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Mapping Quantitative Trait Loci for Waterlogging Tolerance in Cucumber Using SRAP and ISSR Markers

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Abstract: The aim of this study was to map Quantitative Trait Loci (QTL) associated with waterlogging tolerance in cucumber at the early growth stage. A set of 112 $F_{2:3}$ lines derived from the cross between two cucumber inbred lines PW0832 (tolerant) to PW0801 (susceptible), was used to evaluate waterlogging tolerance traits: tolerance score (TOL), Adventitious Root Formation (ARF), waterlogged shoot dry weight (SDWw), waterlogged vine length (VLHw), control Shoot Dry Weight (SDWc) and control vine length (VLHc) using a randomized complete-block design. A genetic linkage map containing 30 ISSR and 32 SRAPs markers was constructed, spanning a total of 992.2 cM with an average interval of 16.0 cM. A total of 25 putative QTL were found to be associated with the six traits studied using Composite Interval Mapping (CIM). Fourteen QTL were detected for the four waterlogging traits (TOL, AFR, SDWw and VLHw) and eleven for the two control traits (SDWc and VLHc). The QTL for the waterlogged traits accounted for 7.9-33.2% of the phenotypic variations while the control traits accounted for 6.9-19.1% of the phenotypic variations. Although the detected regions need to be mapped precisely, the findings and QTL found in this study could provide useful information for future genetic studies in cucumber waterlogging tolerance.

Key words: Cucumber (*Cucumis sativus* L.), waterlogging tolerance, genome mapping, SRAP marker, ISSR marker, QTL

INTRODUCTION

Waterlogging is an important environmental stress that severely limits crop growth and productivity. It has become a major problem in some parts of the world, especially in South China and South East Asia, Australia and some part of United States (Setter and Waters, 2003). In china for example, 24.3 million hectares of waterlogged lowland occupy 20% of cultivated land (FAO, 2004). Consequently, waterlogging has been a major risk for cucumber production in China especially along the Yangtze River basin where low lying areas are often prone to flooding during periods of high rainfall (Jiang *et al*., 2000).

Tolerance of crops to flooding is related to many morphological and physiological traits that are under strong environmental influence (Kozlowski, 1984). Nonetheless, there is evidence for genetic control of flood tolerance. For example breeding of flood tolerance in rice has been has been attempted (Monhanty *et al*., 2000). Some lines with reasonable yield and grain quality have been released such as Prachinburi 2 in Thailand (Lafitte *et al*., 2004). Secondly, a major QTL was mapped at chromosome 9 of rice, designated as sub1 accounting for 70% of the phenotypic variations in flood tolerance (Xu and Mackill, 2004). In Soybean, the Sat_064 QTL marker associated with waterlogging tolerance has also been mapped (Van Toai *et al*., 2001).

Sequence-Related Amplified Polymorphism (SRAP) and Inter-Simple Sequence Repeat (ISSR) markers 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). However, while ISSR is dominant and targets simple sequence repeats (microsatellites) that are abundant throughout the eukaryotic genome

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(Nagaoka and Ogihara, 1997), the SRAP marker, 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).

Cucumber, *Cucumis sativus* L. (2n = 14), a member of the Cucubitaceae family, collectively belongs to a group of vegetables known as cucurbits. The genome of cucumber (750-1000 cm) is estimated to have seven linkage groups (Staub and Meglic, 1993). However different linkages 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. Kennard *et al.* (1994) constructed 50 and 70 point maps of cucumber with ten linkage groups for each map spanning 766 and 480 cM, respectively. However, 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; however some recent works have all supported the seven linkage grouping. For example, Fazio *et al.* (2003) constructed a genetic map of cucumber with seven linkage groups spanning 706 cM with mean marker interval of 5.6 cM using 14 SSR, 24 SCAR, 27 AFLP, 62 RAPD and three morphological markers. Nonetheless, Pan *et al.* (2005) in their studies on first flower nodes in cucumber with SRAP markers, constructed nine linkage groups spanning 1114.2 cM with mean marker interval of 14.5 cM.

Development of molecular-marker linkage map in many species has facilitated the identification of chromosome regions associated with Quantitative Trait Loci (QTL). This has provided the opportunity to gain an accurate understanding into traits inheritance and genome organization (Kearsey and Pooni, 1996; Lynch and Walsh, 1998). QTL detection and characterization has been performed by means of backcross or $F_{2:3}$ populations (Fulton *et al.* 1997; Serquen *et al.* 1997). Employing $F_{2:3}$ for RAPD analysis and mapping in cucumber, Serquen *et al.* (1997) identified QTL for fruit number and weight. Also, in their studies in soyabeans, Van Toai *et al.* (2001) identified a QTL associated with waterlogging tolerance. Although progress has been made in QTL associated with waterlogging tolerance in other crops such as rice (Toojinda *et al.*, 2003), maize (Mano *et al.*, 2005), wheat (Boru *et al.*, 2001) and soybean (Van Toai *et al.*, 2001; Reyna *et al.*, 2003), little is known about QTL for waterlogging tolerance in cucumber.

An indirect selection of waterlogging tolerance using marker assisted selection may prove to be an excellent substitute over traditional phenotypic selection and could improve the efficiency of conventional plant breeding especially as markers are not affected by the environment. Moreover, development of molecular markers for waterlogging tolerance in cucumber would be necessary for future cloning of waterlogging tolerance genes of cucumber. In the present study, we analyzed QTL for waterlogging tolerance of cucumber at the early growth stage, using $F_{2:3}$ population from a cross between susceptible and tolerant cucumber lines.

MATERIALS AND METHODS

Plant materials: Two cucumber inbred lines, PW0832 (P_1) as female parent and PW0801 (P_2) as male parent were used in this study. PW0832 is tolerant to waterlogging whiles PW0801 is susceptible. The two lines were obtained from the School of Horticulture, Yangzhou University, China based on wide variations in their abilities to tolerate flooding conditions (Li, 2007). A population of F_2 plants was created from F_1 seeds obtained from the cross between PW0832 (tolerant) and PW0801 (susceptible). F_2 seeds were planted in the green house of the School of Horticulture, Yangzhou University, China in spring 2005 to produce 112 plants. Each plant was self-pollinated to produce 112 F_3 families, one family from each F_2 plant ($F_{2:3}$). Individuals were genotyped in the F_2 population, while trait values were scored in the F_3 population derived from the F_2 genotyped individuals. The trait values associated with the genotyped individuals was estimated by the mean value of the resulting F_3 family line. Scoring the phenotype as mean of several individuals (as opposed to measurement of a single individual) can offer increased power over the standard F_2 design by reducing sampling variance (Lynch and Walsh, 1998).

Experimental design and waterlogging tolerance evaluation: The experiment was conducted at the experimental farm belonging to the Department of Horticulture of Yangzhou University, Yangzhou, China from September 13th to November 4th of 2006. $F_{2:3}$ lines derived from the F_2 with 6 plants for each line were raised in pots. The experimental design was a Randomized Complete Block Design (RCBD) with six blocks consisting two treatments (control and waterlogged). The plots consisted of a potted plant for each 112 F_3 lines, 10 plants each for the parents and their F_1 hybrid. The control treatment was accomplished by watering plants as required to maintain vigorous plant growth.

Soil waterlogging was simulated by immersing four weeks old potted plants in cement tanks built in a plastic green house and filled with water. Potted plants for the waterlogged treatment were inundated until water level was 3 cm above soil surface in the pots for 10 days before drainage.

One week after removing waterlogged treatment, individual plants were visually scored for tolerance (TOL) using a scale of 0-5 Navazio and Staub (1994), where 0 = dead plants, 1 =100-75% of wilt from base to terminal whorl, 2 = 74-50% wilting of leaves from base to midvine, 3 = leaves between base and midvine undulating and recurved, 4 = recurved leave margins and 5 = green plant with no sign of stress. The higher scale stood for tolerance while the lower scale stood for susceptibility. Adventitious Root Formation (ARF) was also scored visually with 0-3 scale, where 0 = none, 1 = low, 2 = medium and 3 = high, modified from Mano *et al.* (2005). Vine length (VLHw, waterlogged and VLHc, control) and shoot dry weights (SDWw, waterlogged and SDWc, control) were measured two weeks after waterlogging stress was removed to allow enough time for plants to develop new shoots. Shoots were oven-dried at 65°C for 3 days (Fracheboud *et al.*, 2004).

DNA extraction and molecular marker analysis: DNA was isolated from young cucumber leaves according to the protocol of Levi and Thomas (1999). Fifty ISSR primers and 132 SRAP primer combinations (Table 1, 2) were screened for polymorphism using the two parental lines.

For the ISSR analysis, a set of 50 primers representing di, tri, tetra and penta repeats mostly from the University of British Columbia (UBC) primer set #9 were used. Different concentrations of template DNA and Taq DNA polymerase were tested for optimal amplification products. The optimal amplification mixture (25 μL) contained 100 ng DNA, 1 μM ISSR primer (Sangong Inc.), 0.5 mM dNTPs, 1 mM $MgCl_2$, 1x PCR buffer and 1 U Taq DNA polymerase (Sangong Inc.) and sterile double distilled water. PCR amplifications were performed in a Peltier Thermal Cycler PTC-200 (MJ Research) with an initial step at 95°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing temperature of 50°C for 30 sec and elongation at 72°C for 2 min. Finally, an additional extension for 10 min at 72°C was used. Amplified DNA products were denatured at 95°C for 5 min and separated by electrophoresis along with 2000 kb ladder (Sangon Inc.), 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 $AgNO_3$ 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 analysis, two primers were used following the protocol of Ferriol *et al.* (2003). The two primer type comprised of the 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. The forward and the reverse primers amplify the exonic and intronic regions respectively (Table 2). Each PCR contained a reaction mixture (25 μL) made up of 60 ng of genomic DNA, 200 μM of dNTPs, 1.5 mM of $MgCl_2$, 0.3 μM of each primer, 2.5 μL of PCR buffer and 1 unit of Tag DNA polymerase (Sangong Inc.) and sterile doubled distilled water. Samples were also amplified in a Peltier Thermal

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)

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							

Cycler PTC-200 (MJ Research) programmed at 5 min of initial denaturation at 94°C followed by 5 cycles of 1 min denaturation, 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 5 min 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, subscript 1, 2, 3 etc. (starting from the lowest to the highest molecular weight band) were assigned after the primer name.

DMap 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 parent band type. Those with same band type as the PW0832 (tolerant) were given a value of 1, the same band type of PW0801 (susceptible) were given a value of 2, the unclear band types or those lacking data were given the value of 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 (86) dominant markers out of the total of 109 markers that fitted the expected 3:1 Mendelian ratio ($p<0.05$) were used in the linkage analysis. Linkage software MAPMAKER/EXP3.0 (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).

Statistical analysis: The PROC UNIVARIATE procedure of SAS systems for Microsoft Windows, version 9.1 (SAS Institute, 2002) was used to generate normal probability plots, the Shapiro-Wilk statistic was employed to test $F_{2:3}$ family distributions for normality. The PROC MIXED procedure of SAS was used to compute least square means (lsmeans), standard error of generations and broad-sense heritability (h^2) of the $F_{2:3}$ families were estimated with the formula:

$$h^2 = \delta^2_g/(\delta^2_g + \delta^2_e/n)$$

Where:

δ^2_g = Genetic variance
δ^2_e = Environmental variance
n = No. of replications (Falconer, 1989)

QTL analyses for all traits were performed using the window QTL Cartographer version 2.5 (Wang *et al*., 2007) by means of the Composite Interval Mapping method (CIM) proposed by Zeng (1944). The CIM tests the hypothesis that an interval flanked by two adjacent markers contains QTL, while statistically accounting for the effect of other segregating QTL out of the tested interval. The LOD score statistics used is $-\log L_0/L_1$, where L_0/L_1 = ratio of the likelihood under the null hypothesis (no QTL in the interval) and alternative hypothesis (there is QTL in the interval). The parameters were set for map function as Kosambi, distance units as centimorgan (cM), cross information as SF_3 (self cross F_3), background control as 5 of control markers. The significant threshold for each trait was determined by 1000 permutations (Churchill and Doerge, 1994). The Model 6 of the Zmapqtl module of QTL Cartographer was used to scan intervals of 2 cM between markers and putative QTL with a window size of 10 cM. Phenotypic means for waterlogged and control traits were mapped separately in order to identify markers that indicated QTL for traits under waterlogged conditions but had no effect in the control condition (Van Toai *et al*., 2001).

RESULTS

Means, heritability: The frequency distributions of the $F_{2:3}$ families were normal for all the traits by the Shapiro-Wilk test (data not shown). The parents showed statistically significant differences for all the traits under waterlogging stress as well as for the control VLH. But differences between the parents were not significant for the control conditions of SDW. The broad-sense heritability values were generally moderate for waterlogged treatment of TOL (0.70), SDW (0.68) but high for waterlogged VLH (0.88). However, lower broad-sense heritability value of 0.43 was recorded for ARF (Table 3).

DNA markers and polymorphism detection: A total number of 50 ISSR primers (UBC primer set No. 9) and 132 SRAP (each forward primer was combined with each

Table 3: Traits least square mean (lsmean) values for 112 $F_{2:3}$ families, tolerant parent, PW0832 (P_1) and susceptible parent PW0801 (P_2) and their broad sense heritability (h^2) for waterlogging tolerance of cucumber grown in a greenhouse at Yangzhou, China, 2006

	TOL[1]	ARF	SDW (g)		VLH (cm)	
Generation	Waterlogged	Waterlogged	Waterlogged	Control	Waterlogged	Control
P_1	3.14±0.12^a	1.91±0.26^a	2.43±0.11^a	4.47±0.16^b	55.46±2.13^a	101.32±3.76^a
P_2	2.49±0.16^b	0.91±0.21^b	1.97±0.07^b	5.09±0.12^a	50.40±1.20^b	103.94±3.09^a
$F_{2:3}$	3.28±0.16^a	1.94±0.26^a	2.51±0.11^a	4.69±0.16^b	55.61±2.13^a	98.28±3.09^a
h^2	0.70	0.43	0.68	0.78	0.88	0.84

[1]: TOL (tolerance rating) = 0 (dead plant)-5 (green plant with no sign of stress). ARF (Adventitious Root Formation) = 0 none, susceptible)-3(high, tolerant); SDW (shoot dry weight), VLH (vine length). Broad-sense heritability (h^2) was computed as $h^2 = \delta^2_g/(\delta^2_g + \delta^2_e/n)$, where δ^2_g and δ^2_e were the estimates of genetic and residual variances respectively and n, the number of replications. Means within trait followed by the same letter(s) are not significantly different at $p \le 0.01$

reverse primer, Table 2) primer combinations were used for analysis. Five ISSR primers and 15 SRAP primer combinations failed to amplify products of sufficient quality for analysis. However, of 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 (Table 1) and 26 of the SRAP primer combinations (Table 2) were reproducible enough for marker analysis. 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 and 61 SRAP bands.

Linkage map and marker segregation: Eighty-eight (86) dominant markers out of the total 109 markers that fitted the expected 3:1 Mendelian ratio ($p<0.05$) were used in the linkage analysis of which 62 (72%) markers made up of 30 ISSR and 32 SRAP markers were assign to seven linkage groups (LG). Twenty three (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 (Fig. 2).

QTL analysis

General: QTL associated with waterlogging tolerance were mapped in four of the seven Linkage Groups (LG) with 62 informative markers assigned. A total of 25 putative QTL were found to be associated with the six traits studied using CIM, of which 14 were detected for the four waterlogging traits (TOL, AFR, SDWw and VLHw) and 11 for the two control traits (SDWc and VLHc). The QTL for the waterlogged traits accounted for 7.9-33.2% of the phenotypic variations while the control traits accounted for 6.9-19.1% of the phenotypic variations (R^2%). Out of the 14 QTL for the waterlogged traits, 12 QTL individually accounted for more than 10% of the phenotypic variations (R^2%) (Table 4).

QTL for TOL and AFR: Three QTL were found to be associated with TOL (Table 4, Fig. 2). Out of these three QTL, only tol4_1 linked to SA4EM10_5 marker in LG4 was specific to TOL and not associated with other traits. However, it had a negative additive-effect (-0.65) accounting for 33% of the phenotypic variations. Interestingly, this is from the susceptible parent (PW0801) allele therefore indicating that favorable alleles for TOL were dispersed between the two parents. The two other TOL QTL, tol1_1 and tol5_1 linked to UBC807_2 marker of LG1 and ME3EM5_4 marker of LG 5, accounted for 17.0 and 10.4% of the total phenotypic variation respectively with positive additive effects.

ARF had two QTL associated with it; these included arf2_1 and arf5_1, of these QTL arf5_1 was specific to ARF linked to ME2EM7 at LG 5 (Fig. 1). But arf2_1 was mapped with sdw_c1_1 of SDWc at the same locus linked to marker UBC887_1 in LG 2.

QTL for SDWw, VLHw: Four QTL were found associated with SDWw, these include sdw_w1_1, sdw_w2_1, sdw_w4-1 and sdw_w5_1. Two of these QTL, sdw_w1_1 sdw_w4_1, were specific to SDW_w. Of these specific QTL, only sdw_w1_1 showed positive alleles from the tolerant parent, PW0382 and accounted for 10.2% of the total phenotypic variation of SDWw, therefore increasing the value of the trait.

In the case of VLHw, five QTL were detected, these were vlh_w1_1, vlh_w1_2, vlh_w2_1 vlh_w4_1 and vlh_w5_1 (Table 4 and Fig 2.). Two QTL vlh_w4_1 and vlh_w5_1 were specific to VLHw, with vlh_w4_1 showing negative additive effects indicating the susceptible parent was the source of the alleles. However, vlh_w5_1 with positive additive effects contributed only 7.9% of the phenotypic variation (R^2%) for VLHw.

Two QTL, sdw_w2_1 of SDWw and vlh_w2_1 of VLH_w were both linked to the UBC884_2 marker in LG2 (Fig 1) with positive additive effects, accounting for 17 and 27% of the total phenotypic variations of VLHw and SDWw respectively. Also, vlh_w1_1 and vlh_w1_2 of VLHw and tol1_1 for TOL were also mapped in the same locus linked to UBC807_2 marker in LG1 (Table 4, Fig. 1). They all had

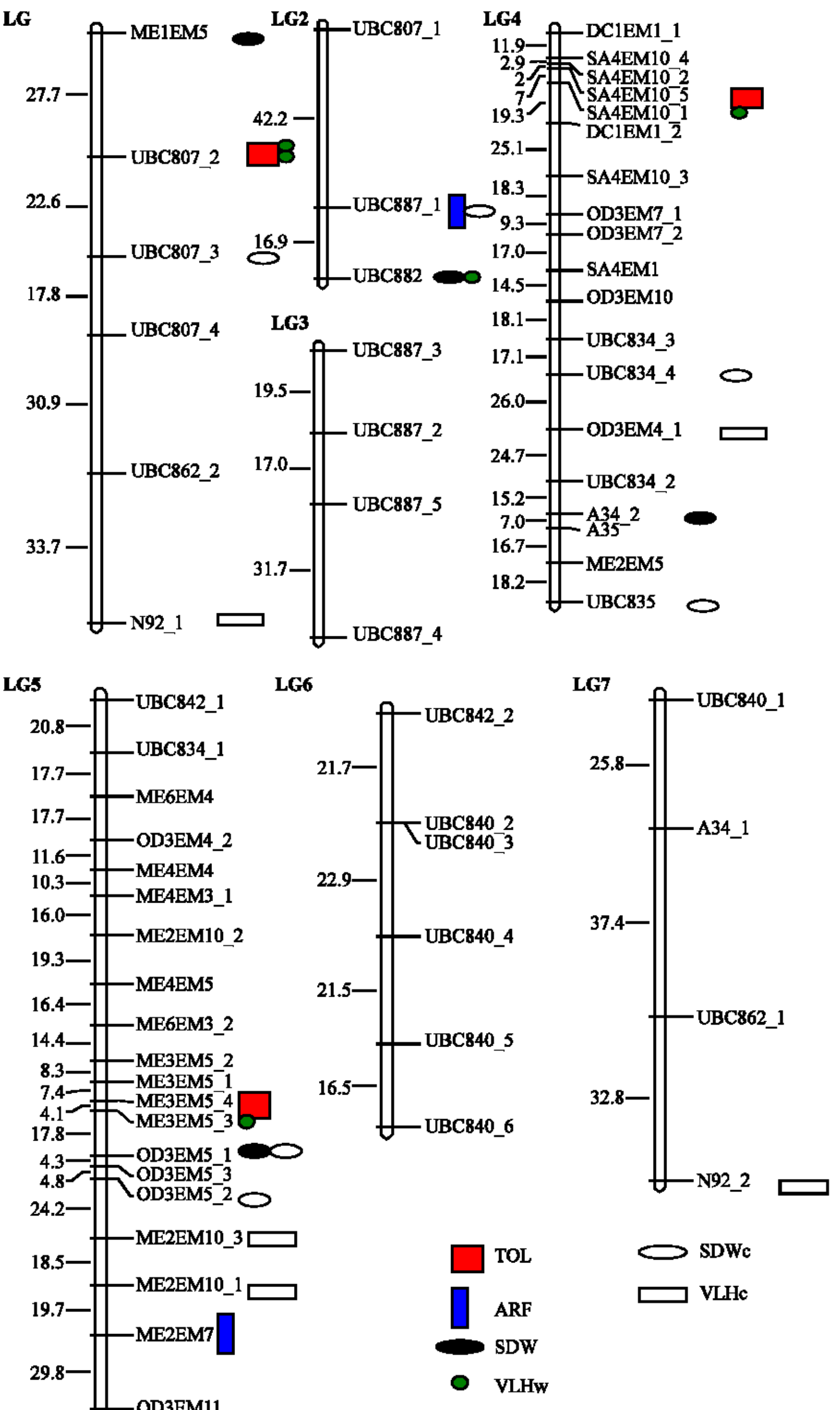

Fig. 1: SRAP and ISSR based molecular linkage map of cucumber derived from F_2 population of a cross between waterlogging-tolerant PW0832 (P_1) and waterlogging-susceptible PW0801 (P_2) and summary of QTL for four waterlogging traits, tolerance score (TOL), adventitious root formation (ARF), waterlogged shoot dry weight (SDWw) and waterlogged vine length (VLHw), control shoot dry weight (SDWc) and control vine length (VLHc). Marker names are at the right, while distances between adjacent markers (in cM) are at the left of each linkage group

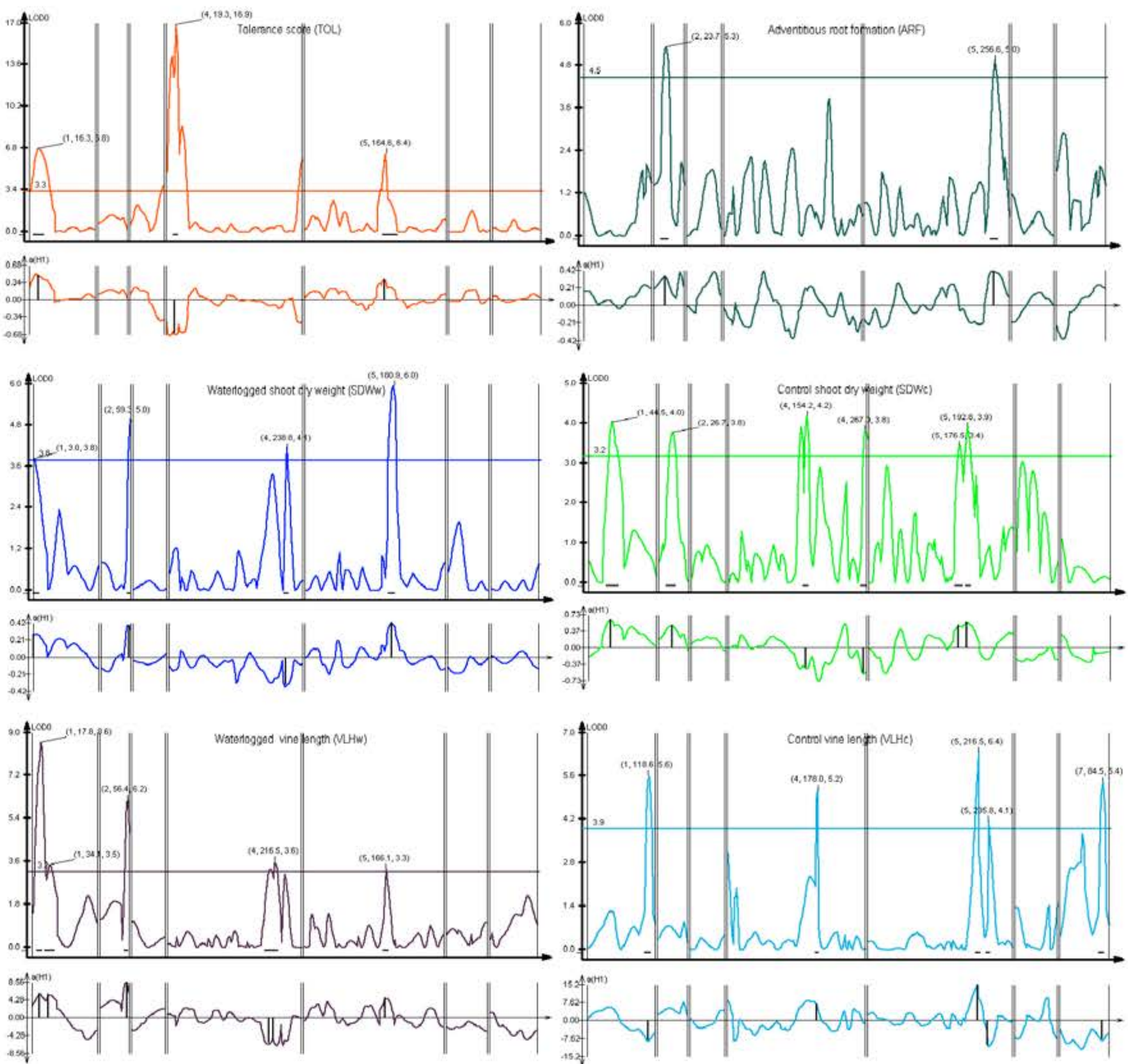

Fig. 2: Genome scans for waterlogging tolerance and control Quantitative Traits Loci (QTL) as identified by Composite Interval Mapping (CIM). Log-likelihood (LOD) scores of six traits made up of tolerance score (TOL), Adventitious Root Formation (ARF), waterlogged shoot dry weight (SDWw), control shoot dry weight (SDWc), waterlogged vine length (VLHw) and control vine length (VLHc) are plotted against seven linkage group (partitioned by vertical grids). Significance of QTL is indicated by LOD score above the threshold values determined by permutation analysis at a significant level of p = 0.05. The graph below shows the additive effects for each of the QTL identified

positive additive effects with R^2% values of 15.1%, 12.0 and 17.0% for vlh_w1_1, vlh_w1_2 and tol1_1 respectively.

QTL for SDW_c and VLH_c: Six and five QTL were detected in SDWc (sdw_c1_1, sdw_c2_1, sdw_c4_1, sdw_c4_2 sdw_c5_1, sdw_c5_2) and VLHc (Vlh_c1_1, Vlh_c4_1, Vlh_c5_1, Vlh_c5_2 and Vlh_c7_1), respectively (Fig. 2). For SDWc, sdw_c1_1, sdw_c4_1, sdw_c4_2 and sdw_c5_2 were specific, but only two of them (sdw_c1_1 and sdw_c5_2) had positive additive effects, the value of the others were negative. All the five QTL associated with VLHc were specific, with Vlh_c4_1, Vlh_c5_1 having positive additive effects and cumulatively contributing 27.1% of the total phenotypic variation of the trait.

However, some QTL from both the control and the waterlogged traits were mapped at the same locus. For

Table 4: Map position and QTL with LOD score ≥2.5 for waterlogging and control traits detected in F_2 population of Cucumber (*Cucumis sativus* L.) derived from a cross between waterlogging-tolerant PW0832 (P_1) and waterlogging-susceptible PW0801 (P_2) grown in the greenhouse at Yangzhou, China, 2006 (Output data from QTL cartographer)

Traits[a]	QTL[b]	Linkage group	QTL position (cM)	Nearest marker	QTL LOD score	Threshold LOD score	R^2 (%)	Additive effect[c]
TOL	tol 1_1	1	16.3	UBC807_2	6.8	3.3	17.0	0.50
	tol 4_1	5	19.3	SA4EM10_5	16.9	3.3	33.2	-0.65
	tol 5_1	5	164.6	ME3EM5_4	6.4	3.3	10.4	0.44
ARF	arf 2_1	2	23.7	UBC887_1	5.3	4.5	14.0	0.36
	arf 5_1	5	256.6	ME2EM7	5.0	4.5	17.2	0.41
SDWw	sdw_w1_1	1	3.0	ME1EM5	3.8	3.8	10.2	0.30
	sdw_w 2_1	2	59.3	UBC882	5.0	3.8	17.3	0.40
	sdw_w4_1	4	238.8	A34_2	4.1	3.8	10.0	-0.34
	sdw_w5_1	5	180.9	OD3EM5_1	6.0	3.8	21.4	0.43
VLHw	vlh_w 1_1	1	17.8	UBC807_2	8.6	3.2	15.1	6.10
	vlh_w 1_2	1	34.1	UBC807_2	3.5	3.2	12.0	5.71
	vlh_w 2_1	2	56.4	UBC882	6.2	3.2	26.9	8.56
	vlh_w 4_1	4	216.5	SA4EM10_1	3.6	3.2	14.3	-5.35
	vlh_w 5_1	5	166.1	ME3EM5_3	3.3	3.2	7.9	4.81
SDWc	sdw_c 1_1	1	44.5	UBC807_3	4.0	3.2	18.0	0.65
	sdw_c 2_1	2	26.7	UBC887_1	3.8	3.2	14.9	0.52
	sdw_c 4_1	4	154.2	UBC834_4	4.2	3.2	8.0	-0.43
	sdw_c 4_2	4	267.0	UBC835	3.8	3.2	11.8	-0.50
	sdw_c5_1	5	176.5	OD3EM5_1	3.4	3.2	13.2	0.58
	sdw_c 5_2	5	192.8	OD3EM5_2	3.9	3.2	10.1	0.52
VLHc	Vlh_c 1_1	1	118.6	N92_1	5.6	3.9	8.9	-7.94
	Vlh_c 4_1	4	179.5	OD3EM4_1	5.2	3.9	8.0	7.62
	Vlh_c 5_1	5	216.5	ME2EM10_3	6.4	3.9	19.1	15.56
	Vlh_c 5_2	5	235.8	ME2EM10_1	4.1	3.9	12.0	-9.84
	Vlh_c 7_1	7	84.5	N92_2	5.4	3.9	6.9	-7.74

[a]: TOL (tolerance score) = 0 (dead plant)-5(green plant with no sign of stress). ARF (Adventitious Root Formation) = 0 none, susceptible)-3(high, tolerant); SDWw (waterlogged shoot dry weight), VLHw (waterlogged vine length) SDWc (control shoot dry weight), VLHc (control vine length). [b]: The first number following the letters represents the linkage group location of the QTL and the second number represents the order of QTL of a trait in the same linkage group. [c]: Percentage of phenotypic variance explained by QTL. Positive additive effect indicates that the high values of the trait is inherited from the tolerant parent (PW0832) while negative additive effect indicates high value of the trait were inherited by the susceptible parent (PW0801)

example, sdw_c2_1 and arf2_1, both showing positive additive effects and accounting for 14.0 and 14.9% respectively of the total phenotypic variations of their respective traits, were mapped in the same locus linked to UBC887_1 marker in LG 2 (Fig. 1). Similar result also occurred with sdw_c5_1 and sdw_w5_1 both linked to the SRAP marker OD3EM5_1 with positive additive effects and accounting for 21.4 and 13.2% of the total phenotypic variations of their respective traits.

DISCUSSION

Our objective in this study was to provide information about the genetic control of waterlogging tolerance and its associated molecular markers and also test the usefulness of ISSR and SRAP markers for QTL studies in cucumber. The degrees of polymorphism exhibited by both markers clearly demonstrate their usefulness in genetic analysis of cucumber. We further speculate that because of their simplicity and accessibility these markers may rapidly become an invaluable tool for cucumber genome analysis. 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. In this study a linkage map of 62 loci spanning a total length of 992.2 cM with an average genetic distance of 16.0 cM between adjacent markers was constructed, 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).

Although the parents differ significantly in the means of TOL, ARF, SDWw and VLHw, a wide range of variations were observed in these traits among the $F_{2:3}$ lines with their ranges indicating occurrence of transgressive segregation. Also, the high to moderate broad-sense heritabilities of the waterlogging traits showed the extent to which the environment influence these traits, with a lower influence over VLHw and SDWw than both TOL and AFR. Understanding the gene action for waterlogging tolerance has been reported in several studies. For example, Boru *et al*. (2001) studied the genetic behavior of waterlogging tolerance in wheat using leaf chlorosis as the indicator for tolerance and found that

waterlogging tolerance was controlled by additive genetic effects. Also Thseng and Hou (1993) reported that the pregermination flooding tolerance of sorghum was controlled mainly by additive gene with heritabilities of 0.97 and 0.75 for both broad-sense and narrow-sense respectively. The high heritability of VLHw and SDWw as well as high additive effects of some of the QTL associated with them in this study indicates that selection in the early generations for these traits would be effective.

The QTL analysis underlying waterlogging tolerance using the $F_{2:3}$ lines derived from a cross between PW0832 (tolerant) and PW0801 (susceptible) parents of cucumber have revealed several features in this study. Four of the seven linkage groups contained waterlogging tolerant QTL. The tolerant parent PW0832 contributed desirable effects to 10 of the 14 QTL detected in the waterlogged traits in this study. The PW0832 allele at these loci could be useful to enhance waterlogging tolerance in cucumber. Out of the 14 QTL for the waterlogged traits, 12 of them individually accounted for more than 10% of the phenotypic variations, therefore confirming their importance to waterlogging tolerance. Also, all waterlogging traits, with exception of ARF, had QTL of both positive and negative additive effects. Of the 14 QTL from waterlogged traits, the susceptible parent PW0801 increased three QTL one each for TOL, SDWw and VLHw. These results indicate that favorable alleles for waterlogging tolerance were dispersed between the two parental lines used in our study. For example TOL had a QTL (tol4_1) with a negative additive-effect (-0.65) accounting for 33% of the total phenotypic variations.

The two major QTL toll_1, tol5_1 of TOL and arf5_1 of ARF contributing substantially to the phenotypic variations of these traits with positive additive effects, appear to specifically control waterlogging tolerance in the $F_{2:3}$ population used in this study. However, toll_1, vlh_w 1_1 and vlh_w 1_2 were mapped in the same locus UBC807_2 in the linkage map with positive additive effects. This could be explained by the relationship between TOL and VLH both physiologically and genetically. It is possible that these are single genes with pleiotropic effects. If these QTL are indeed the same gene, then they may control waterlogging stress tolerance mechanism in the population used in this study. Fine mapping of these clusters would therefore be needed to differentiate between tight linkage and pleiotropy of the genes involved.

Similarly, sdw_w1_1, sdw_w2_1 and sdw_w5_1, with positive alleles of the tolerant parent PW0382 with cumulative R^2% value of 48.9%, increased the value of the SDWw substantially. For VLHw, four QTL vlh_w1_1, vlh_w1_2, vlh_w2_1 and vlh_w5_1, all with positive additive effects, also contributed a total of 61.9% of the total phenotypic variation (R^2%). This is confirmed by the higher heritability of 88% of VLHw in this study. Also the identification of QTL contributing 61.9% of the total phenotypic variance indicates that more of the variations are yet to be exploited. However, the magnitude of variations explained by these markers is substantial in view of the quantitative nature of this trait.

Several QTL of both control and waterlogged traits were located in the same locus. For example, sdw_c2_1 and arf2_1 were both mapped in the same locus linked to UBC887_1 marker in LG 2, while sdw_c5_1 and sdw_w5_1 were all linked to the SRAP marker OD3EM5_1. If these QTL are the same locus, then the gene may be involved with the general growth conditions rather than being specifically for waterlogging tolerance. This is confirmed by the positive additive effects of these QTL indicating that tolerant parent (PW0832) might have conferred better shoot weight under the control conditions.

The untapped variations in this study could be due to various reasons such as incomplete genome coverage, loose linkage between marker loci, interactions between traits and environmental factors. Further analysis of this population under different agroclimatic conditions would be necessary. This could be feasible by evaluating the QTL in PW0832 in a near isogenic background. This could provide a good opportunity for fine mapping of these QTL associated with waterlogging tolerance to confirm and facilitate their rapid utilization. Furthermore, genes in the identified QTL regions in this study may be revealed through comparative expression analysis using our parental lines and populations developed from them.

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