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Population biology and evaluation of genetic resources of *Dasypyrum villosum*

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Dasypyrum villosum (L.) Candargy was known as *Haynaldia villosa* until recently, when the taxonomic priority for the use of *Haynaldia* as a genus for a fungal species was established (Humphries, 1978). *D. villosum* is an allogamous annual diploid species (2n = 14, VV genomes) in the Triticeae tribe of the Poaceae. It is related to a tetraploid perennial species, *D. breviaristatum* (Lindb. f.) Frederiksen, previously known as *D. hordeaceum* (Frederiksen, 1991). This species is believed to be an autoploid, but insufficient evidence has been provided to exclude alloploid origin. The two species have little sympatry, with the tetraploid being found in North Africa and Greece and the diploid more widely distributed in the Mediterranean Basin, both coastal and inland, and in the Black Sea area of West Asia (Frederiksen, 1991).

D. villosum has been the subject of considerable interspecific hybridization studies with cultivated tetraploid and hexaploid wheat and *Secale* and *Aegilops* species. Very little homology of the V genome to the genomes of these species has been revealed by chromosome pairing in F_1 hybrids (Frederiksen, 1991). A 380 bp repeated DNA sequence was identified by De Pace et al. (1992) which did not hybridize to DNAs of several *Triticum* species, including B and D genome relatives, and rye. This confirms the great genetic distance of *D. villosum* from those species.

Despite the genetic differentiation of *D. villosum* from wheat, it is relatively easy to produce doubled amphiploids with both tetraploid and hexaploid wheats, as reported by, for example, by Jan et al. (1986) and De Pace (1987), and disomic addition lines have been produced (Sears, 1953; Blanco et al., 1987) which are stable, further indicating little or no recombination of V and wheat genomes. The *D. villosum*-durum wheat amphiploid is reproductively stable and is a vigorous plant, reminiscent of triticale. It has brittle rachis, as does *D. villosum*. As far as we know, no *D. villosum*-wheat recombinant chromosomes have been produced yet. The *D. villosum*-specific DNA probe mentioned above should be useful in the detection of recombinants by *in situ* hybridizations (De Pace et al., 1992). A method to assign genes to *D. villosum* chromosomes was presented by Zhong and Qualset (1991). In essence, it appears that *D. villosum* may be used as a genetic resource for wheat, although chromosome recombination will require special manipulation.

POTENTIALLY USEFUL TRAITS

Phenotypic evaluations of *D. villosum* have revealed numerous characters that would be useful for wheat. De Pace et al. (1990) reviewed literature which showed that *D. villosum* has resistance to organisms causing powdery mildew and take-all diseases of wheat. *D. villosum* also shows resistance to *Septoria tritici* and barley yellow dwarf virus (BYDV). It grows in water-stressed environments and may be a source of tolerance, although it may be heterogenous in its response (De Pace et al., 1990). Zhong and Qualset (unpubl.) drew similar conclusions for salinity tolerance, based on hydroponic studies. High grain protein concentration has also been reported (Della Gatta et al., 1984; De Pace et al., 1988b). The *D. villosum*-durum amphiploid has good growth characters for a forage crop (De Pace et al., 1990). It should be emphasized that these phenotypic characters may not be expressed in wheat, and their genetic basis in *D. villosum* are generally not known. An exception is the grain storage proteins, which have been studied extensively.

The high-molecular-weight glutenins (*Glu-V1*) are apparently orthologous to the *Glu-A1*, *Glu-B1* and *Glu-D1* loci of wheat in that the subunits have similar molecular weights and are coded by homoeologous group 1 chromosomes (Montebove et al., 1987; Blanco et al., 1991). Gliadin storage proteins were found on *D. villosum* chromosomes 4V and 6V (Montebove et al., 1987). Blanco et al. (1991) and Shewry et al. (1991) confirmed the 4V and 6V locations and also showed that a gliadin locus occurs in chromosome 1V. The loci on 1V and 6V are apparently orthologous to *Gli-1* and *Gli-2* in wheat and are designated *Gli-V1* and *Gli-V2*, respectively. The locus on 4V does not have correspondence to a locus on wheat chromosome group 4. Blanco et al. (1991) and Shewry et al. (1991) hypothesized that this locus (*Gli-V3*) may be the result of a translocation between chromosomes 4V and 6V with subsequent divergence of amino acid sequences in the monomeric proteins. As the storage protein loci have a direct bearing on the rheological properties of flour dough, polymorphism in *D. villosum* at these loci may provide useful alleles for modifying wheat end-use quality.

ISOZYME LOCI

By using wheat-*D. villosum* chromosome addition lines, several enzymatic protein variants have been detected (*see* Table 1). The seven enzymes studied revealed eight loci which could be assigned to all

Table 1 **Enzymatic protein variants detected in the study using wheat-*Dasypyrum villosum* chromosome addition lines**

Locus	Enzyme		Chromosome	Reference
Gpi-V1	GPI-1	glucose-phosphate isomerase	1V	Montebove et al. (1987)
Adh-V1	ADH-1	alcohol dehydrogenase-1	4V	Montebove et al. (1987)
Got-V2	GOT-2	glutamate oxaloacetate transaminase	6V	Montebove et al. (1987)
Got-V3	GOT-3	glutamate oxaloacetate transaminase	3V	De Pace et al. (1988b)
Sod-V2	SOD-2	superoxide dismutase	7V	Montebove et al. (1987)
Est-V1	EST-1	esterase	—	Montebove et al. (1987)
Lpx-2	LPX-2	lipoxygenase	5V	Montebove et al. (1987)
Ndh-1	NDH-1	NADH dehydrogenase-1	4V	De Pace et al. (1988a)
Aadh-2	AADH-2	NAD-dependent aromatic alcohol dehydrogenase-2	6V	De Pace et al. (1988a)

D. villosum chromosomes except 2V. Allelic forms were identified for most loci, as judged by comparisons to the wheat cultivar Chinese Spring, Modoc durum wheat, *D. villosum*, the *D. villosum*-Modoc amphiploid and Chinese Spring-*D. villosum* chromosome addition lines. De Pace (1987) showed that *D. villosum* itself was polymorphic at *Got-2* (2 alleles) and *Est-1* (4 alleles). The isozyme loci may be linked to useful traits, but none has been detected yet. Polymorphism at isozyme loci is useful in studying genetic diversity in and between *D. villosum* populations and for confirming its mating system.

POPULATION BIOLOGY

With respect to its population dynamics and reproductive behaviour, *D. villosum* has not been investigated thoroughly. We have sampled populations along the Adriatic sea coast in Yugoslavia and Italy. Island populations were sampled in the Montenegro area of Yugoslavia and in southern and central Italy. This is only a small part of the range of distribution for *D. villosum* and our results may not be representative of the whole distribution. Certainly, we cannot generalize about genetic differentiation among populations beyond the two areas that we have studied.

Distribution and sampling

D. villosum occurs along roadsides, fence rows and disturbed or uncultivated sites from sea level to about 500 m a.s.l. There are occasional reports of it as a weed in winter wheat or barley fields. For the most part, we observed *D. villosum* as discontinuous populations along the transects which we followed. It is associated with many species of grasses and forbs (De Pace, 1987) and does not appear to be a predominant taxon in these mixed communities. We found one large, rather uniform-appearing stand of *D. villosum* in Italy in an apparently abandoned farmland. This population was sampled and proved to be uniformly early in flowering time compared to other collections, but it was genetically diverse for other characters; it had some characters of a domesticated species. In disturbed-area populations *D. villosum* was easily distinguished as single plants with 3-20 fertile tillers. The spikes were large, with brittle rachis, and the caryopses dimorphic for colour (amber and dark purple or black) on a single spike (De Pace, 1987).

We have used the term 'population' to represent a stand of *D. villosum* at a point where collections were made. These were generally disjointed populations, containing between 10 and several hundred plants. The sampling strategy used was to collect three spikes per plant from at least 10 plants per site. Populations were sampled at roadsides, fence rows, orchards and open areas near the Adriatic Sea. Details of these samples are given in Qualset et al. (1984), De Pace, (1987) and Zhong (1991). In the transects followed, collections were made at irregular points 5-10 km apart.

Single-plant-derived half-sib progenies were studied in laboratory, greenhouse and field experiments at Viterbo, Italy and Davis, California. Qualitative and quantitative traits were studied, as well as isozymes and seed storage proteins, using widely accepted methods. Details are available from research papers and dissertations cited in this chapter.

Mating system

Two enzymes showed allelic diversity in *D. villosum* populations which could be used to estimate outcrossing from half-sib families. The results from *Got-2*, *Got-3* and *Est-1* from six Italian populations

showed a range in outcrossing from 0.509 to 0.999, based on about 10 families per population with 10 plants per family assayed (De Pace, 1987). This gave a mean outcrossing parameter estimate of 0.749, representative of a highly allogamous species. The anthers are large and dehisce after extrusion, and thus outcrossing is favoured. Selfed progenies are easily obtained by pre-anthesis bagging, and the progenies show some indication of inbreeding depression.

Allelic diversity

With the three loci for the two enzymes mentioned above, Shannon information index values calculated on the average gene frequency over all six Italian populations ranged from 0.536 to 0.718. This diversity was distributed as 77% within populations and 23% between populations (De Pace, 1987).

The diversity for the high-molecular-weight (HMW) glutenin locus *Glu-V1* was examined in greater detail than isozyme loci. The HMW glutenin subunits identified migrated into the same region as *Glu-B1* subunits of wheat in SDS-PAGE gels. Fourteen alleles were identified: one null, 10 single subunits, and three with two subunits (Zhong and Qualset, 1993). These alleles were studied in 12 Italian and two Yugoslavian populations; 5-10 alleles (mean = 7) were found in each population, only two alleles were found in one population, and seven were found in 10 or more populations. The weighted allele frequencies ranged from 0.003 to 0.17 over all populations. Nei's (1975) diversity statistics indicated that intra-population diversity for *Glu-V1* ranged from 0.70 to 0.86 (mean = 0.81 over 14 populations). The total diversity estimates in Italy and Yugoslavia were similar (0.88 and 0.82, respectively) as were the partitions of intra-populations (0.82 and 0.78) and inter-populations (0.06 and 0.04). Thus, the pattern of diversity for *Glu-V1* was similar to the results observed for isozymes, but there was less differentiation among populations. There was little divergence of *D. villosum* between Italy and Yugoslavia or between central and southern Italian populations.

Quantitative trait diversity

Most quantitative traits have a multigenic basis and the variation in these traits may be used in wheat breeding. Patterns of variation for multigenic traits may differ from qualitative traits, especially isozyme and storage protein loci for which no direct fitness values have been established. Therefore, sampling and evaluation of populations for multigenic traits is extremely important from the point of view of conservation strategy, species and population fitness, and genetic resources for breeding purposes.

De Pace (1987) and Zhong (1991) studied quantitative trait variation for four and 43 populations, respectively. One of the most important results obtained by De Pace (1987) was that *D. villosum* is polymorphic for growth habit, mainly due to variation in vernalization response. This quantitative trait is controlled mainly by a few loci in wheat, but genetic data have not yet been obtained for *D. villosum*.

Zhong's (1991) field studies included 31 populations from Italy and 12 populations from Yugoslavia, using 8-10 families of 3-5 plants each for each population in two replicates. Six traits were measured on the spikes, three related to flowering and anthesis; in addition, flag leaf length and width and mature plant height were measured. Uni- and multivariate analyses were conducted using data for all traits. Genetic variation was found for all traits. Most interesting was the partition of phenotypic variation. The over-trait mean percentages of variance due to countries, populations, families and plants in families were 38, 28, 9 and 25, respectively, with a mean genetic coefficient of variation of

18%. These results contrast greatly with the protein loci analyses, mainly in that they show greater differentiation between countries and among populations. These results were further analysed by computing Euclidean distances for each pair of populations for all traits. These distances were subjected to the linkage method of hierarchical cluster analysis. Six groups of populations were revealed by the cluster analysis, giving further evidence for genetic divergence in *D. villosum*. One of the clusters (the one with very early flowering time, mentioned earlier) had one population only; Yugoslavian populations formed one cluster; another cluster had one Yugoslavian population and two Italian ones; four Italian populations formed a cluster; and a large group of 22 populations formed a cluster. The results outlined here indicate that geographic genetic differentiation has occurred in *D. villosum*, probably because of natural selection; full results will be published shortly.

EVALUATION AND CONSERVATION

D. villosum is widely dispersed and occurs at sites which are unlikely to be destroyed. Thus, we would suggest that *in situ* conservation is sufficient for these species, but that sampling should be done for research and plant breeding purposes. The enzyme and storage protein results indicate that geographic-based sampling was not very important, whereas the quantitative trait data gave another picture. However, geographic-based sampling is highly desirable in order to identify genotypes with useful traits. The question of quantitative trait allele distribution cannot be resolved by this analysis, but for practical purposes samples of populations in different geographic regions is recommended. Oka's (1975) methods were used to evaluate various sampling strategies. Based on this analysis, as few as five populations with three plants sampled per population and 30 half-sib individuals grown from each family would capture 95% of the total genetic variability in this species. However, more populations would be desirable to extend the geographic distribution range.

While *in situ* conservation is sufficient for the preservation of the genetic variability in this species, the collected samples can be conserved *ex situ* for many years without regeneration. If regeneration is required, we recommend that populations be grown in reproductive isolation if population integrity is to be retained. A large species composite or several regional (or provenance samples in forestry terms) composites of seed would be sufficient for most research and breeding purposes.

Discussion

A. Mujeeb-Kazi: The interpretation of *Dasypyrum villosum* as a genetic resource for *Triticum turgidum*, *T. aestivum* and triticale seems to be based on its hybridization capability. If you were to find another alien source such as *Henrardia*, *Heteranthelium* or *Taeniantherum*, how would you use them in your system?

C.O. Qualset: There are two conceptual bases for considering that a species may be a genetic resource for wheat if the interspecific hybridization cannot be accomplished sexually, by somatic fusion. The first depends on isolation of the gene from the potential donor species, followed by genetic transformation by means of a suitable vector. Unfortunately, genetic transformation cannot be applied routinely at this time. The second possibility is more feasible and has been demonstrated in several taxa, that is, by serial hybridization among species, eventually including wheat as a parent. The problem is identifying the appropriate bridging species.

J.G. Waines: Dasypyrum has a rather curious distribution pattern. It is found over most of the Mediterranean Basin, but does not occur along its eastern shore. How do you explain this?

C.O. Qualset: It has been found in Morocco and along the Black Sea coast. But I have no complete information about the ecogeographic distribution of this species. It would be an interesting problem for a future study.

J. Valkoun: There is a proposal, put forward by Hajichristodolou in Cyprus to use self-regenerating pastures for certain breeding farming systems in the WANA region. Crosses between *Hordeum spontaneum* and *H. vulgare* are being used. Could the amphiploid *T. durum* x *D. villosum* also be used for self-regenerating pastures in high rainfall areas of WANA, such as Cyprus?

C.O. Qualset: This is an interesting idea. The Tritipyrum amphiploid has good forage potential because of high biomass production and, having brittle rachis, could be a self-seeder for rainfed pasture situation. This idea could be tested experimentally in the WANA region. The Modoc x *D. villosum* amphiploid is available from the University of Tuscia, Italy and the University of California, Davis. The performance data as a forage crop was reported by De Pace et al. in *Wheat Genetic Resources: Meeting Diverse Needs*, edited by J.P. Srivastava and A.B. Damania and published by John Wiley & Sons in 1990.

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