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ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2011) **9**(2); 243–246
doi:10.1017/S147926211100030X

Comparison of genomic and EST-derived SSR markers in phylogenetic analysis of wheat

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Abstract

Microsatellite markers (simple sequence repeats, SSRs) are used for a wide range of crop genetic and breeding applications, including genetic diversity assessment, phylogenetic analysis, genotypic profiling and marker-assisted selection. Genomic SSR (gSSR) have attracted more attention because of abundance in plant genome, reproducibility, high level of polymorphism and codominant inheritance. Recently, the availability of data for expressed sequence tags (EST), has given more emphasis to EST-derived SSRs, which belong to the transcribed regions of DNA, and are expected to be more conserved and have a higher transferability rate across species than gSSR markers. In the present study, several gSSR and EST-SSR markers were investigated for their transferability and level of DNA polymorphism in different ancestral tetraploid and diploid *Triticum* and *Aegilops* species. The same gSSR and EST-SSR markers were also evaluated for their applicability in the phylogenetic analysis of wheat. Both gSSR and EST-SSR markers showed differences for the average transferability rate and the number of alleles/locus. Phylogenetic trees based on gSSR and EST-SSR markers were in accordance with phylogenetic relations based on cytogenetic and molecular analyses.

Keywords: expressed sequence tags-simple sequence repeats; genomic simple sequence repeats; phylogenetic analysis; transferability; wheat

Introduction

Molecular markers are used for a wide range of purposes in crop genetics and breeding, including genetic linkage and comparative mapping, positional cloning, genetic diversity assessment, phylogenetic analysis, genotypic profiling, quantitative trait loci and marker-assisted selection. In recent years, genomic microsatellites (or simple sequence repeats, gSSR) have attracted more attention because of abundance in plant genome, reproducibility, high level of polymorphism and codominant inheritance (Nicot *et al.*, 2004). The recent wide availability of data for expressed sequence tags (ESTs) increased EST-derived SSRs, which belong to the transcribed regions of DNA and are expected to be more conserved and have a higher rate of transferability across species than genomic SSR markers (Rudd, 2003). The objectives of the present work were to test the transferability and polymorphisms of gSSR and EST-SSR markers in *Triticum* and *Aegilops* species closely related to cultivated wheats, and to test their applicability for wheat phylogenetic analyses. The use of polymorphic SSR markers either for the characterization and evaluation of germplasm or for phylogenetic analysis of wheat was also discussed.

Materials and methods

Twelve species or sub-species were analyzed: *Triticum aestivum* (AABBDD), *T. turgidum* ssp. *durum* (AABB),

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https://doi.org/10.1017/S147926211100030X Published online by Cambridge University Press

T. timopheevii (AAGG); *T. turgidum* ssp. *dicoccoides (*AABB*), T. monococcum*, *T. urartu*, *Aegilops squarrosa*, *A. speltoides* (S), *A. bicornis*, *A. longissima*, *A. sharonensis* and *A. searsii*. A total of 20 genotypes, including ancestral tetraploid and diploid *Triticum* and *Aegilops* species, were finally used to assess SSR applicability for phylogenetic analysis of wheat.

SSR and EST-SSR primer sequences developed by La Rota *et al.* (2005), annealing temperature and expected PCR product size are reported in the web site http://wheat.pw.usda.gov.

DNA amplifications were carried out as described by Gadaleta *et al.* (2009).

Results and discussion

Primer pairs of 79 gSSRs and 61 EST-SSRs were tested in 20 genotypes belonging to the 12 species or subspecies of the *Triticum–Aegilops* complex under the same PCR conditions as originally applied for amplification in wheat.

The 79 gSSR and 61 EST-SSR markers were polymorphic across the 12 species or subspecies of the *Triticum–Aegilops* complex (Fig. 1(a)). Among the 2800 data points (140 SSR × 20 genotypes), *c.* 10% were missing data, true

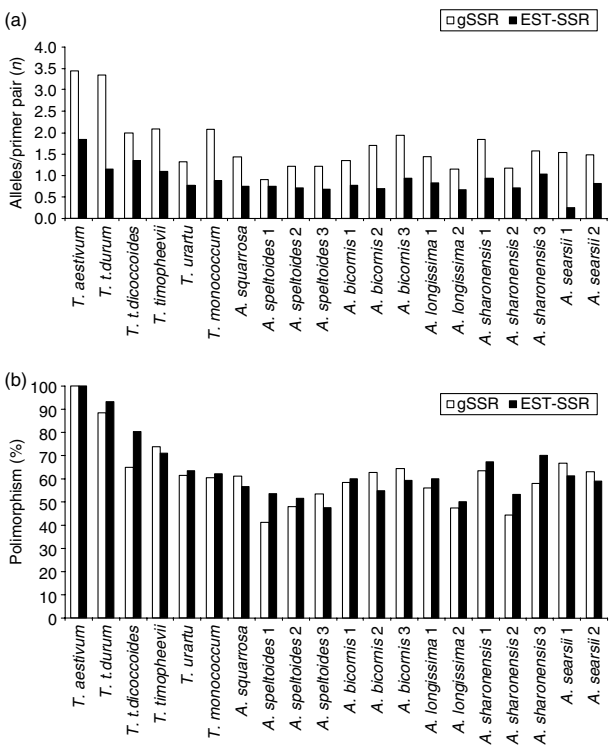

Fig. 1. Average number of alleles/primer pair of wheat gSSR and EST-SSR markers (a) and their transferability in 20 accessions of *Triticum* and *Aegilops* species (b).

null alleles or failed PCR amplifications. The results of DNA amplification from the genotypes of the five *Aegilops* species indicated that the polymorphism was genotype dependent. The mean number of alleles among the species was 1.71 (ranging from 1.90 for *A. speltoides* to 3.43 for *T. aestivum*) for gSSR markers and 1.88 (ranging from 1.26 for *A. searsii* to 1.84 for *T. aestivum*) for EST-SSR markers. Polymorphism was relatively higher in the source species than in related species. As expected, polymorphism of EST-SSR markers was lower than that of gSSR markers, likely because the higher level of conservation of DNA sequences belonging to the transcribed region of the genome, as previously reported (Nicot *et al.*, 2004).

Results of DNA amplification from the genotypes of the *Aegilops* species indicated that the rate of transferability was genotype dependent. Cross-species transferability of EST-SSRs was observed in 670 out of 1115 combinations (60.1%), whereas gSSRs gave amplified products in 845 out of 1407 combinations (60.0%). As expected, transferability was higher in the *Triticum* species with respect to *Aegilops* species (Fig. 1(b)), as also reported by Sourdille *et al.* (2001).

DICE genetic similarity coefficients were used to prepare dendrograms using the UPGMA method.

The dendrograms (Fig. 2) based on gSSR and EST-SSR bands were not significantly different. Each species was separated and the genotypes tested for the *Aegilops* species were always grouped together. Phylogenetic trees were consistent with cytotaxonomical and molecular data on species relationships in the *Triticum–Aegilops* complex. Cluster analysis showed that:

(1) *Triticum species* were separated from *Aegilops* species;
(2) *T. monococcum* was clustered with *T. urartu*, both species having a common A genome;
(3) tetraploid and hexaploid species of *Triticum* formed a close group;
(4) close clustering of *A. bicornis*, *A. longissima* and *A. sharonensis* is consistent with cytotaxonomical data; *A. searsii* was less clustered with the above species;
(5) within the section *Sitopsis*, separation of *A. speltoides* from the remaining four species (*A. bicornis*, *A. longissima*, *A. sharonensis* and *A. searsii*) was consistent with the Eig's classification on morphological traits;
(6) *A. squarrosa* seemed to be closer to the B genome donor species in the EST-SSR dendrogram and to the A genome donor species in the genomic dendrogram.

Thus, we can conclude that wheat EST-SSR markers show a high transferability across a range of

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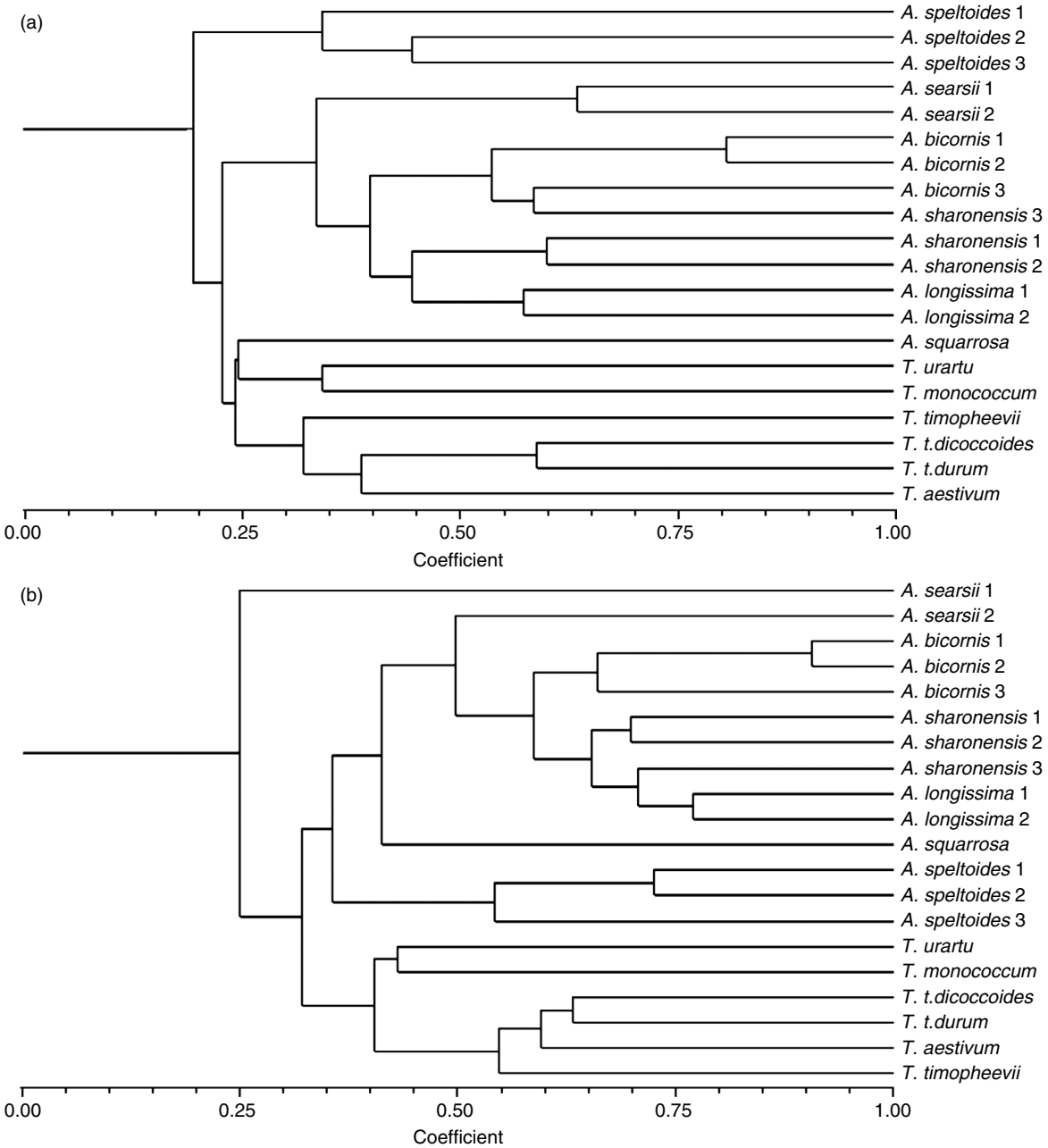

Fig. 2. Dendograms based on (a) genomic SSR and (b) EST-SSR markers.

species. This transferability makes them a powerful tool to work on orphan wild species, where less effort has been devoted to develop genomic resources such as molecular markers. Wild species are an important source of both abiotic and biotic resistances, and molecular markers are precious tools to use and reduce the linkage drag derived from the introgressions of genes (location and size of the introgression) from these species.

Acknowledgements

This research was supported by a grant from Ministero dell'Università e della Ricerca, Italy, project 'AGROGEN'.

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