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Expressed sequence tag-simple sequence repeat-based molecular variance in two *Salicornia* (Amaranthaceae) populations

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ABSTRACT. *Salicornia* spp is one of the most salt-tolerant vascular plants and is native to salt marshes and estuaries. We developed expressed sequence tag derived-simple sequence repeat (EST-SSR) markers for estimating genetic diversity and marker-assisted *Salicornia* breeding. Six polymorphic EST-SSRs of 40 detected 27 alleles, ranging from three to five alleles per locus. The average number of alleles per locus was 4.33 and 4.17, and the major allele frequency at locus DY529765 was high, being 0.859 and 0.857 in *S. bigelovii* and *S. europea*, respectively. Gene diversity, heterozygosity and polymorphism information content were highest at locus DY529950 and similar in

these two species. Gene diversity increased with increase in the number of alleles that had a low major allele frequency at a locus. Six polymorphic loci effectively discriminated 46 taxa into three clusters via different analyses. **S**ignificant deviation of F_{ST} from zero in three suggested populations for six loci indicated population differentiation and limited gene flow among them. A reduced median network established that taxon SB65 is primitive. SMART (simple modular architecture research tool) analysis of peptide sequences of six EST-SSRs showed that loci DY529765, DY529950 and EC906203 contained transmembrane, TLC, AgrB and NTR domains and might be involved in salinity stress tolerance. These EST-SSRs are a valuable resource for marker development and may be useful in marker-assisted *Salicornia* breeding.

Key words: Gene diversity; Gene flow; Allele frequency; Reduced median network; Salinity tolerance

INTRODUCTION

Salicornia, glasswort also known as sea asparagus, is a widespread hygrohalophytic cosmopolitan genus of succulent annual herbs of family Amaranthaceae. The genus is taxonomically described as a nightmare (Kadereit et al., 2007), native to salt marshes and estuaries, arguably, one of the most salt-tolerant vascular plants (Ayala and O'Leary, 1995). Its seeds have been reported to produce oil that is high in polyunsaturated fat and is comparable to soybean (Glenn et al., 1991, 1998) and safflower (Christiansen, 2008). After oil extraction, the remaining oilseed biomass can be used as a high-protein feed for livestock (Glenn et al., 1991).

Although there is an increasing interest in the production of glassworts, taxonomic treatment of tribe Salicornieae especially genus *Salicornia* is still not satisfactory due to various reasons. The tribe is characterized by a simple and similar morphology having highly reduced leaves and flowers. Morphological distinction is only possible in fresh state between flowering and fruiting (Ge'hu et al., 1979). Plants show high levels of phenotypic plasticity (Sagane et al., 2003) and they are mostly cleistogamous (Noble et al., 1992), although hybridization has been suggested as well (Lahondère, 2004). Several authors (e.g., Stace, 1997) favor a wider species concept within *Salicornia*, in which segregates or microspecies, rather than species are distinguished, others (e.g., Ge'hu and Ge'hu-Franck, 1992; Lahondère, 2004) prefer a more narrow species view. To date the genus *Salicornia* contains around 21 species (Anonymous, 2010).

Pickleweed, *Salicornia bigelovii* Torr., is an important species in the genus, which is commercially cultivated as a specialty vegetable for the U.S. and European fresh produce markets (OASE, 2010). It also has potential for production of oilseeds, forage, and biofuels (Glenn et al., 1991, 1999; Weber et al., 2007; Christiansen, 2008). *S. bigelovii* is an out-crossing plant; however, seed setting with self-pollination is possible. Therefore, it can be hybridized among lines and purified through selfing. This species slightly differs from that being harvested along Europe's shores (*S. europea*). Another species, *S. europaea* is treated in a broad sense but its aggregate is represented in Eurasia and North America by several diploid and tetraploid races. In China, most probably Chinese plants belong primarily or exclusively to the Eurasian continental race known as *S. prostrata* Pallas (Ill. Pl. 8. 1803).

Salicornia plants tend to have phenotypic variation partly caused by environmental parameters like temperature, salinity, soil quality, and plant density (Boorman et al., 2001). The specific limits of the classification of *Salicornia* plants based on the morphological features, especially based on dried plant features, are obscure (Ball, 1964). Molecular markers provide an opportunity to prove relevance between genotype and phenotype in the plants. Several anonymous molecular markers, like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), etc., have been developed for studies focused on genetic diversity, population genetics and structure. In contrast to anonymous random DNA markers, functional markers derived from polymorphic sites within genes have been recently used to functionally characterize allelic variation in plants (Andersen and Lübberstedt, 2003; Lübberstedt et al., 2005; Asp et al., 2007).

The SSRs are among the most variable DNA sequences in the genome (Weber, 1990), due to mutations whose rate and type depend mainly on the number of repeat motifs (Wierdl et al., 1997). However, the mutation rates differ among loci and among alleles, and also between species (Ellegren, 2000). Previously SSRs have been isolated from genomic libraries utilizing anonymous DNA fragments but in more recent studies SSRs have been detected through computational methods in sequence databases generated from large-scale expressed sequence tags (ESTs) sequencing projects. About 1 to 5% ESTs from different plant species have been found to contain SSRs suitable for marker development (Kantety et al., 2002). EST-SSR markers have been developed for a number of plant species, including rice (Temnykh et al., 2001), durum wheat (Eujayl et al., 2002), barley (Thiel et al., 2003), wheat (Peng and Lapitan, 2005), and cotton (Han et al., 2006). As EST-SSR markers are directly associated with an expressed gene, thus, completely linked with putative qualitative or quantitative trait locus alleles. Therefore, EST-SSR markers being superior, more informative and conserved than anonymous markers (Andersen and Lübberstedt, 2003), are useful as anchor markers for comparative mapping across species, comparative genomics, and evolutionary studies (Eujayl et al., 2002; Kantety et al., 2002; Thiel et al., 2003). However, EST-SSR's conserved nature may also limit their degree of polymorphism. Several studies showed transferability of SSR loci within a genus to be above 50%, but poor across genera (Eujayl et al., 2002; Thiel et al., 2003).

The taxanomic treatment of *Salicornia* is not satisfactory especially in China where it is in need of revision (Zhu et al., 2003). Therefore, in the present studies, different taxa of genus *Salicornia* were collected at random from the foreshore of the Yellow Sea, China, and classified into two groups based on their morphometric resemblances to either *S. bigelovii* or *S. europaea*. A comprehensive EST collection in the NCBI database (http://www.ncbi.nlm.nih.gov/) from genera *Salicornia* and *Suaeda* was computationally exploited for SSR identification. These EST-SSRs were used to determine genetic diversity within and among various naturally growing *Salicornia* populations. This paper presents EST-SSR-based molecular variance in naturally growing two *Salicornia* populations along the Chinese coast. This paper also identifies functionally associated EST-SSRs for developing useful markers to assist *Salicornia* breeding.

MATERIAL AND METHODS

Plant materials

Fresh young fleshy stems of 100 morphological different taxa of genus *Salicornia*

were collected at random from the foreshore of the Yellow Sea, China. The stems of each taxa were packed separately in polyethylene bags, immediately placed in insulated ice box and kept in this box during their logistic transfer to the laboratory for storage at -70°C until their further processing. Genomic DNA of the 100 samples was extracted individually using CTAB method (Doyle and Doyle, 1987). Good-quality DNA was obtained from 46 taxa only, 32 resembling morphologically to *S. bigelovii* and 14 to *S. europea*.

Design of EST-SSR primers

In total 2280 ESTs, 1103 from genus *Salicornia*, and 1177 from its close relative genus *Suaeda* were downloaded on January 12, 2009 from NCBI (http://www.ncbi.nlm.nih.gov/). These ESTs were analyzed for ≥20-bp SSRs, using the SSRHunter 1.3 software (http://en.bio-soft.net/dna/SSRHunter.html). Forty ESTs of 2280 contained SSRs of size ≥20 bp and more than six of core repeats. Forty EST-SSR sequences were analyzed for their phylogenetic relationship using the Clustalx2 software (http://www.ebi.ac.uk/Tools/clustalx2/index.html). Forty EST-SSRs were used in the present studies and a set of 6 polymorphic EST-SSRs was finally selected to use for further analyses in the present studies. Forward and reverse primers were designed using the Primer Premier 5.0 software (http://www.premierbiosoft.com/primerdesign/index.html) keeping target polymerase chain reaction (PCR) product size between 100-300 bp.

PCR and PAGE

PCR was performed with a 20-µL volume containing 12.8 µL ddH_2O, 1 µL genomic DNA (20 ng/µL), 0.4 µL of each primer (10 µM), 2 µL r*Taq* buffer (10X), 1.6 µL $MgCl_2$ (25 mM), 1.6 µL dNTPs (10 mM), and 0.2 µL r*Taq* DNA polymerase (5 U/µL) (Takara Bio Inc; Shiga, Japan; http://www.takara-bio.com/). PCR was conducted in a thermo-cycler (LabCycler, SensoQuest Biomedizinische Elektronik, GmbH., Göttingen, Germany) set with initial denaturation temperature of 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 50-60°C for 30 s and extension at 72°C for 30 s followed by a final extension at 72°C for 3 min. PCR products were separated on 12% denaturing polyacrylamide gels followed by silver staining to detect and analyze the PCR products according to Bassam et al. (1991) with slight modifications (12% acetic acid, 0.2% silver nitrate, 6% sodium bicarbonate).

Statistical data analysis

Gene diversity, heterozygosity, polymorphism information content (PIC), and allele frequencies were calculated following functions explained in Liu and Muse (2005) and PowerMarker V3.0 Manual using the PowerMarker V3.0 software (see URL; http://www.powermarker.net for Manual and software). Effective loci discrimination was determined through principal component analysis (PCA) using PROC PRINCOMP in the SAS software. Inter-and intra-population structure depicting gene flow was constructed using the Structure V2.3.1 software (http://pritch.bsd.uchicago.edu/structure.html) according to Pritchard et al., (2000). Reduced median network was constructed using the Network 4.5.1.6. software (www.fluxus-engineering.com/network_terms.htm).

RESULTS

Polymorphism, allele distribution and genetic diversity within and among populations

Phylogenetic tree of 40 EST-SSRs was subdivided into three groups (Figure 1). All EST-SSRs in group I and few in group III appeared to be of high similarity. EST-SSRs in group II were more diverse. Of 40 six EST-SSRs, one from each group I and group III, and four from group II, were polymorphic and thus used to genotype 32 *S. bigelovii* and 14 *S. europea* taxa (Table 1). Rest of diverse EST-SSRs from groups II and III did not amplify or become polymorphic within target size. All six polymorphic loci detected 27 alleles in total. Average allele numbers per locus within the population were comparable between two populations, 4.33 and 4.17 in *S. bigelovii* and *S. europea*, respectively (minimum = 3, maximum = 5 alleles per locus in each population; Table 2). Allele E was not detected at respective loci of BE240888, and EC906203 and EC906222 in *S. bigelovii* and *S. europea*. Frequency of major allele C (145 bp) at locus DY529765 was significantly ($P < 0.05$) high among all loci, 0.859 and 0.857 in *S. bigelovii* and *S. europea*, respectively. Conversely, major alleles A (160 bp) in *S. bigelovii* and B (165 bp) in *S. europea* at DY529950 were significantly less frequent among the six loci. Null alleles were observed at locus DY529903 in both populations and at EC906222 in *S. bigelovii* alone. All taxa from both populations deviated significantly from Hardy-Weinberg equilibrium (HWE) at all loci except at DY529765 in *S. europea* (data not shown).

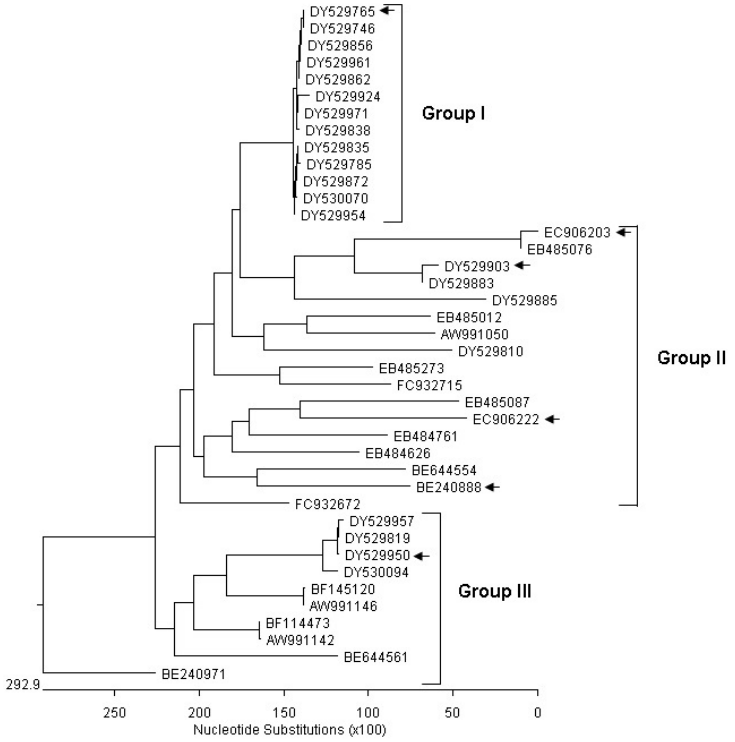

Figure 1. Phylogenetic tree of 40 EST-SSRs. Polymorphic EST-SSRs are pointed with arrows

Table 1. Primer sequences, SSR motifs, PCR product size, source and predicted domains of six polymorphic EST-SSR markers used for genetic diversity analysis of 46 *Salicornia* spp accessions.

Marker name	Forward primer sequences	Reverse primer sequences	SSR motifs	PCR product size (bp)	Source	Predicted domains, motifs
BE240888	5'-GTTATGATTTTGAGAGACCGA-3'	5'-CAGAAGAATTATTAACCGCCA-3'	$(AAC)_7$	174	*Suaeda*	LC
DY529765	5'-GGGGAGACAAGGAGCAAACA-3'	5'-CAATAAGGCAGCACACAGCAA-3'	$(GAAAA)_4$	268	*Salicornia*	LC/TM/TLC/NTR
DY529903	5'-GGGCAGGTACTTTCACAATT-3'	5'-CGTAGAAGGTGTCTCTGCAA-3'	$(GCTA)_5$	291	*Salicornia*	LC
DY529950	5'-CCACCCTCATCATCATTATCAT-3'	5'-GAGACAAGGAGCAAACACCAT-3'	$(TCTTT)_5$	162	*Salicornia*	TM/AgrB
EC906203	5'-GCCAAGATCCACTAGGCTTGTT-3'	5'-GGTCCAATGGGAGGTGGCTT-3'	$(CTG)_{10}$	129	*Salicornia*	LC/TM
EC906222	5'-GGACGAGGAAATCATCT-3'	5'-CGGGAACGACAAATCTAT-3'	$(TCCTC)_5$	289	*Salicornia*	LC

SSR = simple sequence repeat; PCR = polymerase chain reaction; LC = low complexity; TM = transmembrane; Agr = accessory gene regulator; TLC = TRAM, LAG1 and CLN8; NTR = netrin-like.

Table 2. Allele frequency distribution within and between *Salicornia bigelovii* and *S. europea*.

Locus	Allele name	Allele size (bp)	*S. bigelovii*	*S. europea*
BE240888	A	135	0.047	0.071
	B	141	0.156	0.214
	C	147	0.469[a]	0.429[a]
	D	150	0.328	0.214
	E	156	-	0.071
DY529765	A	135	0.047	0.036
	B	140	0.047	0.071
	C	145	0.859[a]	0.857[a]
	D	160	0.047	0.036
DY529903	A	292	0.313	0.357
	B	304	0.625[a]	0.429[a]
	C	Null	0.063	0.214
DY529950	A	160	0.281[a]	0.071
	B	165	0.266	0.250[a]
	C	170	0.094	0.214
	D	180	0.094	0.214
	E	185	0.266	0.250[a]
EC906203	A	113	0.063	0.107
	B	119	0.063	0.071
	C	122	0.297	0.536[a]
	D	131	0.547[a]	0.286
	E	152	0.031	-
EC906222	A	170	0.109	0.143
	B	180	0.344	0.321
	C	185	0.031	0.071
	D	195	0.453[a]	0.464[a]
	E	Null	0.063	-
Average allele numbers per locus			4.333	4.167
Major allele's average frequency			0.539	0.494
SEM			0.222	0.200

[a]major allele = having highest frequency at each locus within population; SEM = standard error of the mean.

Gene diversity, heterozygosity and PIC remained highest at locus DY529950 (Table 3). Ranges of respective gene diversity in *S. bigelovii* and *S. europea* were 0.255-0.762 and 0.258-0.778. Heterozygosity and PIC in respective aforementioned populations ranged between zero and 0.906 and 0.929, and 0.245-0.722 and 0.246-0.741, respectively. Four loci BE240888, DY529903, EC906203, and EC906222 were statistically almost similar ($P < 0.05$) in their depiction of gene diversity and PIC. Significant ($P < 0.05$) positive association was observed between gene diversity and PIC while major allele frequency showed strong negative association with gene diversity and PIC (Figure 2).

Table 3. Gene diversity, heterozygosity and polymorphism information content (PIC) of six loci in *Salicornia bigelovii* and *S. europaea*.

Loci	*S. bigelovii*			*S. europea*		
	Gene diversity	Heterozygosity	PIC	Gene diversity	Heterozygosity	PIC
BE240888	0.646	0.094	0.581	0.714	0.000	0.671
DY529765	0.255	0.188	0.245	0.258	0.214	0.246
DY529903	0.508	0.000	0.428	0.643	0.000	0.567
DY529950	0.762	0.719	0.722	0.778	0.929	0.741
EC906203	0.604	0.313	0.544	0.615	0.071	0.556
EC906222	0.660	0.906	0.600	0.656	0.929	0.595
Average	0.572	0.370	0.520	0.611	0.357	0.563
SEM	0.176	0.363	0.165	0.182	0.450	0.170

SEM = standard error of the mean.

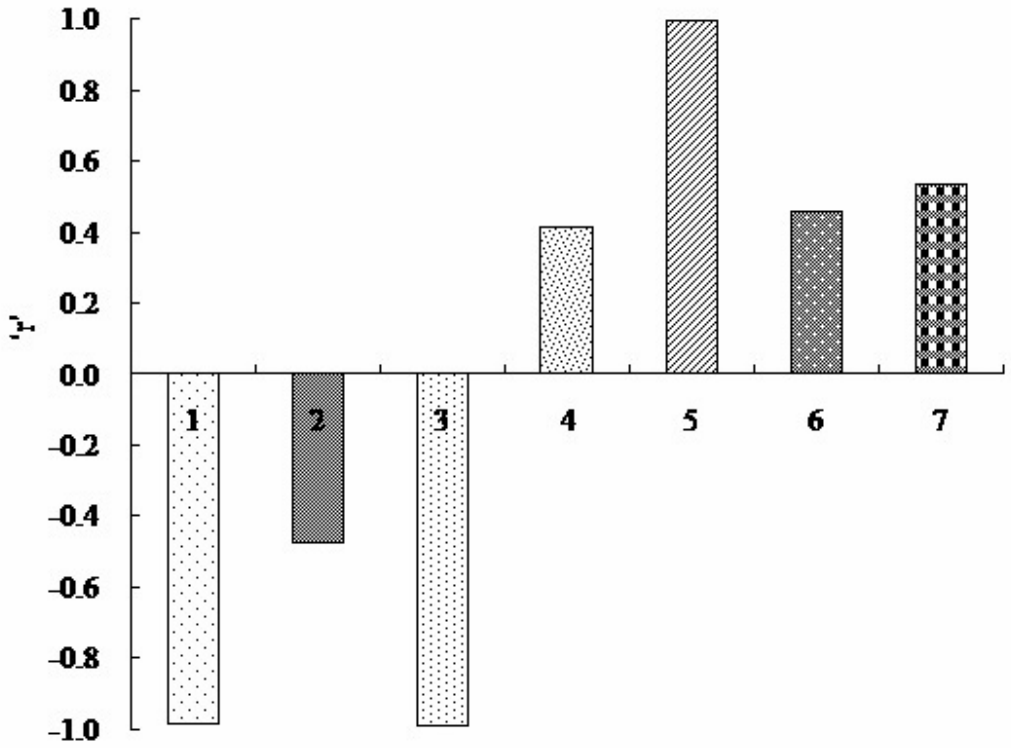

Figure 2. Correlation coefficients 'r' between selective parameters. 1 = Major allele frequency (MAF) *vs* genetic diversity (GD); 2 = MAF *vs* heterozygosity (HET); 3 = MAF *vs* polymorphism information content (PIC); 4 = GD *vs* HET; 5 = GD *vs* PIC; 6 = HET *vs* PIC; 7 = number of alleles *vs* GD.

Gene flowing between populations

Six polymorphic loci effectively discriminated predefined two populations, based on morphological classification, into three clusters via PCA (Figure 3). Considerable overlaps between *S. bigelovii* and *S. europea* populations were evident. The majority of taxa from *S. europea* grouped into clusters II and III, while cluster I contained only two taxa from *S. europea*. Phylogenetic analysis and UPGMA tree view constructed via Ni's estimates also substantiated the results of PCA (data not shown).

The pairwise global estimate of F_{ST} indicated significant differentiation between populations after correction for multiple comparisons, with F_{ST} values ranging from 0.189 to 0.481. The Bayesian clustering procedure detected the maximum likelihood for a model of three genetically distinct populations (K = 3, ln P(D) = -438.3) via STRUCTURE 2.3 basic algorithm described by Pritchard et al. (2000). For K >3, the clustering process failed to calculate a homogeneous posterior probability of the data between each iteration (Figure 4A). The STRUCTURE algorithm showed that a parti-

tioning of the genetic variation into three clusters was most probable when independent and correlated allele frequencies were applied (Figure 4B). A large proportion of the taxa have a genetic signature typical for one specific cluster. However, some taxa have a divided membership between two and three clusters.

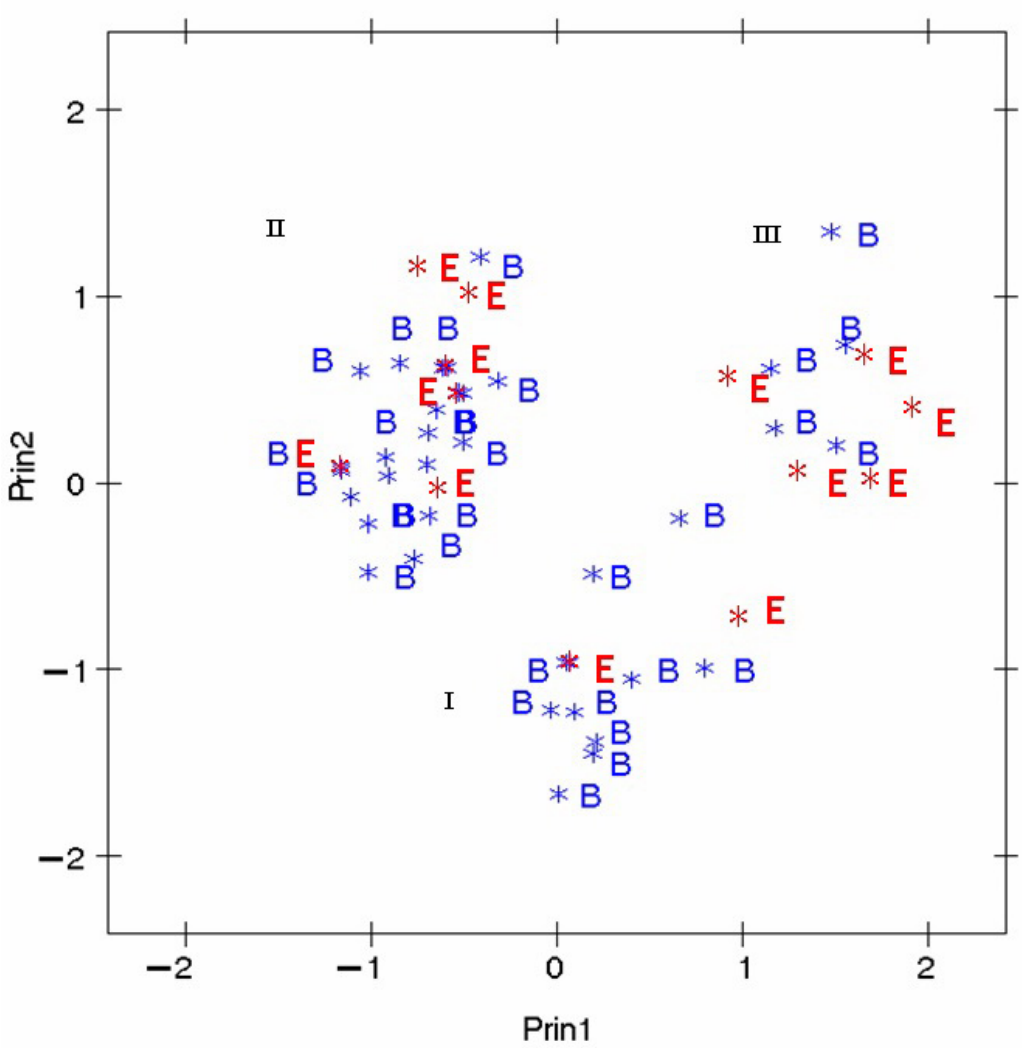

Figure 3. Effective loci discriminating predefined two populations into three clusters revealed by principal component analysis. 'B' represents *Salicornia bigelovii* and 'E' *S. europea*.

Detection of mutation in allele and taxa phylogenies via network analysis

Twenty-three of 27 alleles appeared in reduced median (RM) network analysis (Figure 5A). BE8885 appeared as consensus and/or reference allele, the rest of alleles showed deviations from the reference allele. DY9502 and DY9505 shared high similarity of nucleotide

sequence and high similarity was shared between EC2224 and DY7653 as well. RM network analysis of 46 taxa exhibited parallelism in 3 taxa only while 43 taxa were apparent in the network (Figure 5B). Figure 5B depicts SB65 as consensus or reference taxon. Descendants of node SB69 are grouped into two clades. One clade is descended from SE61 while the second clade is from node SB68. Clade from node SE61 includes ~50% of taxa from morphologically categorized *S. europea* while clade from node SB68 includes only one taxon SE10 from *S. europea*.

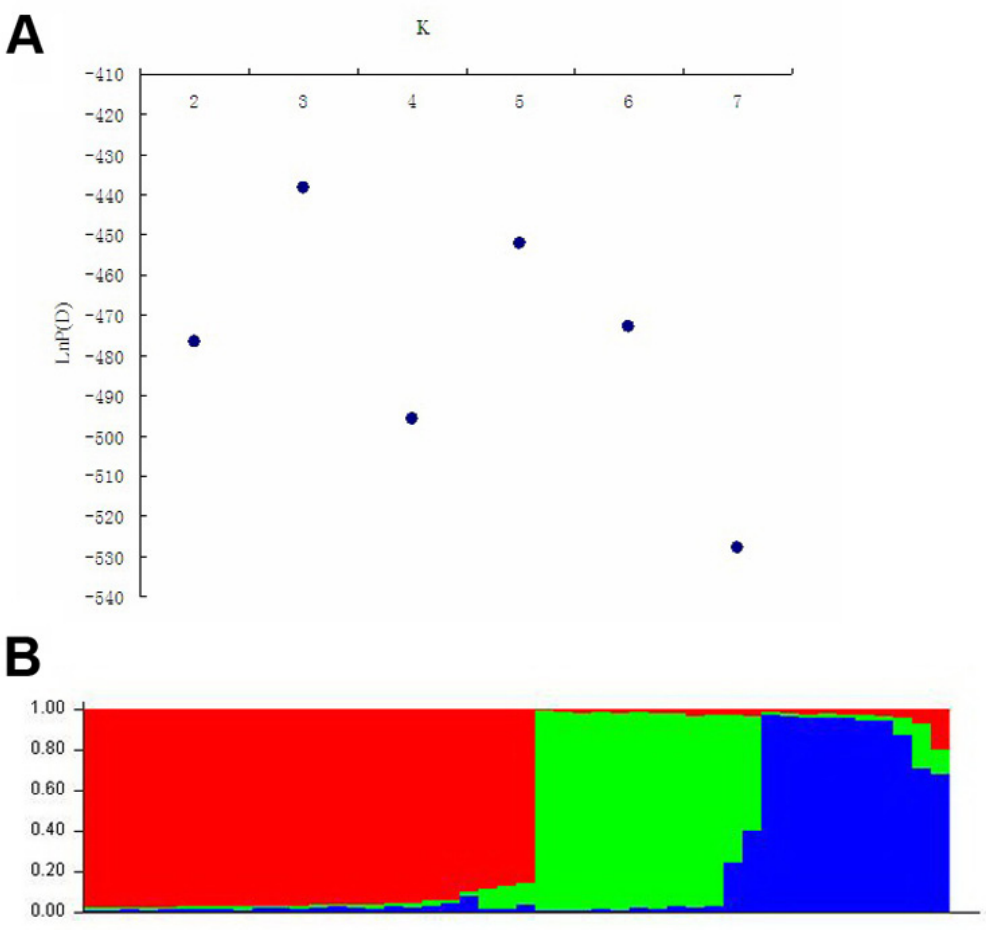

Figure 4. Structure of inter- and intra-populations. **A.** Estimated logarithms of data [LnP(D)] against the number of populations tested (K). **B.** Estimated population structure inferred from the whole *Salicornia* taxa for K = 3. Each taxon is represented by a thin vertical line, which is partitioned into K colored segments representing the taxon's estimated membership fraction in K clusters.

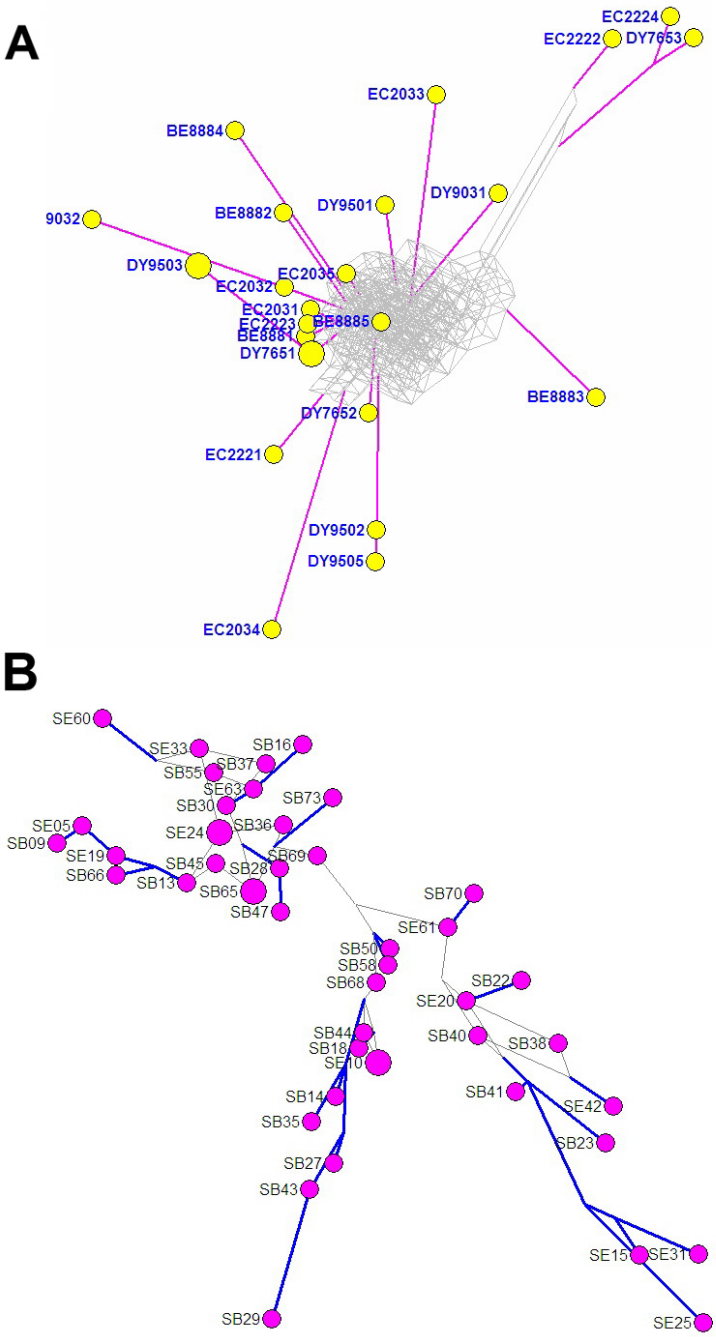

Figure 5. Reduced median network analysis. **A.** Networking among 27 alleles; names contain the first two letters and the last 3 digits from the respective EST-SSRs while the last digit of the name showed the respective allele number at the locus. **B.** Networking among 46 taxa, SB stands for *Salicornia bigelovii* and SE for *S. europea*.

DISCUSSION

Genetic diversity within and among populations

EST-SSR marker system is increasingly being used for diversity analyses in breeding and natural populations or genbank materials due to their power of functional diversity examination (Asp et al., 2007; Salem et al., 2010) and inexpensive development costs (Varshney et al., 2005). Computational analysis depicted that only 40 (1.75%) ESTs found in two genera, *Salicornia* and *Suaeda*, contained SSRs of size ≥20 bp and six (15% of 40) EST-SSRs were polymorphic and thus used for further analyses in the present study (Figure 1). Of six EST-SSRs, five were from *Salicornia* and one from *Suaeda* (Table 1). High level of polymorphism associated with SSRs is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Varshney et al., 2005). These polymorphic markers were able to discriminate among various taxa of *Salicornia*.

Gene diversity, heterozygosity and PIC are high at locus DY529950 but its major allele frequency is less and vice versa at locus DY529765 (Tables 2 and 3). Former locus has more number of alleles (5) compared to the latter ones (4 alleles). A positive significant association between number of alleles and gene diversity has been previously reported, and the use of number of alleles to evaluate the genetic diversity has been suggested (Salem et al., 2010). The correlation between gene diversity and the number of alleles at six polymorphic loci is moderate ($r = 0.533$) and less than $r = 0.741$ reported earlier in barley (Salem et al., 2010). Results at two aforementioned loci agree with the suggestion of Salem et al. (2010) but our cumulative data at six loci do not support this suggestion. However, large number of alleles with low major allele frequency at a locus produced high gene diversity and PIC values, and vice versa. Therefore, the use of the number of alleles together with their major allele frequency at a particular locus is suggested in order to evaluate the genetic diversity.

Average gene diversity, heterozygosity and PIC within each population were high and also remained comparable between two populations morphologically categorized (Table 3). Large number of alleles and high gene diversity/PIC confirm broader genetic base of two populations. This might be due to out-crossing nature of the plants and equal distribution of salt-tolerant genes among the two populations. It is quite possible that these two populations might have common ancestors and share many genes in common. Average PIC values of 0.520 and 0.563 in *S. bigelovii* and *S. europea*, respectively, confirmed that EST-SSR markers are highly informative in the two populations. Strong association between gene diversity and PIC indicated that gene diversity is essentially the same as PIC (Botstein et al., 1980; Anderson et al., 1993).

The genus *Salicornia* is native to salt marshes and estuaries, arguably, one of the most salt-tolerant vascular plants (Ayala and O'Leary, 1995). Simple modular architecture research tool (SMART) analysis of amino acid sequences coded by nucleotide sequence of ESTs showed involvement of domains responsible for salt tolerance. Three *Salicornia* loci DY529765, DY529950 and EC906203 contained transmembrane domains, DY529765's TLC domain and DY529950's AgrB domain have transport activities and may be involved in abiotic stress particularly salinity stress tolerance. Hence, these EST-SSRs are valuable resource for marker development and may be useful in marker assisted *Salicornia* breeding aimed at improving salt tolerance. As allele C at locus DY529765, which contains transmembrane, TLC and NTR domains, is most frequent among salt tolerant 46 taxa collected from the Yel-

low Sea coast, thus it may be used for development of an important salt-tolerant marker. However, further investigation to explore its expression and tolerance mechanisms is needed.

Gene flowing between populations

Phenotypic variation in *Salicornia* plants might be caused by environmental parameters like temperature, salinity, soil quality, and plants density (Boorman et al., 2001). Morphological features were suggested to be obscure for classification of *Salicornia* plants (Ball, 1964). Molecular discrimination of two morphological distinct populations into three distinct clusters via PCA, phylogenetic analysis and UPGMA tree view, and considerable overlaps between *S. bigelovii* and *S. europea* populations evidenced the involvement of common genes (Figure 3). Out-crossing nature of the plant, anthesis over a period of 30-60 days (Zerai et al., 2010) and co-existence of taxa within the same locality might have promoted the gene flow between the two populations. There were several deviations within each population due to this gene flow. The gene flow also resulted in evolution of a third molecular group whose members morphologically resemble the two populations. Some of the taxa appeared to be admixture of two populations. Previous studies reported deviation between molecular- and morphological-based classification of *Salicornia* plants, e.g., morphologically distinct two species of *Salicornia* in Japan consisted of five molecular groups (Sagane et al., 2003).

Previously, genetic differentiation between the six homogeneous clusters of spotted gum species obtained from STRUCTURE mirrored geographically separated sampling localities (Ochieng et al., 2010). In the present study, significant deviation of F_{ST} from zero over three populations and six loci indicated population differentiation and limited gene flow among the three suggested populations. Specific typical genetic signature of most of the members of each cluster supports division of 46 taxa into three distinct populations (Figure 4B). However, divided membership of some taxa between two and/or three clusters substantiated the gene flow among the taxa and it appeared to be more appropriate that these three clusters/populations are microspecies rather than distinct species.

RM network analysis showed three different clades/branches (Figure 5B). Major clade containing SB65 as reference taxon also contains SB69, which may be evolved from SB65. Other two clades appeared to be descendent from SB69. SE61 originating from SB69 descends 50% *S. europea* and 50% *S. bigelovii* while SB68 descends the majority of *S. bigelovii* types. SE61 clade showed high gene diversity and PIC compared to the rest of two clades (data not shown) and thus have more broader genetic base. Distances of various taxa from the respective reference taxon showed the magnitude of mutations and deviations of respective taxon from the reference or consensus taxon (see for explanation, Bandelt et al., 1995). This analysis suggested the existence of three different clades or microspecies within *S. bigelovii*, as the majority of taxa resembled with this species found at the coast of the Yellow Sea.

CONCLUSIONS

The present study highlights the discrimination power of EST-SSR marker system to analyze functional diversity in breeding and natural populations or genbank materials for its exploitation in crop improvement as well as *ex situ* conservation strategies of plant genetic resources. These EST-SSRs that have major domains involved in salinity tolerance are a useful

resource for salt-tolerant marker development. Three *Salicornia* loci, DY529765, DY529950 and EC906203, contained transmembrane, TLC, AgrB, and NTR domains, and might be involved in salinity stress tolerance and are thus potentially useful in marker-assisted *Salicornia* breeding. Gene diversity increased with an increase in number of alleles having low major allele frequency at a locus and vice versa. Molecular data explained the high gene diversity among the 46 *Salicornia* taxa; however, it did not support the morphological classification of 46 *Salicornia* taxa found at Chinese coast of the Yellow Sea into two populations but rather explained the existence of the three populations.

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