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Genetic resources of wild emmer wheat revisited: genetic evolution, conservation and utilization

E. Nevo
Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31999, Israel

The multidisciplinary studies conducted during 1984-1987 at the Institute of Evolution, University of Haifa, of wild emmer wheat, *Triticum turgidum* var. *dicoccoides*, the progenitor of cultivated wheats, are reviewed. The following aspects are discussed: (i) Population genetics and ecology at the micro- and macrogeographical scales in Israel and Turkey; (ii) Genetic resources of disease resistance; (iii) Wheat storage proteins: (a) protein content; (b) diversity of HMW glutenin subunits (c) rDNA diversity; (iv) Plant genetic resources: Predictability by isozyme markers and ecology. It is concluded that the rich genetic diversity of wild emmer for multiple disease resistances, elite agronomic traits and environmental adaptations is geographically structured and predictable by ecology, allozyme and DNA markers. Consequently, sampling strategies in nature could be optimized by following ecological and genetic factors as effectively predictive guidelines for conservation and utilization in wheat improvement.

Genetic diversity in nature is the basis of evolutionary change and of domestication. Natural populations harbour rich genetic diversity (reviewed in Nevo et al 1984) which is ecogeographically structured and largely adaptive, locally, regionally and globally (Nevo 1988). Consequently, the conservation and utilization of genetic diversity should be used in crop improvement, not only as genetic markers but, also, for direct amelioration of cultivars. This is crucial in view of the generally higher genetic homogeneity of cultivars, than their ancestors (Brown and Clegg 1983), due to modern pure line breeding, making them increasingly susceptible to pathogens, pests and environmental stresses (Plucknett et al 1983).

The best hope for future crop improvement lies in rationally exploring and exploiting the rich gene pools of the plant's wild relatives (e.g. Zohary 1970, Harlan 1976, Feldman 1979, Feldman & Sears 1981, Nevo et al 1982, Nevo 1983). Crossing techniques are available and success has already been achieved in transferring wild to cultivated gene pools for wheat improvement (reviewed in Grama et al 1983). This becomes particularly promising through the transference of particular genes of interest, from the ancestors, to their cultivars (Hohn & Dennis 1985).

The Institute of Evolution at the University of Haifa started, in 1975, a multidisciplinary research programme of wild cereals, including wild emmer, *Triticum dicoccoides* (*Triticum turgidum* var. *dicoccoides* in Kimber & Feldman 1987). The 1979-1983 studies of wild emmer involved the genetic analysis of 12 Israeli populations (Nevo et al 1982); phenotypic and genotypic agronomic traits, and disease resistance to mildew and several rusts (reviewed in Nevo 1983). Here, I review our wild emmer wheat studies conducted during 1983-1987, i.e., between the 6th and 7th International Wheat Genetics Symposia, and outline our future research programmes.

Wild emmer wheat, *T. dicoccoides*, genomic constitution AB, is the tetraploid wild progenitor from which all wheats originated

(Zohary 1970, Kimber & Feldman 1987). It hybridises with cultivated tetraploid wheats, and gene transfer from wild to hexaploid cultivated wheats is possible through a partially fertile, pentaploid bridge (Grama & Gerechter-Amitai 1974). Wild emmer is distributed over the Near East Fertile Crescent (Harlan & Zohary 1966) but its centre of distribution is in the catchment area of the Upper Jordan in Israel. For its ecology see Nevo et al (1982).

RESULTS AND DISCUSSION

1. Population genetics and ecology: micro- and macrogeographical scales:

A. Microgeographical differentiation

Microgeographic allozymic differentiation can take place over short distances, despite considerable gene flow, and is therefore adaptive at the morphological, physiological (Bradshaw 1972) and allozymic (Nevo et al 1977, 1981, 1983, 1986) (reviewed in Brown 1979) levels. Little is known about the affecting factors of local environmental heterogeneity, either physically or biotically (e.g. in barnacles, Nevo et al 1977). Our studies in microspatial differentiation of *T. dicoccoides* involved spatial factors of soil type, yearly and slope differences, as well as microclimatic variation. In these cases, presumably, the aridity index played a major factor in microsite genetic differentiation. We conducted two microsite population genetic studies and Golenberg (1986a) conducted an in-depth study on the multilocus structure and dynamics of wild emmer. I will describe each briefly.

Microspatial genetic differentiation by edaphic, topographical and temporal factors (Nevo et al 1988a) We analysed electrophoretically allozymic variation in proteins, encoded by 47 loci, in 356 individual plants of wild emmer collected in 1983/84 and 1984/85 at Tabigha, north of the Lake of Galilee, Israel. The test involved, in each year, two 100 metre transects, each equally subdivided into basalt and terra rossa soil types. Significant genetic differentiation, genetic phase disequilibria, and genome organization, according to soil type, was found over very short distances. Our results suggest that allozyme polymorphisms in wild emmer are partly adaptive and differentiate at both single and multilocus structures, primarily by ecological factors including soil type, topography and temporal yearly changes probably through aridity stress. The Institute now participates in a multidisciplinary study at the nearby Amiad microsite.

Microspatial genetic differentiation by climatic factors (Nevo et al 1988b) Allozymic variation in proteins, encoded by 49 loci was analysed electrophoretically in 137 individual plants of wild emmer, *T. dicoccoides*, collected in 1984 and 1985, from a microsite in Yehudiyya, northeast of the Lake of Galilee, Israel. The test involved two climatic microniches (i) *sunny* between trees, and (ii) *shady* under tree canopies, in the open Tabor oak forest. Significant genetic differentiation at one-, two- and multilocus structures was found between the neighbouring climatic niches, separated by only a few metres. Our results suggest that allozyme polymorphisms in wild emmer are partly adaptive and differentiate here, primarily at the multilocus level due to climatic factors, presumably related to the aridity stress.

Multilocus structures and dynamics in wild emmer (Golenberg 1986a) Two multilocus genotypes of wild emmer as defined by eight loci, *Mdh*-1, *Ipo1*, *β-Glu*, *Pept*-1, *Pept*-3, *6Pgdh*-2, *Hk* and *Rc* were identified in the area of Yehudiyya Nature Preserve (Nevo et al 1982) and were found to be distributed in a step cline along a short 10km transect (Golenberg & Nevo 1987). This microsite was the focus of intense study (Golenberg 1986a). The linkage relations and chromosomal locations of the 8 loci were determined by analysis of crosses at Yehudiyya, and by comparison with isozyme patterns of ditelocentric accessions of *T. aestivum* cultivar Chinese Spring (Golenberg 1986a,b). It was determined that *Mdh*-1 and *Hk* are linked and probably located on the chromosome 1B. *Pept*-1 and *Rc* are also linked and probably located

on the chromosomal arm 6Bq. Gametic phase disequilibrium between loci was tested on four generations and found to increase. Two cases of probable epistatic selection were noted, but no evidence suggesting the regeneration of the original multilocus structures was found.

Significant differences were found among populations of the Yehudiyya and Qazrin genotypes for morphological, germination and phenological characters when grown under standardised greenhouse conditions. Reciprocal transplantation and replacement series competition experiments did not, however, reveal any local adaptations in the year studied. Outcrossing rates were estimated to be well below 1%. Gene flow rates were found to be low in general. Genetic neighbourhood area was estimated to be about 5 metres in diameter, implying that the mean gene flow distance per generation was about 1.25 metres. In conclusion, there was no strong evidence indicating that multilocus structures are the units of selection in wild emmer here. Sporadic selection, high selfing rates, and limited gene flow, appear to be of major importance in multilocus evolution in wild emmer.

B. Macrogeographical differentiation (Nevo & Beiles 1988)

The population genetics of wild emmer comprising 37 populations, 33 Israeli and 4 Turkish, involved 1815 plants. Each plant was typed electrophoretically at 42 isozyme loci. The results indicated that (a) 36 loci (86%) were polymorphic across the range studied. (b) The proportion of polymorphic loci per population, *P*-1% averaged 0.22 (range,0.050-0.415), and the genetic diversity index, *He* averaged 0.059 (range, 0.002-0.119). (c) Genetic diversity varied significantly between ecological regions, close populations, soil types and different population sizes. (d) There were 118 alleles, 114 in Israel and 61 in Turkey. (e) Out of the variant alleles, 67 were local or sporadic, indicating considerable differences between populations in allelic content, i.e., displaying a population genetic "archipelago" structure. (f) Genetic distances between populations were high, averaging D=0.134, (range, 0.018-0.297), indicating sharp genetic differentiation over short distances. (g) Discriminant analysis succeeded in classifying plants according to country, region, soil type and population size, with 86-97% accuracy. (h) Genetic diversity and allele frequencies were significantly correlated with, and partly predictable by, temperature, water and soil variables, and combinations thereof, much above that expected by chance; (i) Genetic differentiation among populations was 60%, and within populations 40%; (j) A considerable amount of significant and differential gametic phase disequilibria and genome organization characterized ecological regions and soil types. These results support earlier conclusions that genetic differentiation is primarily the result of diversifying selection through climatic and soil variations. The genetic structure based on ecology and allozymes provides optimal sampling guidelines for the conservation and utilization of the wild genetic resources in wheat improvement.

Genetic resources of disease resistance

Wild emmer harbours high amounts of disease resistant genes. We tested reactions of wild emmer to leaf rust, *Puccinia recondita tritici* (Moseman et al 1985) and to stripe rust, *Puccinia striiformis* (Nevo et al 1986a). In the leaf rust study, reactions were determined from 687 Israeli accessions of wild emmer, to infection with culture PRTUS of *P. recondita tritici*. Resistant and moderately resistant accessions were obtained among 353 accessions collected at 180 sites across the range in Israel. In the stripe rust study, we tested 114 accessions from 11 sites both in the seedling and adult stages. We found large amounts of disease resistant genes. Elite disease resistant genotypes can be effectively screened in nature (Nevo et al 1985, 1986a, Nevo 1987). The resistant accessions identified in these and earlier studies are being used to develop enhanced hexaploid and tetraploid wheat germplasm resistant to *P. recondita tritici*, *Erysiphe graminis tritici* and *P.*

striiformis, at Beltsville, Md.

Genetic resources of wheat storage proteins

(a) Protein content (Nevo et al 1986b).
Protein percentage, kernel and protein weight, vary within, but particularly between, populations. Notably, ecologically marginal populations exhibit high protein content but low kernel weight as compared with central populations. Climatic factors and allozyme variation can significantly predict protein weight and kernel weight (R^2=0.60-0.70). Thus, wild emmer contains large amounts of yet untapped genes for elite protein and high seed weight, predictable by ecology and allozymes.

(b) Glutenins (Nevo & Payne 1987).
The diversity of HMW glutenin subunits was studied electrophoretically in 231 individuals, representing 11 populations of wild emmer from Israel. The results show that (a) The two HMW glutenin loci, *Glu*-A1 and *Glu*-B1, are rich in variation, having 11 and 15 alleles, respectively. (b) Genetic variation in HMW glutenin subunits is often severely restricted in individual populations. (c) Significant correlations were found between glutenin diversity and physical (climate and soil) and biotic (vegetation) variables. Our results suggest that: (a) at least part of the glutenin polymorphisms in wild emmer can be accounted for by environmental factors and (b) the endosperm of wild emmer contains many allelic variants of glutenin storage proteins that are not present in bread wheat, and these could be utilised in breeding varieties with improved breadmaking qualities.

(c) rDNA diversity (Flavell et al 1986).
The variation in the intergenic spacer of ribosomal DNA (rDNA) of wild emmer in Israel was examined in 112 plants, of 12 populations tested earlier for allozymic variation, encoded by 50 gene loci. The variation detected, by means of restriction endonucleases, results in part, from variation in the number of 135-bp repeats that are in tandem array in the intergenic DNA spacer. Populations of *T. dicoccoides* display a wide spectrum of rDNA spacer-length variation. Some populations are very homogeneous, whereas others are heterogeneous. Allozymes and rDNA diversities within populations are highly and significantly intercorrelated, and both are predictable by climatic variables.

Plant genetic resources: prediction by isozyme markers and ecology

Linkage or association of genetic markers to quantitative traits of agronomic importance can substantially simplify the genetic analysis of complex quantitative traits. Enzyme genes and DNA markers are ideal candidates for quantitative genetic analysis. We have recently applied this methodology to the analysis of genetic resources of wild cereals in Israel (reviewed in Nevo 1987). Allozyme markers and ecological factors provide an important predictive method for identifying elite genotypes characterised by single or multiple disease resistances, high protein content and a variety of quantitative traits of agronomic importance including germination, earliness, biomass, and yield variables. Our predictive methodology could be improved by additional crossing tests, in an attempt to verify the results derived from the correlation analysis and thus, establish the linkage relationships between the isozyme or DNA (RFLP) genetic markers and the quantitative trait.

CONCLUSIONS AND PROSPECTS

Multidisciplinary studies of wild emmer, *T. dicoccoides*, in Israel, conducted at the Institute of Evolution are reviewed here as means for wheat improvement. The Near East in general, and Israel in particular (Nevo 1986), are the centres of origin and diversity of wild emmer, where it developed wide genetic adaptations, against multiple pathogens, and diverse ecological stresses. Genetic variation is transferable from the wild to the cultivated gene pool, thus genes of wild emmer are optimal for future wheat improvement.

The rich genetic diversity of wild emmer described here, previously (Nevo 1983) and elsewhere (Grama et al 1983, Feldman 1979) for multiple disease resistances, agronomic traits of economic significance, and environmental adaptations, is geographically structured, and is predictable by ecology and allozyme markers. Consequently, conservation and utilization programmes should maximise sampling strategies by following the ecological-genetic factors and allozyme/DNA markers as effectively predictive guidelines. Our predictive methodology, by ecology and allozymes, is correlative, and should be improved by using isozyme and DNA genetic markers, linked to traits of agronomic importance. We recently started using DNA restriction polymorphisms of chromosomally located clones. Our predictive methodology, if further developed by additional isozyme and DNA markers, verified by crossing tests and gene mapping, could substantially contribute to optimise sampling, conservation, and utilization of the as yet largely untapped genetic resources of wild gene pools for crop improvement.

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