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Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin

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Summary

Glutenin and phenotypic diversity within Syrian landraces composed of *Triticum turgidum* var. *durum* and *T. aestivum* was compared and related to some geographical and climatological characteristics.

Glutenin diversity was determined on the basis of the composition of the glutenin fraction in kernels using Gregorius' level of population differentiation (δ_T) – being a modified Nei's measure of gene diversity (H) – and the Shannon-Weaver variation index (I). These two indices were highly correlated. A total of five Glu-A1 and nine Glu-B1 alleles that were not yet described were found. The phenotypic diversity was determined on the basis of the coefficients of variation of ten phenological and morphological traits.

A highly significant positive correlation coefficient between the glutenin and phenotypic diversity indices was found. Both diversity indices were positively correlated with annual precipitation, minimum January temperature and altitude of the collection site. There was a negative correlation between genetic diversity and the maximum August temperature. Correlation of site characteristics was stronger with the glutenin diversity indices than with the phenotypic diversity index.

Further could be concluded that all common glutenin alleles can be found in the landraces from the western coastal and mountainous part of Syria.

Introduction

Several approaches for the assessment of genetic diversity are available. Sometimes agronomic and morphological data are used for this purpose (Ceccarelli et al., 1987; Konishi, 1987; Tolbert et al., 1979). They are relatively easy to collect, and often useful for other purposes as well. Molecular markers like isozymes and storage proteins (Jana & Pietrzak, 1988; Nevo, 1988; Nevo et al., 1988; Asfaw 1989) reflect the genotype more directly, independent of environmental influences (Brown & Weir, 1983; Gepts, 1989). In this study data of the two categories are compared, viz. phenotypic diversity expressed as coefficients of variation of quantitative traits, and glutenin banding pattern diversity.

In total 185 landrace populations of durum wheat (*Triticum turgidum* var. *durum*) were collected in 1987 and 1988 in the Syrian Arab Republic (van Slageren et al., 1989). This country is part of the center of origin for this species (Zeven & de Wet,

1982). Some of the populations contained admixtures of *T. aestivum*. Based on the available information it is believed that 87 populations originated at or in the vicinity of the collection site. These sites represented a wide range of agro-ecological areas (Elings & Nachit, 1991). The phenotypic diversity of the populations was assessed for 10 quantitative characteristics of single-head progenies (Elings, 1991). Glutenin diversity of the material was determined on the basis of the composition of the glutenin fraction in the kernels. This fraction represents 10% – 40% of the total endosperm storage proteins, and is composed of different subunits, varying in molecular weight from 11,000 to 136,000 Dalton. Here the high molecular weight (HMW) glutenins (molecular weight above 70,000 Dalton) were studied (Levy et al., 1988).

This study was aimed at relating genetic diversity to geographical and climatological characteristics of the collection site, and at relating glutenin allelic composition of the landraces to geographical distribution of durum wheat in Syria. It was done in the context of a programme to improve techniques for sampling genetic variation which might increase efficiency of conservation strategies of genebanks.

Material and methods

The 87 populations which were supposed to have originated locally (Elings & Nachit, 1991) were used for phenotypic diversity assessment and electrophoretic study. Of each population between 26 and 50 (average 46.8) single-head progenies were examined: the composition of high molecular weight (HMW) subunits of the storage protein glutenin was analysed via Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE).

The method described by Payne et al. (1979) was used for protein extraction and protein reduction to subunits. Fractioning was carried out with SDS-PAGE in 8.33% acrylamide. Samples were run on gels of 14 cm for 5–5$^1/_2$ hours at a voltage of 400 volts, and subunits were classified according to the nomenclature proposed by Payne & Lawrence (1983). However, due to the gel concentration, distinction between HMW subunits 2 and 2* was not possible.

The *T. aestivum* varieties Granada, Bastion and Tenor (characterized by subunits 2*, 5 + 10 and 6 + 8, subunits, 1, 2 + 12 and 7 + 9, and subunits 1, 5 + 10 and 20, respectively) were selected as standards. This choice, however, proved to cause difficulty in identification in the zone between subunits 19 and 8.

Taxonomic classification in *T. turgidum* var. *durum* and *T. aestivum*, as based on the genetic interpretation of the glutenin banding pattern conflicted in 224 cases (5.5%) with the morphological field identification used previously by Elings (1991). Chromosome numbers of these lines were determined. The results justified renaming to *T. turgidum* var. *durum* 32 cases and to *T. aestivum* in 42 cases, and led to reinterpretation of a proposed Glu-D1 allele, appearing in 83 cases, as a new Glu-A1 allele. In 164 cases identical banding patterns were interpreted as having different ploidy levels, these were removed from the data set.

Genetic diversity within populations was calculated as the 'level of population differentiation' (δ_T) as described by Gregorius (1987):

$$\delta_T = \frac{N}{N-1} \frac{\sum_j (1 - \sum_i (p_{ij}^2))}{N_j}$$

where

N is the number of single-head progenies in the population,

p_{ij} is the relative frequency of type i of character j, and

N_j is the number of characters.

This is equal to Nei's (1973) measure of gene diversity (H), if frequencies are gene frequencies and population size is infinite. For comparison the Shannon-Weaver variation index (I) was also calculated (Shannon & Weaver, 1949):

$$I = \sum_j \frac{(-\sum_i p_{ij}^2 \log(p_{ij}))}{N_j}$$

where

p_{ij} is the relative frequency of type i of character j, and

N_j is the number of characters.

Most calculations were performed with the GENSTAT statistical package and GWBASIC programs.

Two typifications, established on the basis of banding patterns and alleles respectively, were analyzed. First electrophoretic patterns were considered: objects were considered identical if glutenin banding patterns were the same. Secondly, the genetic background of each banding pattern was described and used in the assessment of diversity. Since durum wheat is a tetraploid species, a single-head progeny could be characterized by two genes each with a distinct set of alleles. The hexaploid *T. aestivum* lines, which were not excluded from the analysis, had one more gene on the third genome, but since this gene appeared to be fixed this genome was not considered.

At Tel Hadya in Syria, Elings (1991) scored the same populations for 10 phenological and morphological traits, viz. days to heading, flag leaf length and width, plant height, awn and spike length, number of spikelets per spike, quantified awn and spike color, and degree of seed shrivelling. Coefficients of variation per trait per population were calculated from the original evaluation results. These coefficients of variation were then linearly rescaled through division by the maximum value per trait, making the highest occurring value for each characteristic equal to 1.0. Per population, rescaled coefficients of variation were averaged over traits, resulting in one phenotypic variation score per population.

Results

Nineteen HMW subunits were detected, of which 12 had been previously described by Payne and Lawrence (1983), viz. HMW subunits 1, 2 or 2*, 5, 6, 7, 8, 9, 10, 12, 13, 19 and 20. The 19 HMW subunits combined into 48 distinct combinations. After elimination of HMW subunits that corresponded to known alleles, the remaining subunits could be explained by assuming five new Glu-A1 and nine new Glu-B1 alleles.

Eight hybrids in as many populations were identified, which all had two Glu-B1 alleles, in populations where these alleles are common. In addition two glutenin banding patterns caused difficulties; one possessed only the Glu-D1a allele, whereas the other possessed besides a Glu-B1 allele two Glu-A1 alleles, one of which did not appear in the rest of the population. These two cases and the eight hybrid lines were removed from the data set.

The calculated values of Gregorius' level of population (δ_T) for both typifications, banding patterns and alleles, and the index of the phenotypic diversity are presented per population in Table 1.

The correlation coefficient between Gregorius' index (δ_T), based on banding patterns or alleles, and the Shannon-Weaver variation index (I) was very high – more than 0.98 (Table 2). The correlation coefficient between the glutenin diversity index and the phenotypic diversity index was significantly positive at $P < 0.01$.

Populations with highest diversity indices originated from coastal areas and adjacent mountains, the northwestern part of the country, and in the area around Damascus to the southwest. Lowest genetic diversity was found in populations from eastern regions. This geographical distribution has its parallel in environmental site characteristics. Correlation coefficients between the diversity indices and four characteristics of the collection site (Table 3) indicated a significant positive correlation of genetic diversity with annual precipitation, and a nearly equal but negative correlation with maximum August temperature. The correlation coefficient between annual precipitation and maximum August temperature was -0.796. Genetic diversity correlated positively with minimum January temperature and altitude of the collection site. The total amount of variation in glutenin diversity that could be explained by these four site characteristics in a multiple regression analysis (adjusted R^2) was 30.3%, adding only little to the 22.4% explained by maximum August temperature alone.

Correlation coefficients between site characteristics and glutenin diversity indices were much

Table 1. Estimates of the diversity within the populations

Obj	N HP	N DU	N AE	δ_T Band.	δ_T Alle.	CV Phen.	Obj	N HP	N DU	N AE	δ_T Band.	δ_T. Alle.	CV Phen.
ID001	26	24	2	0.578	0.258	0.544	ID236	49	49	0	0.279	0.139	0.225
ID004	49	49	0	0.156	0.080	0.308	ID245	44	43	1	0.285	0.147	0.613
ID025	49	44	5	0.327	0.180	0.582	ID246	48	48	0	0.042	0.021	0.468
ID032	44	43	1	0.172	0.088	0.623	ID247	50	50	0	0.117	0.059	0.407
ID040	49	46	3	0.265	0.155	0.455	ID250	44	39	5	0.402	0.211	0.423
ID042	48	46	2	0.491	0.283	0.546	ID251	42	41	1	0.465	0.215	0.535
ID044	46	46	0	0.000	0.000	0.565	ID255	45	45	0	0.210	0.128	0.439
ID051	49	47	2	0.324	0.165	0.550	ID269	37	36	1	0.467	0.252	0.642
ID052	47	45	2	0.512	0.264	0.429	ID270	39	25	14	0.707	0.416	0.609
ID054	49	46	3	0.361	0.150	0.437	ID272	40	23	17	0.809	0.553	0.539
ID056	32	24	8	0.738	0.423	0.423	ID274	24	21	3	0.692	0.507	0.571
ID058	22	22	0	0.385	0.193	0.397	ID275	46	28	18	0.771	0.396	0.440
ID070	48	47	1	0.160	0.080	0.430	ID276	40	25	15	0.772	0.442	0.544
ID072	48	48	0	0.301	0.156	0.338	ID277	47	33	14	0.681	0.323	0.462
ID076	49	49	0	0.041	0.020	0.417	ID283	31	26	5	0.447	0.182	0.679
ID079	32	0	32	0.063	0.031	0.440	ID286	45	45	0	0.512	0.256	0.570
ID094	48	48	0	0.330	0.306	0.442	ID287	39	22	17	0.742	0.638	0.461
ID095	47	47	0	0.043	0.043	0.518	ID290	49	49	0	0.226	0.223	0.419
ID097	44	44	0	0.133	0.068	0.369	ID300	50	50	0	0.117	0.059	0.433
ID099	42	42	0	0.048	0.024	0.489	ID301	50	50	0	0.274	0.137	0.442
ID100	48	48	0	0.042	0.021	0.516	ID319	49	49	0	0.193	0.099	0.410
ID101	42	42	0	0.094	0.047	0.542	ID329	50	50	0	0.154	0.079	0.397
ID102	48	48	0	0.082	0.041	0.389	ID332	50	50	0	0.118	0.060	0.402
ID103	45	45	0	0.044	0.022	0.408	ID335	50	50	0	0.291	0.152	0.373
ID117	42	42	0	0.139	0.071	0.437	ID336	50	50	0	0.280	0.143	0.412
ID119	34	32	2	0.169	0.085	0.482	ID342	50	50	0	0.000	0.000	0.416
ID127	41	40	1	0.049	0.024	0.447	ID348	49	49	0	0.376	0.198	0.413
ID128	40	40	0	0.596	0.298	0.586	ID351	44	30	14	0.660	0.182	0.680
ID135	39	39	0	0.051	0.026	0.218	ID357	49	49	0	0.352	0.176	0.402
ID136	49	49	0	0.372	0.193	0.481	ID358	49	49	0	0.000	0.000	0.446
ID163	50	50	0	0.040	0.020	0.336	ID362	49	49	0	0.153	0.077	0.473
ID167	33	33	0	0.174	0.087	0.368	ID364	49	49	0	0.194	0.099	0.421
ID172	49	49	0	0.081	0.041	0.411	ID369	50	50	0	0.000	0.000	0.453
ID174	46	46	0	0.086	0.043	0.400	ID381	48	48	0	0.230	0.115	0.401
ID203	42	42	0	0.048	0.024	0.589	ID385	49	49	0	0.000	0.000	0.416
ID205	47	47	0	0.235	0.121	0.359	ID386	48	48	0	0.345	0.193	0.463
ID208	48	46	2	0.762	0.400	0.517	ID389	44	41	3	0.593	0.541	0.582
ID209	42	42	0	0.257	0.128	0.566	ID393	47	47	0	0.509	0.263	0.417
ID218	41	40	1	0.637	0.332	0.508	ID402	49	48	1	0.298	0.225	0.512
ID220	42	42	0	0.180	0.090	0.410	ID406	50	50	0	0.153	0.076	0.461
ID222	50	50	0	0.153	0.077	0.547	ID410	48	48	0	0.566	0.304	0.616
ID224	49	49	0	0.120	0.061	0.404	ID411	49	49	0	0.000	0.000	0.442
ID225	45	45	0	0.206	0.105	0.512	ID412	49	48	1	0.447	0.261	0.496
ID235	42	2	40	0.654	0.438	0.518							

N HP: number of single-head progenies in the population
N DU: number of durum single-head progenies in the population
N AE: number of aestivum single-head progenies in the population
δ_T Band.: level of population differentiation based on banding patterns
δ_T Alle.: level of population differentiation based on Glu alleles
CV Phen.: average normalized phenotypic coefficient of variation

Table 2. Coefficients of the correlation between the diversity indices

	δ_T Band.	δ_T Alle.	I	CV Phen.
δ_T Band.		0.940**	0.982**	0.400**
δ_T Alle.	0.940**		0.989**	0.348**

** Significant at $P < 0.01$

δ_T Band.: level of population differentiation based on banding patterns

δ_T Alle.: level of population differentiation based on Glu alleles

I: Shannon-Weaver index based on corresponding typification

CV Phen.: average normalized phenotypic coefficient of variation

higher than correlation coefficients between site characteristics and phenotypic diversity, both for diversity in individual characteristics and averaged coefficient of variation.

When only the durum lines in the landraces were considered, correlations between diversity indices and collection site characteristics were found to be in the same magnitude (Table 3).

Four types of geographic distribution of alleles were distinguished: alleles that are common and widely distributed (six alleles), alleles that are common in a restricted area (seven alleles), alleles that appear in two seemingly random populations (three alleles) and alleles that are very rare and appear in only one population (six alleles). Alleles common in only one restricted part of the country always appeared in the western mountainous areas.

Discussion and conclusions

Little difference was detected between the 'level of population differentiation' (δ_T) as described by Gregorius (1987), which is a modified Nei's (1973) measure of gene diversity (H), and the Shannon-Weaver variation index (I) (Table 2). This was expected under these circumstances of relatively many populations and the absence of fractions smaller than 0.02 (Hennink & Zeven, 1991). Interpretation of the glutenin banding patterns in the respective alleles did not improve accuracy (Table 2), possibly due to an extra error introduced with interpretation.

The low but highly significant positive correlation coefficients between the glutenin and phenotypic diversity indices (Table 2) suggest that both measure genetic diversity, although it is obvious

Table 3. Coefficients of the correlation between estimates of diversity within the populations and geographical and climatological characteristics of the collection sites

	Altitude	Precipi.	T_{august}	$T_{january}$
Entire population				
δ_T Band.	0.259*	0.408**	−0.480**	0.249*
δ_T Alle.	0.193	0.482**	−0.483**	0.319**
CV Phen.	0.065	0.222*	−0.255*	0.138
Durum fractions only				
δ_T Band.	0.275*	0.322**	−0.417**	0.120
δ_T Alle.	0.223*	0.420**	−0.457**	0.231*
CV Phen.	0.055	0.225*	−0.227*	0.106

* Significant at $P < 0.05$

** Significant at $P < 0.01$

δ_T Band.: level of population differentiation based on banding patterns

δ_T Alle.: level of population differentiation based on Glu alleles

CV Phen.: average normalized phenotypic coefficient of variation

Altitude: altitude at the collection site

Precipi.: annual precipitation at the collection site

T_{august}: maximum August temperature

$T_{january}$: minimum January temperature

that the genetic base of each index is different. The lower correlation of geographical and climatological site characteristics with the phenotypic diversity index as compared with the glutenin indices (Table 3) can probably be explained by an error in the phenotypic scores. Possible causes are genotype × environment interaction and environmental heterogeneity during evaluation at Tel Hadya, increasing the error in the phenotypic diversity index.

The glutenin diversity index showed higher correlations with site characteristics as compared to the phenotypic diversity index. Glutenin diversity is also much easier to determine, no expensive field evaluations are needed and the results are reproducible and independent of environment. So, although only few loci on few chromosomes are assessed, glutenin diversity seems most suitable for diversity studies. This is of particular interest to germplasm curators, who aim to optimize diversity of collections. It could also be of use to breeders, since the glutenin diversity indices, apart from quantifying diversity of a character related to bread-making quality (Payne et al., 1979), also correlate with diversity of other agronomical important traits, as was shown by the correlation with diversity of phenotypic traits.

The positive correlations of diversity with annual precipitation and minimum January temperature and the negative correlation with maximum August temperature deserve extra attention (Table 3). In Syrian arid conditions, water shortage and terminal heat are predominant stress factors for grain production. The calculated correlation coefficients suggest that these growth-limiting conditions are negatively related to both phenotypic and glutenin diversity. This corresponds with the observation of Elings (1991) that populations collected in non-mountainous desert areas with low winter temperature and high summer temperature are relatively homogeneous morphologically. The western areas, showing the highest degree of genetic variability, are less arid and have a less extreme temperature regime. Although August is outside the growing season of wheat in Syria, the maximum August temperature can clearly serve as a measure for terminal heat, shortening the growing season.

This correlation of glutenin diversity with environmental parameters supports the findings of Nevo (1988). However care should be taken in attributing these correlations solely to adaptiveness of seed storage protein polymorphism: these correlations can also be attributed to several other causes (Gepts, 1989), like stochastic processes (e.g. migration or genetic drift) or linkage between the glutenin loci and actual adaptive loci.

The rare alleles, each only occurring in one or two apparently random populations can be considered to be mutants, or the results of other coincidental events. Alleles common in a restricted area occur always in the western coastal and mountainous part of Syria. This could indicate that genetic material is introduced into the eastern part of the Mediterranean basin from other regions, from where alleles are disseminated further. Migration of landraces into new regions, followed by some degree of contamination by or mixture with other landraces can be expected in a country like Syria, where germplasm movement is only limited to some extent by agro-ecological boundaries. Alleles, as was shown with most alleles studied, will thus not be fixed to one area but will spread over a wide area.

Under circumstances like the Syrian, only agro-ecological boundaries for germplasm movement, most genetic variation can be found in the regions with the most favourable growing conditions. The areas with a high level of stress will present interesting, tolerant to stress, but homogeneous genotypes, and need therefore less extensive sampling for genetic resources conservation purposes.

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