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BRIEF COMMUNICATION

Population genetic diversity in the polyploid complex of wheatgrasses using isoenzyme and RAPD data

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Abstract

Thirty five bands (alleles) from six enzyme systems and fifty seven random amplified polymorphic DNA (RAPD) fragments were selected to analyse the genetic diversity of 33 polyploid wheatgrasses (*Triticeae*) populations of species *Thinopyrum junceiforme* and *Elytrigia pycnantha*, and two hybrids, one pentaploid and one novel 9-ploid. Dice's similarity coefficient, the UPGMA-derived phenograms from RAPD, and allozymes markers showed that the clustering of wheatgrass populations was based on ploidy level. These markers had similar levels of diversity between populations, with high genetic similarity within the same ploidy-level and within population's individuals. The tetraploid *Th. junceiforme* populations are closely related, with a large similarity distances varied from 0.8 to 1. Based on the isozyme and RAPD analyses, diploid taxa are related to polyploids with similarity coefficients 0.4.

Additional key words: *Elytrigia pycnantha*, isoenzymes, pentaploid hybrid, 9-ploid hybrid, RAPD, *Thinopyrum junceiforme*.

The wheatgrasses (*Triticeae*) including the *Agropyron* group *sensu lato* form a polyploid complex well known for giving rise to phylogenetic and taxonomic problems because of the existence of considerable natural interspecific and intergeneric hybridization and the absence of obvious generic characters (Cauderon 1966, Melderis 1978, Copigny 1988). On the Brittany coastline, the wheatgrasses belong to the tetraploid species *Thinopyrum junceiforme* (Löve and Löve) (2n=4x=28) and to the hexaploid species *Elytrigia pycnantha* (Godr.) Löve (2n=42). However, the widely distributed populations exhibited pentaploid hybrid forms (2n=5x=35). Less abundant and more restricted in occurence, the only 9-ploid form (2n=9x=63) was first collected on the Isle of Houat situated off the coast of Southern Brittany and was identified by genomic *in situ* hybridization (GISH) technique as a new hybrid genotype in the genus *Elytrigia* Desv. (Refoufi *et al*. 2001b).

Random amplified polymorphic DNA (RAPD) markers were widely used in plants to assess the genetic diversity between and within populations of the same species *Coscinium fenestratum* (Narasimhan *et al*. 2006), in different *Mucana pruriens* caltivars (Padmesh *et al*. 2006), or to evaluate a genetic relationship in *Typhonium* species (Rout 2006), in rose cultivars (Mohapatra and Rout 2006). Also, RAPD markers were used to carry out a relationship analyses of diploid perennial species in *Triticeae* (Wei and Wang 1995).

In addition to RAPD markers, we used allozymes to estimate and compare genetic variations within several wheatgrasses populations. RAPD profiling has many advantages over allozyme analysis, including the increased number of polymorphic markers available as well as the fact that the DNA-level variation is revealed, which provides a less biased estimator of genetic variation than allozymes variation. The main dis-advantage of RAPD markers compared with allozyme markers is that the former products are dominant and the latter codominant.

The aim of this study was to assess the genetic diversity of wheatgrass populations at different intra- and inter-specific levels using different molecular markers, isozymes and RAPDs.

Several populations, 13 for *Thinopyrum junceiforme* (Löve and Löve), 5 for *Elytrigia pycnantha* (Godr.) Löve, 14 for the pentaploid and 1 for the 9-ploid hybrids

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Abbreviations: AAT - aminoaspartate transferase; AMY - amylase; EST - esterase; MDH - malate dehydrogenase; PGI -phosphoglucose isomerase; PGM - phosphoglucomutase; RAPD - random amplified polymorphic DNA.
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were collected on coastal sandy sites located at Saint Malo in the North up to the South the Morbihan Gulf in Brittany (France). Some populations were originated from Houat isle. Also, five diploid parent species (2n=14), *Thinopyrum elongatum* (Host) D.R. Dewey, *Th. bessarabicum* (Savul. & Rayss) A. Löve, *Pseudoroegneria stipifolia* (Czern. ex Nevski) Löve, *Agropyron cristatum* (L.) Gaertn and *Critesion californicum* (Covas and Stebbins) Löve were used as a base line for measuring diversity among the polyploidy wheatgrass populations. These species do not occur in Brittany so were kindly provided by J. Jahier (INRA, France) for the first four diploids samples and the latter one by R-C.C. Wang (USDA, USA).

For isozymes extraction, 300 mg of young leaves of plants were ground in 1 cm^3 of cold extraction buffer of Wyatt *et al.* (1992) modified by Corradini *et al*. (1999). Enzyme systems investigated were esterase (EST, E.C.3.1.1.1), aminoaspartate transferase (AAT = GOT, E.C.2.6.1.1), malate dehydrogenase (MDH, E.C.1.1.1.37), phosphoglucose isomerase (PGI, E.C.5.3.1.9), phosphoglucomutase (PGM, E.C.2.7.5.1), glucose-6-phosphate dehydrogenase (G6PDH, E.C.1.1.1.49), and amylase (AMY, E.C.3.2.1.1). The extracts obtained after centrifugation were electrophoresed through discontinuous acrylamide gels following the procedure of Laemmli (1970). A mixing acrylamide bis-acrylamide running gel (pH 8,8) formed by linear gradient ranging from 6 to 10 % was used for GOT and AMY, from 6 to 13 % for PGI, PGM, MDH and EST. A 6 % stacking gel at pH 6,8 was used for all systems. Electrophoresis buffer was Tris-glycine (0.025 M Tris, 0.192 M glycine), pH 8.6. Enzymatic activities were resolved in gels using protocols described by Pasteur *et al.* (1987). For MDH, the protocol was modified, substrates and reactifs were included in 1 % agarose gel in a similar way than PGI and PGM protocols.

Total genomic DNA was extracted from young leaves of plants using a modified Doyle and Doyle (1987) procedure by Ainouche and Bayer (1997). Of the 16 primers screened for polymorphisms, four revealed consistent profiles, OPC-01 (TTCGAGCCAG), OPC-12 (TGTCATCCCC), OPC-13 (AAGCCTCGTC) and OPC-16 (CACACTCCAG). These primers were used to amplify RAPD fragments using a RAPD protocol. After that, amplified DNA fragments were been visualized on vertical acrylamide gels (7 %).

Ten individuals for each polyploid population (330 individuals in total) were screened for protein electrophoresis and RAPD profiles. The natural wheatgrasses populations studied are polyploid and related to alloploid taxa and hybrids. Thus, the gene frequencies for enzymatic data cannot be estimated. For each individual, the electrophoretic profiles for both enzymatic and RAPD markers, corresponding to reproducible band states, were characterized by the presence/absence of bands and the data scores coded as +/-. Each individual was definied by a set of variable series (bands being either present or absent) associated to phenotypic profiles for each markers. The data were represented as a binary matrix (1/0), with the individuals on lines and the analytical data on columns. This matrix was transformed to a genetic similarity matrix between individuals. The similarity between individuals was estimated with Nei and Li's (1979) genetic distance (S) using the Dice coefficient of similarity (D).

The unweighted pair group method with arithmetic average (UPGMA) procedure of Sneath and Sokal (1973) was used to produce a phenogram illustrating population versus species relationships based on the matrix of Nei and Li's (1979) D values for each RAPD and isozymes data point (Option SIMQUAL for qualitative data). Both types of analysis were phenetic, and the data treatment was performed using the *NTSYS-PC* package version *2.0* (Rholf 1998).

In order to estimate the genetic diversity within populations, the Shannon's index I as defined in Hutcheson (1970) was calculated: $I = - \sum pi \times \ln pi$, where pi is the frequency of the i^{th} isozyme band.

We selected eight of the loci developed from the six enzyme systems. Thirty-five bands or alleles (each band or allele is treated as a character) were counted on the eight loci.

From the cluster analysis, the UPGMA-derived phenogram (Fig. 1) separates the diploid species and the polyploid (33 populations) into 3 main groups. Differenciation is based on ploidy level. The first homogeneous grouping (G1), an outlying group, comprises the five diploid species which exhibits an average simile values close to 0.5 along with the remaining polyploid populations. The second major grouping comprises another dichotomy that separates two main groups (G2 and G3). The G2 group includes all the tetraploid populations of *Th. junceiforme* (T_1 to T_{13}) with an important similarity distance between them ($S \geq 0.8$). The G3 more heterogeneous than G2 group contains the hexaploid *E. pycnantha* populations (E_1, E_4-E_5, E_2 and E_3) with the greater part of pentaploid ones (H_{12}-H_{13}, H_4, H_{11}, H_1, H_7, H_5, H_9, H_{14}, H_6, H_{13}) intercalated in between, plus the 9-ploid hybrid (H9X). The genetic affinity seems to be higher at different ploidy level with a similarity index S ranged from 0.73 to 1 for the pentaploid and $S = 1$ for the hexaploid populations (E_4 and E_5) (Fig. 1). The genetic diversity assessed by the Shannon index I (data not shown) within each population was lower specially for the tetraploid populations (value < 1), more higher for the pentaploid, hexaploid and 9-ploid populations (value ≥ 1).

Based on the observations of RAPD profiles, we were able to score 57 RAPD fragments. As with the isozyme markers, the UPGMA-derived phenogram from RAPD data shows that the clustering of wheatgrass populations is also controlled by ploidy level, thus separating the populations into three groups, but in different combinations (Fig. 2). The first cluster group (G1) includes the diploids taxa, whereas the second one (G2) includes all tetraploid populations of *Th. junceiforme* (T_1, T_{11}, T_5, T_3, T_7, T_6, T_{13}, T_4, T_{10}, T_8-T_9, T_{12} and T_2) as

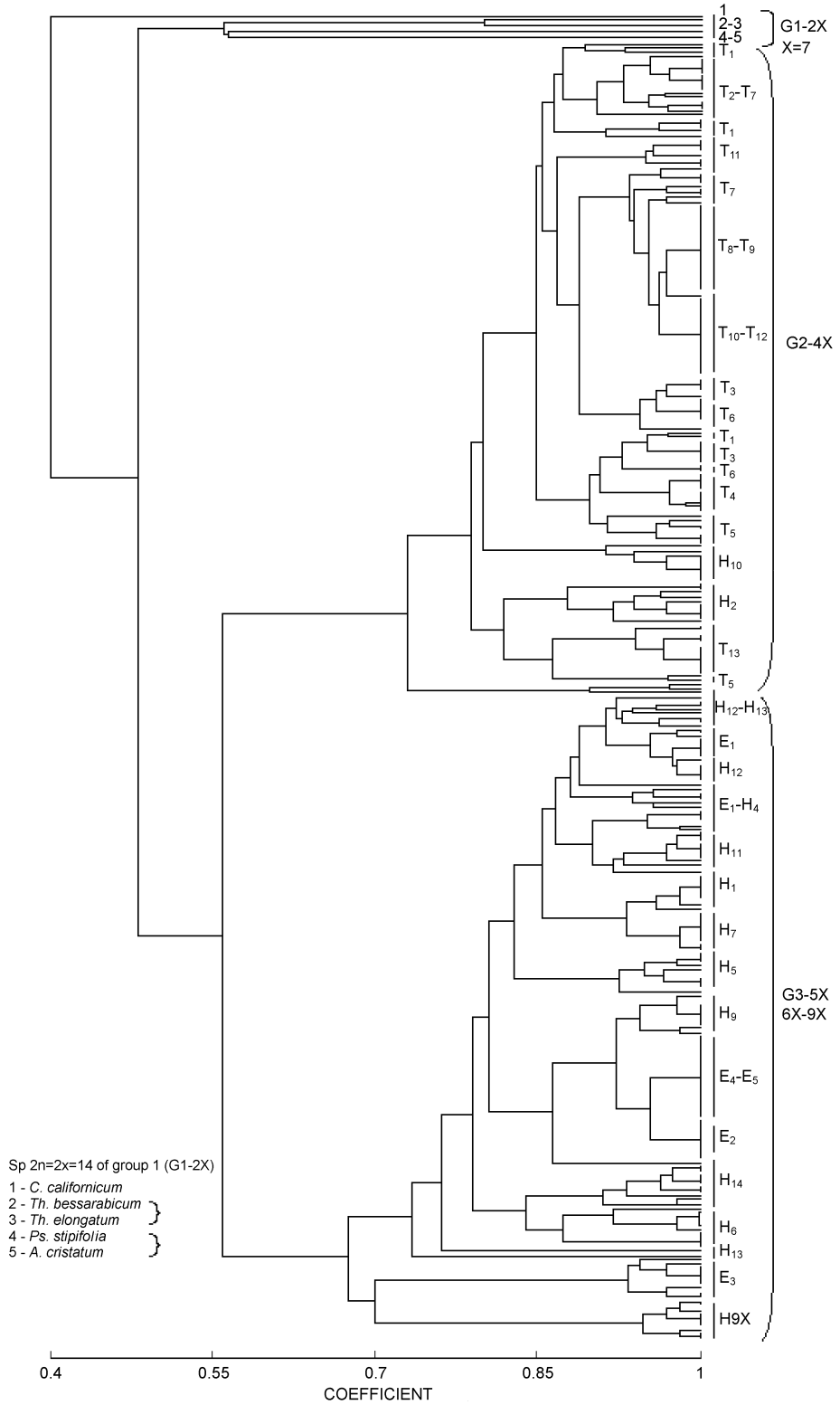

Fig. 1. UPGMA phenogram generated from isozymes data for 335 individuals of diploids and polyploids wheatgrasses analysed. The scale is Dice's genetic similarity coefficient. The diploid species corresponding to the group 1 (G1-2X, X = 7) are: *C. californicum* (denoted as 1), *Th. bessarabicum* (2), *Th. elongatum* (3), *Ps. stipifolia* (4) and *A. cristatum* (5). The group 2 (G2-4X) includes all *Th. junceiforme* tetraploid populations (T_1 to T_{13}), plus 2 pentaploid hybrid populations (H_2 and H_{10}). The group 3 (G3-5X/6X/9X) includes all *E. pycnantha* hexaploid populations (E_1 to E_5), pentaploid hybrid populations (H_1 to H_{14}, none H_2 and H_{10}) plus the 9-ploid hybrid H9X.

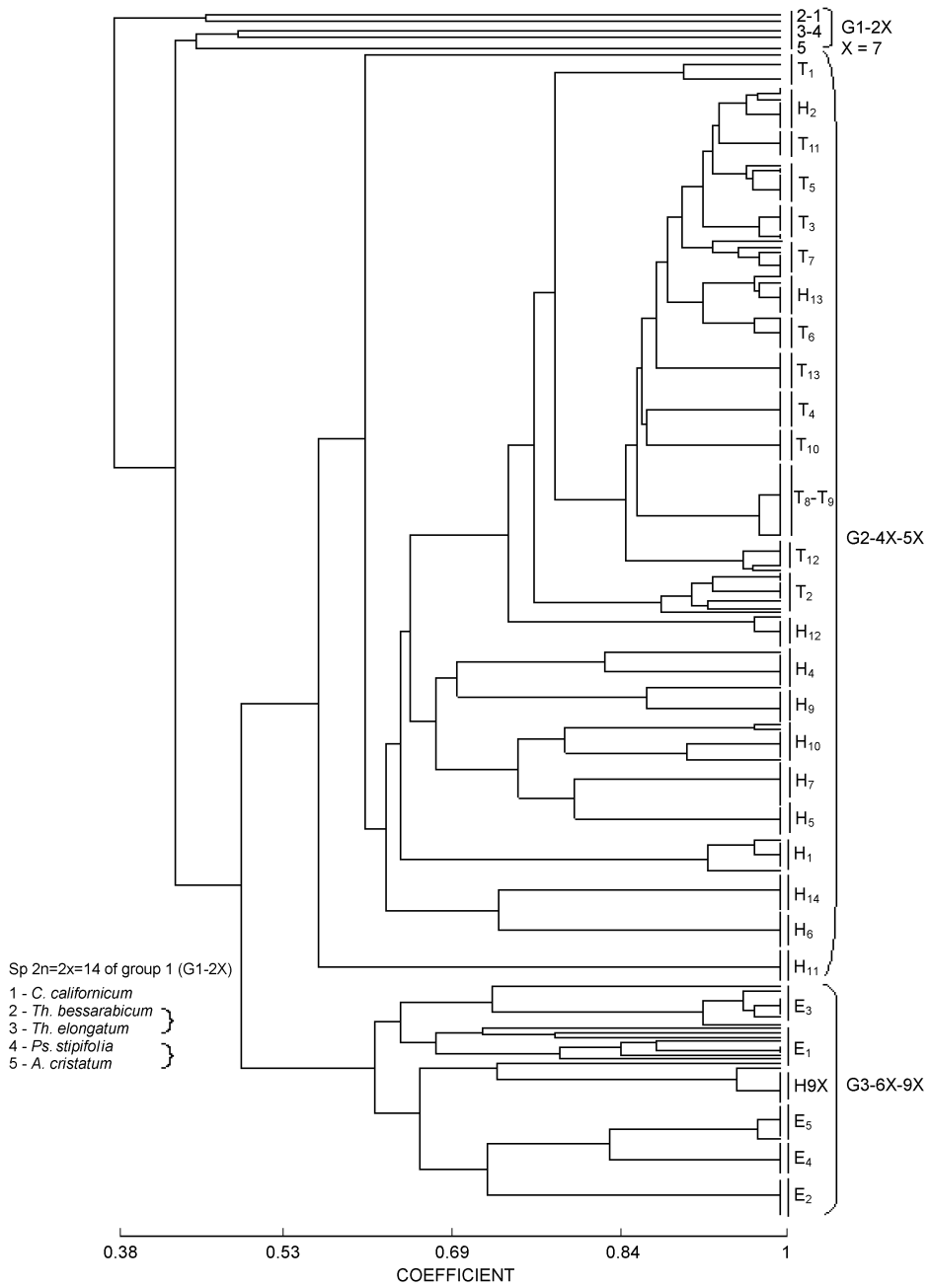

Fig. 2. UPGMA phenogram generated from RAPD data for 335 individuals of diploids and polyploids wheatgrasses analysed. The scale is Dice's genetic similarity coefficient. The diploid species corresponding to the group 1 (G1-2X, X = 7) are : *C. californicum* (1), *Th. bessarabicum* (2), *Th. elongatum* (3), *Ps. stipifolia* (4) and *A. cristatum* (5). The group 2 (G2-4X/5X) includes all *Th. junceiforme* tetraploid populations (T_1 to T_{13}) plus all pentaploid hybrid populations (H_1 to H_{14}) The group 3 (G3-6X/9X) includes all *E. pycnantha* hexaploid populations (E_1 to E_5) plus the 9-ploid hybrid H9X.

well as the pentaploid (H_2, H_{12}, H_4, H_9, H_{10}, H_7, H_5, H_1, H_{14}, H_6, H_{11}) (Fig. 2). They form two subgroups of unequal size, with a discriminant separation situated at a similarity coefficient of 0.65. On the contrary, with enzymatic data, the pentaploids are separated from the tetraploid cluster. The affinity between pentaploid populations is lower than between tetraploids. Between the last ones, the similarity degree is high ($S \geq 0.84$), even more highest within the same population ($S = 1$). The third group's (G3) clusters, includes all the hexaploid populations plus the 9-ploid hybrid. On average, the populations are moderately similar whithin this group, with intra-population similarity coefficient equal to unity, except for the E1 population that exhibits the highest individual diversity.

In the *Triticeae*, genomic evolution as well as phylogenetic relations between several species were assessed using isozymes markers (McIntyre 1988) and

RAPDs markers (Wei and Wang 1995). In the present study, based on the isozyme and RAPD phenograms diploids taxa are related to polyploids with average similarity coefficients near 0.4. However, the analyses show that the RAPD - to a greater extent than isozyme markers - reflect a genetic structure of the population in accordance with the population ploidy level and clearly divergent from diploid group species.

For *Th. junceiforme*, genetic similarity is high both within inter and intra-population's species. This low genetic diversity exhibited may be correlated with the clonal and selfpollinating origin of this species (Dewey 1984). Moreover, this taxa possesses well developed rhizomes which will ensure an enhanced level of asexual reproduction.

The population differentiation was principally caused, on the one hand, by variations in ploidy level and, on the other hand, by species origin. However, both the isozyme and RAPD data reveal an intercalated position of the pentaploid populations related to hybrid form. They are grouped between the tetraploid *Th. junceiforme* and the hexaploid *E. pycnantha* clusters, hence suggesting an intermediate genetic position for the pentaploid form. The hypothesis corroborate our GISH analysis indicating that both species are parents of the pentaploid hybrid (Refoufi *et al*. 2001a). As well as with the 9-ploid hybrid form (H9X) and the hexaploid *E. pycnantha*, the previous GISH data exhibited a close relationships between them (Refoufi *et al*. 2001b). Finally, GISH analysis agree with genetic relationships suggested by phenetic classification and genomic similarity exhibited by isozymes patterns and RAPD markers.

The aim of the present study was to evaluate the potential of isozyme and RAPD markers for assessing the population genetic diversity of Brittany's wheatgrasses. Differences between RAPDs and isozymes are moderate and they exhibit comparable low genetic diversity levels between and within wheatgrasses populations analysed. So, the estimation of genetic variation in such polyploid populations and hybrids could be more efficient if highly polymorphic markers are used like microsatellite polymorphism.

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