Genetic variation of Aegilops cylindrica Host. from Iran, based on RAPD-PCR and HMW glutenin subunits diversity

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ABSTRACT

Genetic variation of 28 populations of Aegilops cylindrica Host., collected from different parts of Iran, were determined with random amplified polymorphic DNA (RAPD) and diversity of high molecular weight (HMW) subunits of glutenin by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method. The diversity within and between populations for the three-band HMW glutenin subunits pattern were extremely low. Out of 15 screened primers of RAPD, 14 primers generated 133 reproducible fragments of which 92 fragments were polymorphic (69%). Genetic similarity calculated from the RAPD data ranged from 0.41 to 0.84. A dendrogram was prepared on the basis of a similarity matrix using the unweighted pair group method with arithmetic averages (UPGMA) algorithm and separated the 28 populations into two groups. Confusion among the population can be happened because of weedy characteristic of A. cylindrica, that as a result the possibility of transportation and cross fertilization between populations across Iran can be high.

Keywords: Aegilops cylindrica, genetic diversity, RAPD, SDS-PAGE

INTRODUCTION

Genetic resources are the gene pool available for breeders and other scientists, and in the Triticeae tribe several pools are recognized (Von Botmer et al., 1992). Aegilops is characterized as a Mediterranean–Western Asiatic element and its center of diversity follows the central part of the Fertile Crescent are in West Asia (Kihara, 1944). A. cylindrica Host. (genome formula: CCDD) is an amphiploid, resulting from hybridization between the diploids A. markgrafii (Greuter) (CC) and A. tauschii Coss. (DD) (Harish et. al.2005). This species (A. cylindrica) is, however, frequent in the Mediterranean area and in the Middle East, which is its center of distribution (Guadagnuolo, et al., 2001, a). It is known to hybridize spontaneously with wheat (Gandilyan & Jaaska , 1980; Snyder et al,. 2000). The chromosomes of the D genome of A. cylindrica and the D genome of T. aestivum are homologous and pair at metaphase I in artificially produced T. aestivum x A. cylindrica hybrids (Kihara, 1944). Naturally formed hybrids between A. cylindrica and wheat occasionally produce seeds, presumably via spontaneous cross-pollination, which could facilitate gene flow between the D genomes of the two species (Gandilyan & Jaaska , 1980; Guadagnuolo et al., 2001, b). A. cylindrica, known as Jointed goatgrass interferes with winter wheat growth and development; thus reducing grain yield and quality (Anderson, 1993). Therefore, understanding the genetic structure of these troublesome weedy populations may facilitate designing new ways to control them, especially when most other conventional methods of weed control have failed to contain their spread (Donald & Ogg, 1991).

Although many landraces of wheats were collected in Iran but genetics of wild wheat and Aegilops from Iran are still largely unknown.. The objective of this study was to understand the extent and pattern of genetic diversity in 28 populations of Jointed goatgrass, collected from roadside of Iran, using HMW glutenin subunits and RAPD markers.

MATERIALS AND METHODS

Plant material

Twenty eight populations of A. cylindrica collected throughout Iran in 2004, including 10 provinces were used in this study.

Storage protein analysis

In order to evaluate genetic diversity of HMW glutenin subunits within and between populations, three samples were taken and analyzed from each of the 28 populations. Chines spring wheat cultivar was used as a standard to compare subunit composition. After removal of the embryo, HMW glutenin subunits were separated from single-grain flour. Proteins were fractionated by SDS-PAGE according to methodology of Laemmli,1970.

RAPD assay

Total genomic DNA was isolated from young leaves of greenhouse-grown plants according to the CTAB protocol (Saghai-Maroof et al., 1984) with minor modifications. To reveal the level of genetic variation for each population, DNA of five plants were bulked and analysed. A total of fifteen decamer oligonucleotides primer from UBC (University of British Colombia)

series were selected according to the number and consistency of amplified fragments.

Data analysis

Polymorphic RAPD fragments were scored as either present (1) or absent (0) across all populations. Only distinct, well-resolved fragments were scored. Binary matrix was used to estimate the genetic similarities between pairs, by employing Dice index (Nie and Li, 1979). These similarity coefficients were used to construct dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.02 (Applied Biostatistics) program (Rohlf, 1998).

RESULTS

Protein analysis

The patterns of HMW glutenin subunits among 28 populations of jointed goatgrass detected that diversity in the three-band HMW glutenin subunits pattern was extremely low. In other words, there was very low diversity within and between studied populations. In a previous study, Wan et al. (2002) identified 3 subunits bands as 1Cx, 1Cy and 1Dy in all accessions of A. cylindrica. In this study, we also found 3 subunits bands for all within and between populations except one sample of AC16 population. In this population, two samples contained the 3 subunits identified by Wan at al. (2002), but one sample showed an extra band at the top of gel (Fig. 1).

RAPD analysis

In the RAPD analysis, 15 decamer oligonucleotides primers were used to assess of the genotypes; 14 of these showed informative polymorphic products resolvable by gel electrophoresis. A total of 133 bands were screened (average of 8.9 bands per primer) among which 92 were polymorphic (69 %). The number of fragments generated per primer varied between 3 and 16. The highest and the lowest number of polymorphic bands per assay unit were 0 (primer OPA-15) and 13 (primer UB-9), respectively.

Estimates of genetic similarity of RAPD based on 92 polymorphic markers between 28 populations of A. cylindrica ranged from 0.41 for AC17/AC24, AC13/AC17 and AC12/AC15 to 0.84 for AC20/AC23 with an average of 0.63. This Value for Chinese winter common wheat and spring common wheats were 0.784 and 0.879, respectively (Sun et al., 1998).

Genetic similarity values were used for cluster analysis through UPGMA, resulting in a dendrogram (Fig. 2). Cluster analysis revealed that populations Ac7 and Ac17 from the Mahabad and Shahrod sites, respectively, were more genetically different from the others (Fig. 3) and the most similar pairs were Ac20 and Ac23.

DISCUSSION

Because A. cylindrica hybridizes spontaneously with wheat, understanding its genetic population structure is fundamental to the full use of the vast A. cylindrica germplasm for wheat breeding (Snyder et al., 2000). In this study, two molecular methods, HMW glutenin subunits and RAPD, were used to investigate the genetic diversity in the 28 populations of A. cylindrica. HMW glutenin subunits analysis showed very low genetic diversity in studied populations, which agrees with isozyme analysis conducted by Watanabe et al. (1994) and Hegde et al. (2002) that revealed little or no variation among accessions of jointed goatgrass. We did find an extra band at top that it can maybe produc by mutation or hybridization A. clingrica with other species of Aegilops.

Compared to HMW glutenin subunits, RAPD markers revealed more polymorphic fragments. This is expected, as proteins markers reflect only variation in the coding parts of the genome, which is by nature more conservative and thus less polymorphic. While RAPDs can detect variation in both coding and non coding sequences, the length of the primers allows the amplification of a large number of fragments with a single primer (Guadagnuolo et al., 2001, a.).

In this study, 92 RAPD loci were used to investigate the genetic diversity in the populations of A. cylindrica. The amount of polymorphism found in our research (69%) was more than reported in previous studies (Okuno et al., 1998; Guadagnuolo et al., 2001, a.; Pester et al., 2003). These differences might be related to the utilization of different A. cylindrica germplasms as well as the use of different primers sequences.

In this research we found that there was little relationship between genetic divergence and geographical origins, so that populations from similar geographical places (AC1 with AC11 or AC27) belonged to separate clusters. Conversely, populations from different geographical conditions (such as AC7 and AC17) were clustered in one part of the dendrogram.

One reason for this trend may be the weedy characteristics of A. cylindrica and its presence as a common weed in wheat fields. The seeds of A. cylindrica are often harvested with wheat grains and transported across a wide geographical area, which provides more opportunity for hybridization among accessions.

REFERENCES

Anderson, R.L. 1993. Jointed goatgrass (Aegilops cylindrica) ecology and interference in winter wheat. Weed Science 41: 388–393.

Donald, W.W., Ogg, A.G. 1991. Biology and control of jointed goatgrass (Aegilops cylindrica), a review. Weed Tech. 5: 3–17.

Gandilyan, P.A., Jaaska, V.E.A. 1980. Stable introgressive hybrid from hybridization between Aegilops cylindrica host and Triticum aestivum L. Genetika 16: 1052–1058.

Guadagnuolo, R., Savova, D., Bianchi, Felber, F. 2001. a. Specific genetic markers for wheat, spelt, and four wild relatives: comparison of isozymes, RAPDs, and wheat microsatellites. Genome 44: 610–621.

Guadagnuolo, R., Savova-Bianchi, D., Felber, F. 2001. b. Gene flow from wheat (Triticum aestivum L.) to jointed goatgrass (Aegilops cylindrica Host.), as revealed by RAPD and microsatellite markers. Theor. Appl. Genet. 103: 1–8.

Harish, T., et al., 2005. Chloroplast and nuclear microsatellite analysis of Aegilops cylindrica. Theor. Appl. Genet, 111:561-572.

Hegde, S.G., Valkoun, J., Waines, J.G. 2002. Genetic Diversity in Wild and Weedy Aegilops, Amblyopyrum, and Secale Species—A Preliminary Survey. Crop Science 42: 608-614.

Kihara, H. 1944. Discovery of the DD-analyser, one of the ancestors of Triticum vulgare (Japanese). Agric. and Hort. (Tokyo) 19: 13–14.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of resistance to disease. Annual Review of Phytopathology 14: 211-245.

Nie, M., Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. **76:** 5269–5273.

Okuno, K., Ebana, K., Voov, B., Yoshida, H. 1998. Genetic divers of central Asian and north Caucasian Aegilops species as revealed by RAPD markers. Gen. Res.Crop Evol. 45: 389-394.

Pester, T.A., Ward, S.M., Fenwick, A.L., Westra, P., Nissen, S.J. 2003. Genetic diversity of jointed goatgrass (Aegilops cylindrica) determined with RAPD and AFLP markers. Weed Science 51: 287-293.

Rohlf, FJ. 1998. ntsys-pc. Numerical taxonomy and multivariate analysis system, version 2.00. Exeter Software, Setauket, NY.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W. 1984. Ribosomal spacer length polymorphism in barley: mendelian inheritance, chromosomal location and population dynamics. Proc Natl. Acad. Sci. USA 83: 1757–1761.

Snyder, J.R., Mallory-smith, C.A., Balter, S., Hansen, J.L., Zemetra, R.S. 2000. Seed production on Triticum aestivum by Aegilops cylindrica hybrids in the field. Weed Science 48: 588–593.

Sun, Q., Ni, Z., Liu, Z., Gao, J., Huang, T. 1998. Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica 99: 205–211.

Von Botmer, R., Seberg O., Jacobsen, N. 1992. Genetic resources in the Triticeae. Hereditas 116: 141-150.

Wan, Y., Wang, D., shewry, P.R., Halford, N.G. 2002. Isolation and characterization of five novel high molecular weight subunit of glutenin genes from Triticum timopheevi and Aegilops cylindrica. Theor. Appl. Genet. 104: 828–839.

Watanabe, N., Mastui, K., Furuta, Y. 1994. Uniformity of the alpha amylase isozymes of Aegilops cylindrica introduced into North America: comparison with ancestral Eurasian accessions. In: Wang, R.R.C., Jensen, K.B., Jaussi, C. (eds), Proc 2nd Intl Triticeae Symp., Logan, UT, June 20-24, pp. 215-218.

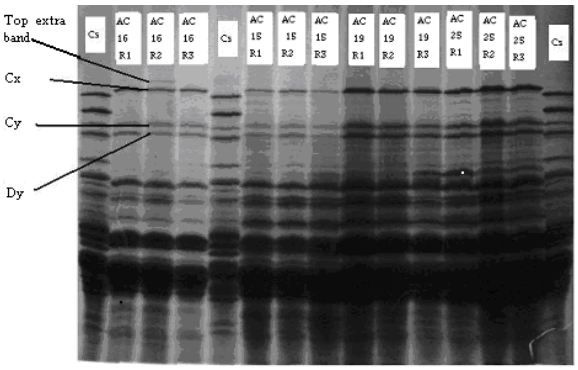

Fig. 1. Example of HMW glutenin subunits among some populations of A. cylindrica. Cs is Chines spring wheat; R1, R2 and R3 are three samples taken from each population.

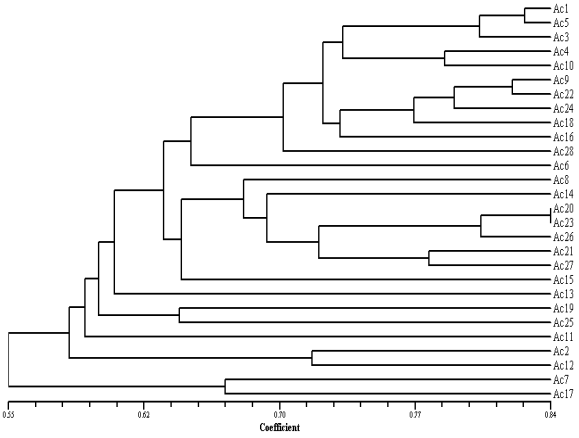

Fig.2. UPGMA dendrogram showing genetic relationships among the 28 populations of *A. cylindrica* used in this study. The dendrogram was constructed based on genetic similarity calculated according to Dice coefficient.