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Screening a European *Avena* landrace collection using morphological and molecular markers for quality and resistance breeding

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SUMMARY – The genetic diversity of about 1,000 *Avena sativa* and *A. byzantina* landrace accessions from European genebanks has been studied. Morphological and agronomic characters have been recorded under in diverse environments and controlled disease resistance screenings were performed for crown rust, stem rust and powdery mildew. Only few landraces were found to be immune or very resistant to the above mentioned pathogens. Protein content for all accessions ranged from 9.8% to 19%. A single AFLP primer pair provided enough polymorphic bands to discriminate more than 180 accessions.

Introduction

Different definitions for landraces have been given through time, the most comprehensive being "landrace is a highly diverse population or mixture of genotypes naturally developed in a certain region under the influence of the regionally prevailing conditions of climate, soil and management, without or with only little mass selection". Given modern breeding approaches, it is of great importance to preserve, conserve, characterize and utilize the proper plant material that can provide useful genes in the genetic pool of the cultivated oats. A large number of modern cultivars have been derived from individual selections from landraces or crosses involving these selections. More than 100 cultivars have been developed worldwide from "Red Rustproof" oat landrace.

The main objectives of the project, funded by the EU under Genetic Resources (GENRES CT99-106), were to establish a database containing passport and evaluation data for a large number *Avena sativa* and *A. byzantina* landraces held at the European genebanks. Furthermore, disease resistance screenings, protein content and molecular characterization of the material was accomplished.

Materials and methods

A total of 1023 accessions from 40 different countries all over the world have been included in the present study. Most of these entries were *Avena sativa* and *A. byzantina* landraces, old varieties and few modern cultivars that were acquired from the European genebank collections. The entries were evaluated in four different countries with diverse environmental conditions (Aberystwyth, United Kingdom-IGER; Alnarp, Sweden-NGB; Braunschweig, Germany-BAZ; Thessaloniki, Greece-AUA),

during 2001-2004, in unreplicated field plots. Twenty-nine primary descriptors (Table 1) have been recorded from at least 30 plants within each entry. The material was also screened for crown rust (*Puccinia coronata*), stem rust (*Puccinia graminis*) and powdery mildew (*Blumeria graminis*) resistance under control environment (greenhouse screenings), and for protein content using a Near Infrared analyzer NIRS System Foss 6500.

Table 1. Primary descriptors used to characterize oat landraces with their corresponding frequencies

| Character | Scoring system and frequencies |
|--|--|
| 1 Growth habit | 3 erect (52%) – 5 semi prostrate (32%) – 7 prostrate (16%) |
| 2 Plant height | mean 123.7 cm, min. 40.3 cm, max. 211.5 cm |
| 3 Stem thickness | 3 thin (24%) – 5 intermediate (61%) – 7 thick (15%) |
| 4 Node hairiness | 0 glabrous (70%) – 3 slightly hairy (18%) 5 moderately hairy (8%) – 7 highly pubescent (4%) |
| 5 Angle of flag leaf to culm | 3 acute <90° (69%) – 5 intermediate about 90° (10%) – 7 obtuse >90° (21%) |
| 6 Rigidity of flag leaf | 3 bent (30%) – 5 slightly bent (43%) – 7 stiff (27%) |
| 7 Angle to culm of leaves | 3 acute <90° (66%) – 5 intermediate about 90° (7%) – 7 obtuse >90° (27%) |
| 8 Rigidity of leaves | 3 bent (31%) – 5 slightly bent (47%) – 7 stiff (22%) |
| 9 Hairiness of leaf margin | 0 glabrous (81%) – 3 slightly hairy (10%) 5 moderately hairy (7%) – 7 highly pubescent (2%) |
| 10 Shape of panicle | 1 unilateral (8%) – 2 equilateral (92%) |
| 11 Erectness of panicle | 3 drooping (6%) – 5 semi-erect (19%) – 7 erect (75%) |
| 12 Angle of panicle branches to main axis | 1 extremely low (16%) – 3 acute (36%) 5 intermediate (42%) – 7 obtuse (6%) |
| 13 Erectness of spikelet | 3 drooping (83%) – 5 semi-erect (10%) – 7 erect (7%) |
| 14 Waxiness of the panicle | 0 absent (39%) – + present (61%) |
| 15 Lemma color | 1 white (27%) – 2 yellow (32%) – 3 grey (12%) 4 red (6%) – 5 black (7%) – 6 other (16%) |
| 16 Hairiness of lemma | 0 glabrous (87%) – 3 slightly hairy (10%) 5 moderately hairy (2%) – 7 highly pubescent (1%) |
| 17 Kernel covering | 0 grains naked (3%) – + grains covered (97%) |
| 18 Awedness (basal floret) | 0 no awns (53%) – 3 weak awns (23%) – 5 strong awns (24%) |
| 19 Hairiness at basal part of primary grain | 0 glabrous (61%) – 3 slightly hairy (13%) 5 moderately hairy (20%) – 7 highly pubescent (6%) |
| 20 Length of second leaf from top | mean 33.3 cm, min. 11.0 cm, max. 58.0 cm |
| 21 Width of second leaf from top | mean 1.7 cm, min. 0.5 cm, max. 3.5 cm |
| 22 Number of tillers | 3 few (17%) – 5 intermediate (63%) – 7 many (20%) |
| 23 Lodging at immature stage | 0 upright (65%) – 3 minor lodging (24.5%) – 5 intermediate (8%) 7 lodged (2%) – 9 extremely lodged (0.5%) |
| 24 Lodging at mature stage | 0 upright (24%) – 3 minor lodging (18%) – 5 intermediate (25%) 7 lodged (22%) – 9 extremely lodged (11%) |
| 25 Days to heading | Counted as days from sowing to 50% of panicles fully emerged |
| 26 Days to harvest | Counted as days from sowing to harvest |
| 27 Relation between maturity of grains and straw | 3 grains ripe before straw (40%) – 5 simultaneously (57%) 7 straw ripe before grain (3%) |
| 28 Number of grains in spikelet | An average of 5 spikelets |
| 29 Shedding at maturity | 3 low (80%) – 5 intermediate (13%) – 7 high (7%) |

Molecular characterization and genetic relationships among the entries was done by Amplified Fragment Length Polymorphism (AFLP) using a single primer pair. The PCR products were then

separated in the Genetic Analyzer ABI 310. Scoring of polymorphic bands was done automatically using suitable software.

Results and discussion

The landraces and the old varieties are rarely cultivated nowadays in developed countries, except in some remote areas. However, they can be a valuable source of alleles, especially for oat breeding programs due to the crossability difficulties faced during interspecific crossing. Landraces and old varieties are usually named after the regions where they were cultivated and/or collected ('Novisad 2', 'Saint Nizier 2' etc), the presence of unique distinguishable characters ('Multiflore', 'Extra Prolifique Blanche' etc) or named by the farmer that made some selection ('Potato' –named after the potato field that was initially collected from). A large number of the entries acquired from the genebanks were not named (45 entries) or they were called 'Local' (48 entries), 'Landsorte' (20 entries) or 'Landhafer' (3 entries). In some cases entries coming from different genebanks, having different accession number, had the same name, i.e. 'Yielder' originating from the U.K. had accession numbers GBR005 01274 (UK genebank) and POL003 50227 (Polish genebank), revealing duplications amongst genebanks. At least 46 entries were present more than once under the same name and having different accession numbers either in different genebanks or even within a single genebank.

All primary descriptors were recorded in the four locations and their frequencies are presented in Table 1. In most cases, primary descriptors for each entry appeared uniform under a single environment but different scores were obtained between the four testing sites within a single entry (Table 2). This phenomenon can be related to the phenotypic plasticity of any given trait, indicating that any trait which has a genetic basis and which may or may not be adaptive can alter its phenotype and can evolve in response to selection depending on the scale of spatial or temporal heterogeneity (Callahan, 2005). Furthermore, it is known that oat landraces are mixtures of homozygous genotypes with certain external uniformity, that can alter their genetic composition (percentage-wise) under different cultivation practices and environmental changes (Zeven, 1998). In 1847 the awnless oat landrace 'Cumberland' was reported to have a tendency to degenerate and to 'acquire' an awn when grown from farm-saved seed on the same ground (Zeven, 1999). However, some of the traits, such as 'shape of panicle' (descriptor 10) and 'kernel covering' (descriptor 17), scored the same in all four testing sites, indicating a rather constant phenotypic expression. Overall frequencies of the traits recorded also indicate the selection tendencies of farmers and breeders. Thus, erect plants (52%) are favored over semi-prostrate (32%) and prostrate (15%) plants; intermediate stem thickness (61%) is favored over thin and thick stems; equilateral (92%) and erected (75%) panicles are preferred to unilateral (8%) and drooping (6%) or semi-erect (19%) panicles; and, covered kernels (97%) over naked oats (3%), to name a few (Table 1). Most of these selection tendencies have evolved through centuries of practical experience. For example, erect plants can be planted in dense pattern and have better air circulation among them, reducing the chance of fungal disease spreads etc.

Table 2. Primary descriptor scores for landrace 'Fahnenhaut' under four different environments

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|------|---|-------|---|---|---|---|---|---|---|----|----|----|----|----|
| AUA | 3 | 170.0 | 7 | 0 | 3 | 7 | 3 | 7 | 0 | 1 | 5 | 1 | 3 | 1 |
| BAZ | 5 | 86.4 | - | 0 | 3 | 5 | 3 | 5 | 0 | 1 | 7 | 1 | 3 | 0 |
| IGER | 3 | 165.0 | 7 | 0 | 7 | 3 | 3 | 3 | 0 | 1 | 7 | 1 | 3 | 1 |
| NGB | 3 | 145.0 | 3 | 0 | 3 | 7 | 7 | 5 | 0 | 1 | 5 | 3 | 5 | 1 |

| | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
|---|----|----|----|----|------|-----|----|----|----|-----|-----|----|-----|----|----|
| 2 | 0 | 1 | 0 | 3 | 39.7 | 2.8 | 7 | 0 | 5 | 169 | 209 | 7 | 2 | 3 | |
| 1 | - | 1 | 5 | - | 26.6 | 1.6 | 5 | 3 | - | 66 | - | . | 2 | 7 | |
| 2 | 0 | 1 | 0 | 0 | 41.6 | 1.7 | 5 | 0 | 5 | 128 | - | 3 | 1.8 | 3 | |
| 2 | 0 | 1 | 0 | 0 | 30.0 | 1.8 | 5 | 3 | 3 | 50 | 119 | 5 | 2 | 3 | |

Controlled disease resistance screenings were performed using a virulent race from each pathogen; i.e. crown rust, stem rust and powdery mildew. Most of the landraces proved to be susceptible to all diseases investigated (Table 3). Only 2 landraces were immune to stem rust and 3 to crown rust, while few more were scored as very resistant (2 for stem rust, 20 for crown rust and 1 for powdery mildew). It can be concluded from the disease screenings that landraces are not a good source for resistance genes and methods for transferring genes from diploid or tetraploid species with ease have to be developed. Reaction to the presence of natural infestations during the cultivation period was also recorded. In general, more landraces were scored as resistant ones compared to the artificial inoculations.

Protein content analysis was performed using seeds planted within a single site. The average protein content of the landraces tested was 14% with a minimum of 9.8% and a maximum of 19%. Thus, landraces can be used in conventional breeding programs in order to increase protein content. Finally, a single AFLP primer pair produced more than 295 polymorphic bands, separating about 180 entries. These entries were clustered according geographical origin.

Table 3. Overall resistance scorings of landraces to controlled screenings

| Reaction | Stem rust | Crown rust | Powdery mildew |
|------------------------|-----------|------------|----------------|
| Immune | 2 | 3 | 0 |
| Very resistant | 2 | 20 | 1 |
| Resistant | 6 | 9 | 3 |
| Moderately resistant | 24 | 9 | 15 |
| Moderately susceptible | 4 | 17 | 23 |
| Susceptible | 956 | 606 | 931 |
| Total | 994 | 664 | 973 |

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