC882	ISSR	12	2	22.2	1	1	0
A35	ISSR	10	3	30.0	1	1	0
A34	ISSR	12	4	33.3	2	2	0
N92	ISSR	13	2	15.4	2	2	0
Total	-	**206**	**48**	**-**	**30**	**21**	**9**

ISSR = Inter-simple sequence repeat.

Table 4. Primers used for SRAP analysis and their polymorphism levels.

Primer	Primer[1] type	No. of Amplified bands	No. of Polymorphic bands	% polymorphism	Mapped markers	Origin of amplicon	
						PW0832	PW0801
ME1EM5	SRAP	20	2	10.0	1	0	1
ME1EM9	SRAP	11	1	9.1	0	0	0
ME2EM5	SRAP	9	2	22.2	1	1	0
ME2EM7	SRAP	9	2	22.2	1	1	0
ME2EM10	SRAP	12	3	25.0	3	0	3
ME3EM3	SRAP	10	1	10.0	0	0	0
ME3EM5	SRAP	15	4	26.6	4	0	4
ME4EM3	SRAP	15	1	6.7	0	0	0
ME4EM4	SRAP	16	2	12.5	0	0	0
ME4EM5	SRAP	15	1	6.7	1	0	1
ME5EM4	SRAP	17	1	5.9	1	0	1
ME6EM3	SRAP	10	3	30.0	2	2	0
ME6EM4	SRAP	9	2	22.2	1	0	1
ME7EM5	SRAP	12	2	16.7	0	0	0
OD3EM2	SRAP	16	2	12.5	0	0	0
OD3EM4	SRAP	15	4	26.7	2	2	0
OD3EM5	SRAP	11	5	45.5	3	1	2
OD3EM7	SRAP	17	2	11.8	2	2	0
OD3EM8	SRAP	14	2	14.3	0	0	0
OD3EM10	SRAP	11	2	18.2	1	1	0
OD3EM11	SRAP	13	1	7.7	1	1	0
SA4EM1	SRAP	15	3	20.0	1	1	0
SA4EM8	SRAP	7	3	42.9	0	0	0
SA4EM10	SRAP	18	6	33.3	5	5	0
DC1EM1	SRAP	9	3	33.3	2	2	0
DC1EM4	SRAP	13	1	7.7	0	0	0
Total	-	**339**	**61**	**-**	**32**	**19**	**13**

SRAP = Sequence-related amplified polymorphism.

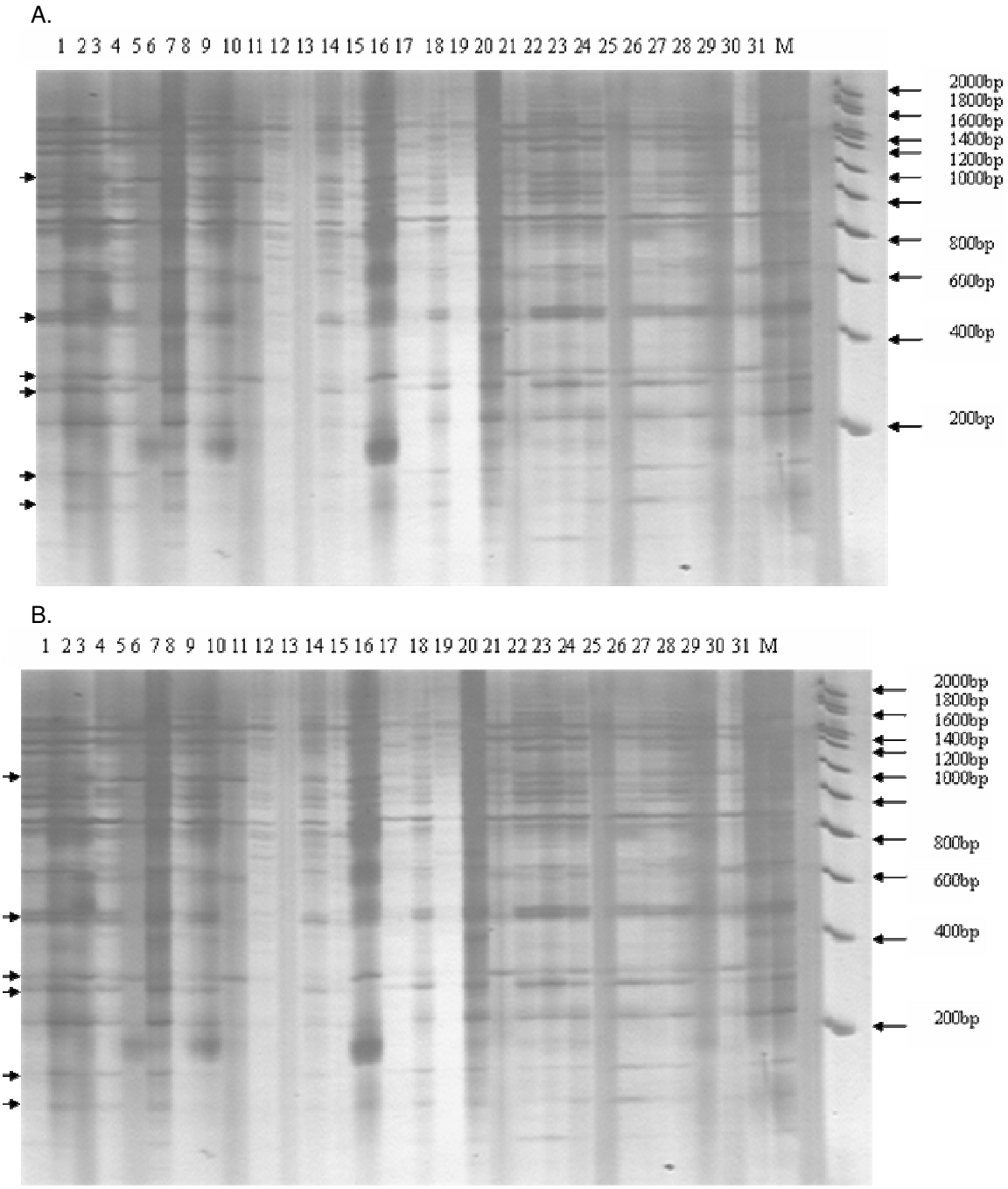

Figure 1. A representative profiles F2 progenies from cross between parental lines PW0832 (P1) and PW0801 (P2). **A.** An ISSR profile generated with UBC834. **B.** A SRAP profile generated with OD3EM5.M is a marker and arrows on the left indicate polymorphic bands.

primer SA4EM10 generated six polymorphisms each. However, ISSR primers UBC835, UBC858 and SRAP primers ME1EM9, ME3EM3, ME4EM3, ME4EM5, ME5EM4, OD3EM11 and DCIEM4 yielded one polymorphic bands each. Overall, there were 545 bands and 109 (20%) polymorphisms generated between the two parents, of these 48 (44%) were ISSRs and 61 (46%) were SRAPs. Although, this is the first studies in cucumber mapping both ISSR and SRAP markers together, the results presented in our studies agrees with previous studies that demonstrate the reproducibility of bands profile generated by SRAP (Li and Quiros, 2001) and ISSR (Fang and Rose, 1997) markers when PCR products are fractionated on polyacrylamide gel.

Generally, dinuclotide ISSR primers produced the highest number of bands and were frequent in the cucum-

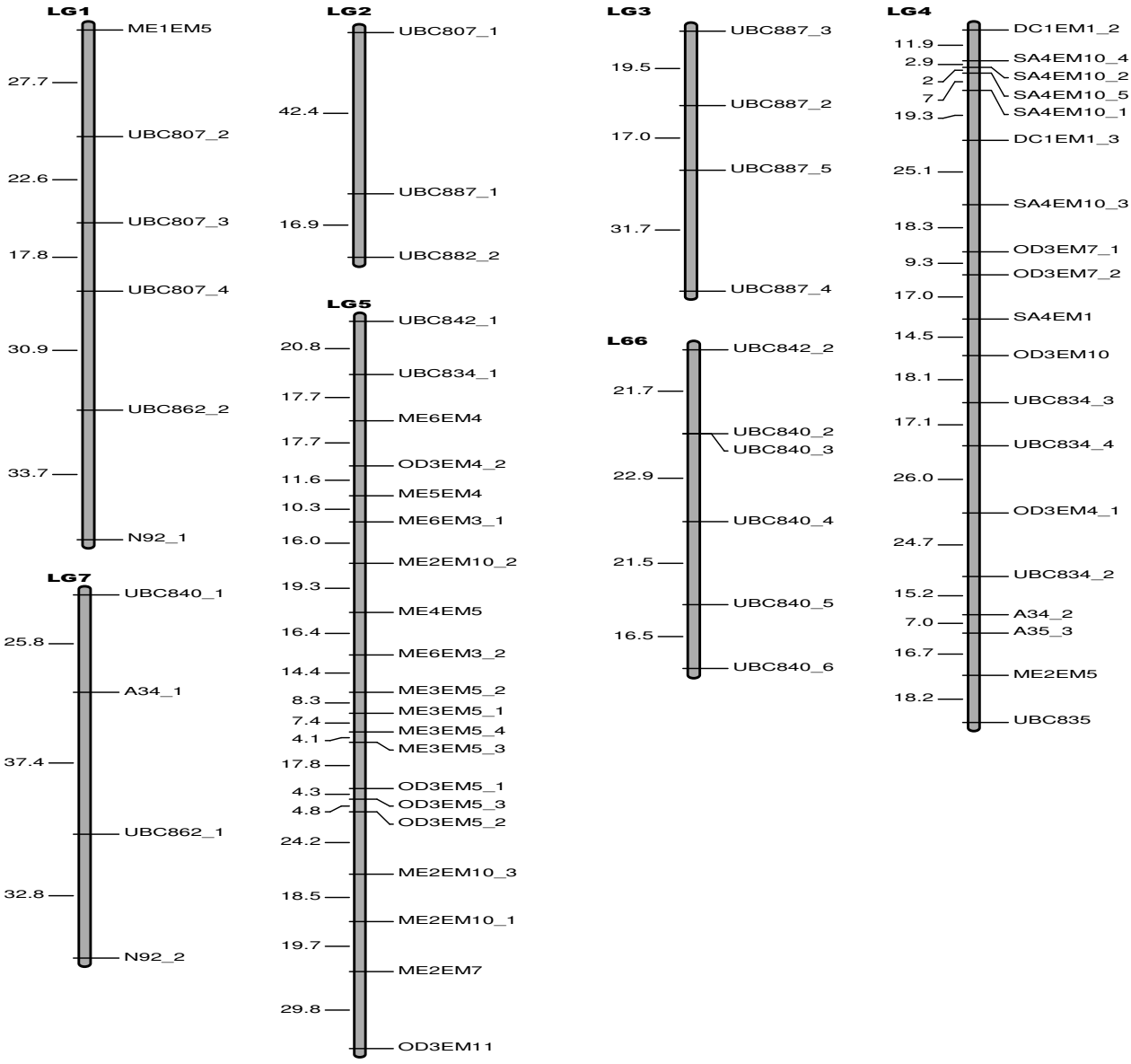

Figure 2. A linkage map including SRAP and ISSR markers developed from F_2 population of a cross between cucumber inbred lines PW0832 and PW0801 including seven linkage group (LG). Marker names are at the right, while distances between adjacent markers (in cM) are at the left of each linkage group.

cucumber genome than the higher repeats. Also, the 3'-end anchor primers of ISSR yielded the highest number of bands in our study, than the 5'-end anchored primers. This is in contrast with observation by Bornet and Branchard (2001) that primers anchored at 5'-end generates higher number of fragments because of their broader specificity. The plausible explanation of our results might be due the degree of density of these repeats in the cucumber genome. This is supported by the fact that the higher the density of repeats in a genome, the more specific primers and stringent PCR conditions needed to optimized resolution on a gel (Fang et al., 1997).

For the SRAP marker, the number of polymorphic and scorable bands was not as high as expected. For example the range of polymorphic bands per primer was 1 - 5 with an average of 2 bands, which is relatively smaller compared with the ISSR marker with an average of 4 bands (Figure 1, Tables 3 and 4). Although the advantages of both ISSR and SRAP markers stems from their simplicity and reproducibility, high number of polymorphisms and the fact there is no need for prior knowledge of DNA sequence, their major limitations could be lack of the number of useful primers that could generate useful polymorphism as observed in our studies and supported by Cekic et al. (2001) and Nagoaka and Ogihara (1997).

Map construction and marker segregation

Seventy-six (86) dominant markers of the 109 that fitted the expected 3:1 Mendelian ratio (P<0.05) were used in the linkage analysis of which 62 markers made up 30 ISSR and 32 SRAP markers were assign to seven linkage groups (Figure 2). The 23 polymorphic markers were excluded from the linkage analysis because of segregation distortions. The linkage map had 62 loci spanning a total length of 992.2 cM with an average genetic distance of 16.0 cM between adjacent markers. However, with this large average distance, greater saturation would be needed for practical application especially for marker assisted selection (MAS). This is because the presence of a tight linkage (<10cM) between a trait and genetic marker may be beneficial for MAS in order to increase the benefit to be derived from selection (Staub et al., 1996).

We could not compare our linkage map with the previously published SRAP linkage map of cucumber by Pan et al., 2005 and Gang et al. (2005) because few markers were common. Gang et al. (2005) constructed molecular linkage map with seven linkage groups spanning 1164.2 cM in length with an average genetic distance of 12.6 cM.

Conclusion

In conclusion, this study has identified primers that generated substantial polymorphism in our population. The study therefore demonstrated the use of ISSR and SRAP markers as potential tools for linkage mapping in cucumber. The degrees of polymorphism exhibited by both markers clearly demonstrate their usefulness in genetic analysis of cucumber. The ISSR-PCR products can be cloned and sequenced to convert the ISSR markers in this study into sequence-characterized amplified regions (SCARS) this would increase the detection of co-dominance to improve the utility of the results of ISSR analysis in this study. Also, amplified fragments from SRAP markers could be recovered from the acrylamide gel, re-amplified and sequenced. These sequences could then be compared with other sequences in the databases for annotation. We further speculate that because of their simplicity and accessibility these markers may rapidly become an invaluable tool for cucumber genome analysis.

ACKNOWLEDGEMENTS

We express our sincere gratitude to the vegetable section of the School of Horticulture and Plant Protection, Yangzhou University for the provision of seeds of the two parental lines used in the study. This work was financially supported by the Foundation of Jiangsu Agricultural High Technology (BG2004313) and by the Chinese Government Scholarship.

REFERENCES

Bornet B, Branchard M (2001). Non-anchored inter-simple repeat (ISSR) marker:reproducible and specific tolls for genome fingerprinting . Plant Mol. Biol. Rep., 19: 209-215.

Bradeen JM, Staub JE, Wye C, Antonise R, Peleman J (2001). Towards an expanded and integrate linkage map of cucumber (*Cucumis sativus* L.). Genome 44: 111-119.

Cekic C, Battey NH, Wilkinson MJ (2001). The potential of ISSR-PCR primer-pair combinations for genetic linkage analysis using the seasonal flowering locus in *Fragaria* as a model. Theor. Appl. Gnet. 1003: 540-546.

Fang DQ, Rose ML (1997). Identification of closely related citrus cultivars with inter-simple sequence repeat markers. Theor. Appl. Genet. 95(3): 408-417.

Fang DQ, Rose ML, Krueger RR, Federici CT (1997). Fingerprinting trifoliate orange germplasm accessions with isoenzymes, RFLPs and inter-simple sequence repeat markers. Theor. Appl. Genet. 95: 211-219.

Fazio G, Staub JE, Stevens MR (2003). Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. Theor. Appl. Genet. 107(5): 864-74.

Ferriol M, Pico B, Nuez F (2003). Genetic diversity of germplasm collection of Cucubita pepo using SRAP and AFLP markers. Theor. Appl. Genet. 107: 271-282.

Gang W, Junsong P, Xiaozun L, Huanle H, Hizhong W, Cai R (2005). Construction of cucumber genetic linkage map with SRAP markers and location of genes for lateral branch traits. Chin. Sci. Bull. (in Chinese) 48(19): 2063-2067.

Kantey RV, Zeng XP, Bennetzen JL, Zehr BE (1995). Assessment of genetic diversity in Dent and Popcorn (*Zea mays* L.) inbred lines using Inter-simple sequence repeat (ISSR) amplification. Mol. Breed 1: 365-373.

Kennard WC, Poetter K, Dijkhuizen A, Meglic V, Staub JE, Havey MJ (1994). Linkages among RFLP, RAPD, isozyme , diseases resistance , and morphological markers in narrow and wide crosses of cucumber. Theor. Appl. Genet. 89: 42-48.

Knerr LD, Staub JE (1992). Inheritance and linkage relationships of isozyme loci in cucumber (*Cucumis sativus* L.). Theor. Appl. Genet. 84: 217-224.

Kosambi DD (1944). The Estimation of map distance s from recombination values. Ann. Eugen. 12: 172-175.

Kumar LS (1999). DNA markers in plant improvement: An overview. Biotech. Adv. 17: 143-182.

Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L (1987). MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Larsen SO (1979). A general problem for estimation of haplotype frequencies from population diploid data, Comput. Programs Biomed. 10: 48-54.

Levi A, Thomas CE (1999). An improved procedure for the isolation of high quality DNA from watermelon and melon leaves. Cucurbit Genetic Coop. Rpt. 22: 41-42.

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.

Lincoln S, Daly M, Lander E (1992). Constructing genetic maps with MAPMAKER/EXP 3.0 .Whitehead Institute Technical Report, Cambridge, MA.

Liu BH (1998). Statistical genomic: linkage,mapping and QTL analysis, CRC press Washington D.C., p. 611.

Liu RH, Meng JL (2003) Map draw: a Microsoft Excel macro for drawing genetic linkage map based on a given genetic linkage data. Hereditas (Beijing) 25: 317-321.

Meglic V, Staub JE (1996). Inheritance and linkage relationships of isozyme and morphological loci in cucumber (*Cucumis sativus* L.). Theor. Appl. Genet. 92: 865-872.

Nagaoka T, Ogihara Y (1997). Applicability of Inter-Simple Sequence Repeat polymorphism inwheat for use as DNA markers in comparison to RFLP and RAPD markers.Theor. Appl. Genet. 94: 597-602.

Pan J, Wang G, Li X, He H, Wu A, Cai R (2005). Construction of genetic map with SRAP markers and localization of genes responsible for the first flower node trait in cucumber (*Cucumis sativus* L.). Prog. Nat. Sci. (in Chinese) 15(5): 407-413.

Staub JE, Serquen FC, Gupta M (1996). Genetic markers, map construction and their application in plant breeding. HortScience 31: 729-741.

Staub JE, Sequen FC, Mccreight JD (1997). Genetic dversity in Cucumber (*Cucumis sativus* L.), iii An evaluation of Indian germplasm. Genet. Res. Crop Evol. 44(4): 315-326.

Staub JE Meglic V (1993). Molecular genetic markers and their legal relevance for cultivar discrimination: a case study in cucumber. HortTechnol. 3: 291-300.

Tatiloglo T (1993). Cucumber, in G. Kalloo & B. O. Bergh (Ed.). Genetic Improvement of Vegetable Crops. Perganon press, New South Wales, Australia.

Tsumura Y, Ohba K, Strauss SH (1996). Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas fir (*Pseudotsuga menziesii*) and Sugi (*Cryptomeria japonica*). Theor. Appl. Genet. 92: 40-45.

Yun-Tao J, Chang-Qing, Bin-Yun C (2007). An optimal method of silver staining in Polyacrylamide Gels. Electrophoresis 28(8): 1173-1175.