Assessment of genetic diversity in European emmer wheat populations

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ABSTRACT

The emmer wheat (*Triticum turgidum* L. spp. *dicoccum* Schrank ex Schübler) cultivation has been revaluated in the 1990s for its health characteristics; this has determined an increase in its cultivation. A total of 38 emmer wheat accessions, collected around Europe, were evaluated employing both agro-morphological characteristics and molecular markers. The agronomic traits evaluated were: vernalization response, winter hardiness, date of heading and flowering, lodging, plant height at harvest and resistances against powdery mildew, leaf rust and yellow rust. Quality control was also performed measuring the protein content, gluten quality and quantity; moreover a baking test was carried out. The assessment of genetic variability was carried out at the molecular level utilizing 6 SSR, 6 EST-SSR and 6 ISSR primers for a total of 107 loci. The protein content for both winter and spring emmer was considerably influenced by both environment and genotype, but wet gluten content revealed always high values. Nearly all spring emmer accessions showed resistance to powdery mildew. The molecular analysis showed a great genetic distance between the evaluated material; the expected heterozygosity and the variance between accessions were consistent, indicating an equal distribution of the alleles and the presence of great differences in the analyzed material. The molecular markers employed were capable to discriminate the country of origin, even if some miss-classification was present. Finally, no defined clusters were obtained considering winter versus spring accessions.

INTRODUCTION

Hulled wheats are species in the bridge between cultivated (bread and durum) and wild wheats. Their spikes are not fragile, but they are hulled. Moreover, they are the first domesticated wheat species. There are hulled wheats at all possible ploidy levels: diploid (2x), tetraploid (4x) and hexaploid (6x). At the tetraploid level *Triticum turgidum* L. spp. *dicoccum* Schrank ex Schübler (emmer wheat) is the domesticated form derived from spp. *dicoccoides* (wild emmer wheat), and from it the spp. *durum* (Desf) Husn. (durum wheat) has been originated. Hulled wheat cultivation has decreased drastically during the 1960s due to its low productivity and hulled kernel; but in the 1990s, the increase in interest in natural and organic products has led to a "rediscovery" of this hulled wheat, which has health characteristics associated with high starch-resistant content[1]. Moreover, it has an aptitude to grow at low temperatures, in soils with limited fertility, and utilizes low input techniques. It is also a source of genes for breeding wheat[2,3]. The interest toward *T. dicoccum* has determined an increase of the cultivated areas to about 2000 ha[4,5] and it has contributed to safeguard and to evaluate the available germplasm[6], to select strains among old landraces and to develop new genotypes between interspecific crosses[7]. For these reasons it has become of interest to know the amount of diversity existing both between and within emmer populations. In recent times, as in many other crops, several kinds of molecular markers (RAPD, AFLP, RFLP, ISSR, SSR) have been utilized to assess genetic diversity in emmer wheat accessions[4,5,8,9,10]. Since results obtained by molecular markers might not adequately reflect the pattern of variation related to agronomic traits[11], in the present study, European emmer wheat accessions were evaluated for agro-morphological characteristics and their genetic variations have assessed by EST-SSR, ISSR and SSR molecular markers.

MATERIALS AND METHODS

A total of 38 emmer wheat (*T. dicoccum*) accessions, collected around Europe from: Germany (15 accessions), Austria (2 accessions), Italy (9 accessions), Switzerland (4 accessions), Spain (3 accessions), Slovakia (2 accessions), Kosovo (1 accession) and Israel (1 accession) (Table 1), were evaluated for agro-morphological characters. The crop was sown both in autumn (10–18 October) and in spring (1–18 March). The field trials were carried out at the experimental station of BOKU University, Vienna. Emmer was sown in 2000 in Raasdorf I (RA00)(16° 35′ E, 48° 14′ N), 2001 in Raasdorf I (RA01), and in Glinzendorf (GL01) (16° 39′ E, 48° 15′ N), 2002 in Groß-Enzersdorf (GE02)(16° 33′ E, 48° 11′ N), and 2003 in Groß-Enzersdorf and Raasdorf II (RA03). All field trials were sown as row-column designs with 2 replicates. Plot size was 1.8 and 2.5 m² in 2000 and 2001, 5.0 and 6.1 m² in 2002, and 6.4 m² in 2003. The agronomic traits evaluated were: vernalisation response, winter hardiness, date of heading and flowering, lodging, plant height at harvest, and resistances against powdery mildew (*Erysiphe graminis*), leaf rust (*Puccinia recondita*), and yellow rust (*Puccinia striiformis*). Quality assessments were also carried out by measuring the protein content, gluten quality and quantity, and a baking test. The assessment of genetic variability among the 38 accessions were performed at the molecular level utilizing 6 EST-SSRs, which present homology with

genes of know function such as: ABA induced protein, PM-19, alcohol dehydrogenase, ADP/ATP translocator gene and CERI/like 3' gene (Dupw 04, Dupw 23, Dupw 38, Dupw 124, Dupw 167, Dupw 254); 6 ISSRs (811, 841, 847, 848, 856, 857) (University of British Columbia) and 6 SSRs (WMS 372, WMS 328, WMS 294, WMS 92, WMS 95, WMS 319) primers. The presence/absence of bands produced were converted in a 0-1 matrix and utilized in the statistical analyses, which was performed by GDA software, in order to determine Nei[12] genetic distances, heterozigosity, genetic variation and the percent of polymorphism, using a 95% criteria.

Table 1. Plant material

Accessions	Collection site	Accession	Collection site
TRI 628	Prizren, Kosovo	EM 01	Switzerland
Kahler	Germany	EM 10	Switzerland
EM 02	Germany	EM 15	Austria
EM 05	Germany	EM 17	-
EM 09	Germany	TRI 5329	Switzerland
EM 20	Germany	TRI 17251	Schweiz,CH
EM 23	Germany	TRI 11283	SK
TRI 16881	Germany	TRI 11295	Lazy, SK
TRI 1776	Germany	TRI 15125	Latali GE
TRI 1778	Germany	TRI 16444	Capracotta IT
TRI 4747	Germany	TRI 16723	Leonessa, IT
TRI 4753	Germany	TRI 16724	Leonessa, IT
TRI 4755	Germany	TRI 16726	Spoleto,IT
TRI 5127	Germany	TRI 16768	Urbino, IT
TRI 16775	Senigallia, IT	BVAL 212002	Germany
TRI 16796	Verniano,IT	BVAL 212011	Germany
TRI 17753	Garfagnana, IT	TRI 17213	Spain
TRI 16812	Italy	TRI 17700	Spain
TRI 16879	Israel	BVAL 212013	Austria
TRI 16883	Spain		

RESULTS

Agronomic traits

Analysis of variance revealed significant environmental (P<0.0001) and genotypic (P=0.028) effects for yield. Mean yields, obtained in 4 consecutive years but in different locations, for each winter emmer accessions are presented in Table 2. Spearman rank correlation analysis between the environments indicated interaction between genotypes and environments. From all 15 possible combinations between environments only 3 were significant: RA00–RA01 (r_s = 0.67*), RA00–GL01 (r_s = 0.88***), and RA01–GL01 (r_s = 0.75*). TRI 16768 and TRI 5127 showed highest yields in the better performing environments, but had below-average yields in the low performing environments. The highest yields were reached in the environments GE03 and RA01, which received also the highest rates of N fertilizer, while the lowest yields were observed in the organic and low N input trials. Despite the increase of yield with increasing N fertilization, a significant leveling off was observed. It is indicating that emmer wheat is not suitable for too high N fertilization rates. Most probably because high N input results in severe lodging, hence in ear breakage and poor grain filling, and, therefore, yield loss. Also the yield of spring emmer accessions analysis had significant environmental (P=0.0005) and genotypic (P=0.0245) effects. Mean yields of the individual accessions in the tested environments are given in Table 2. Highest yields in almost all environments were recorded for BVAL 212002, ranging from 285.56 to 485.15 g m^{-2}. Generally, the average yields of spring emmer wheat were found to be lower than those of winter emmer. The main reason is most probably drought during juvenile phase in case of insufficient rainfall in spring.

Table 2. Mean yield of hulled grains (g m^{-2}) for spring emmer (a) and winter emmer (b) accessions in the respective environments.

Accession	2000	2001	2002	2003	Mean
TRI 628	164.94	197.81	-	250.73	217.50
TRI 5329	209.44	208.02	268.29	-	200.47
TRI 11283	178.44	292.52	372.91	334.03	294.48
TRI 11295	-	284.68	362.28	354.02	305.55
TRI 15125	235.39	244.39	271.35	351.92	275.76
TRI 16723	187.94	359.81	360.09	336.80	311.16
TRI 16724	234.17	341.04	322.44	316.33	303.50
TRI 16726	261.06	237.16	432.25	319.02	312.37
TRI 17213	119.67	381.27	-	231.95	257.30
TRI 17251	119.39	191.57	294.03	306.48	227.87
TRI 17700	-	390.06	134.77	237.35	225.95
BV 212002	285.56	334.46	485.15	367.79	368.24
EM 01	203.89	328.64	368.46	327.20	307.05
EM 10	121.94	247.50	350.10	300.42	254.99
EM 15	198.44	149.24	-	327.99	238.23
EM 20	148.89	240.06	179.88	294.02	215.71
EM 23	187.89	276.36	251.69	260.52	244.12
b)					
BV 212011	360.08	247.33	149.65	297.67	263.68
TRI 16768	560.24	644.93	313.90	216.53	433.90
TRI 16775	401.64	652.97	352.49	191.36	399.61
TRI 16796	418.72	554.47	301.64	192.72	352.47
TRI 16881	358.20	342.08	286.33	423.27	352.47
TRI 16883	290.84	299.27	431.72	217.53	309.84
TRI 1776	470.64	398.89	269.56	327.04	366.53
TRI 4747	420.52	606.99	369.47	246.81	410.69
TRI 4755	402.64	352.38	253.85	346.28	338.78
TRI 5127	511.28	626.87	220.87	245.16	401.04

For 1000 kernel weight (TKW) analysis of variance of winter emmer accessions showed highly significant (P<0.0001) environmental and genotypic effects. In contrast to yield, TKW was stable over environments and no serious cross-over interactions were observed. Rank correlation coefficients between the environments were all significant ranging from 0.68 to 0.94. Genotypic mean comparisons clearly identified TRI 16796 and TRI 16768 having the highest TKW (>40 g). No genotypic influence was observed for kernel fraction, however, the environmental effect was highly significant (P<0.0003), with the 2003 trials showing significantly lower kernel fractions (GE03: 68.7%; RA03: 67.4%)

than the trials in 2000 (RA00: 74.3%) and 2002 (GE02: 72.6 %). Analysis of variance for TKW of spring emmer accessions showed highly significant environmental and genotypic effects (P<0.0001). The significantly highest TKW (>40 g) were recorded for TRI 17213 and TRI 17700, showing best performances over all environments. Susceptibility to diseases was only evaluated in case of incidence. Almost all winter emmer accessions showed acceptable to excellent resistance to yellow rust and leaf rust. Only TRI 16768 was highly susceptible to yellow rust during the epidemics in the years 2000 and 2001. Compared to winter emmer the spring sown crops headed in average 10 days later and were about 15–20 cm shorter in plant height. The recorded lodging scores were low to medium-high (1.3–6.3). The protein content of both winter and spring emmer was significantly effected by environment and genotype (P<0.0001). Although rank correlations between the environments were not significant, and, therefore, indicate cross-over interaction, at least two winter emmer genotypes with high and stable protein contents could be identified: TRI 16883 and TRI 4755. Also BVAL 212011 showed high protein contents in all environments except for GL01.

Molecular analysis

The molecular markers revealed a great Nei genetic distance between the analysed accessions. The expected heterozygosity (He=0.41) and the variance between accessions were high (MSP=0.84), indicating a presence of about 70% for the most common allele and the existing of great differences in the analysed material. The molecular analysis showed different pictures if in the analysis were considered only the microsatellites or both kind of markers (SSR and ISSR). The microsatellites were able to discriminate between country of origin, even if some miss-classifications were present. On the other hand, no consistent clusters were obtained considering winter versus spring accessions.
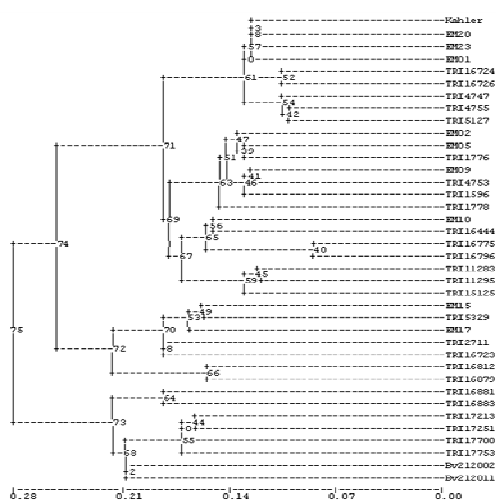

Figure 1. Genetic distance (Nei, 2002) obtained with EST_SSR, ISSR and SSR molecular markers

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