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Wheat storage proteins: glutenin diversity in wild emmer, *Triticum dicoccoides*, in Israel and Turkey. 2. DNA diversity detected by PCR

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Abstract Germplasm of *Triticum dicoccoides* collected from different environments in Israel was evaluated by using the PCR as a molecular marker. Two pairs of primers were used in the PCR in amplifying the DNA tracts coding the high- and low-molecular-weight glutenin subunits. Analyses reveal great variability within and between populations indicating the high values of this germplasm for future breeding programs to improve the protein quality in wheat.

Key words Genetic polymorphism · Genetic resources · Glutenin · Molecular markers · PCR · Wild emmer

Introduction

Modern plant breeding and agronomic practices have regrettably eroded the genetic diversity of cultivated plants. Consequently, it has also increased the crop vulnerability to pathogens and climatic variation (Plucknett et al. 1983). The genetically rich gene pools of the wild relatives of cultivated plants are the best hope for future crop improvement (Feldman and Sears 1981; Nevo 1986; Plucknett et al. 1987). In the wheat group the basic elements of information for planning a comprehensive utilization of the wild gene pool are available (Zohary 1970). In this context, *Triticum dicoccoides* is an important source of genetic variation which could be inserted in breeding programs to improve the quality (protein content and resistance) of present wheat varieties (Nevo 1995), especially in view of its close genetic affinity with common wheat (Zohnary 1970).

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The study of the genetic diversity and genetic structure of wild emmer wheat is important from the (1) theoretical point of view, in order to understand the evolutionary process of this important wheat progenitor and (2) practically, in order to evaluate the potential of this genetic resource for future crop improvement.

Previous studies on Israel's natural populations of *T. dicoccoides* cover the following aspects: (1) population genetics and ecology at the micro- and macro-geographical scales; (2) genetic resources of disease resistance; (3) wheat storage protein; (4) tolerance against ecological stresses of drought, salt, herbicides; (5) photosynthetic yield, and (6) plant genetic resources for other agronomically important traits (Carver and Nevo 1990; Nevo 1983, 1988, 1993).

In the previous studies the markers used were essentially allozymes (Nevo 1987; Nevo and Beiles 1989; Nevo et al. 1982, 1988 a, b, 1991), wheat storage proteins, i.e. high-molecular-weight (HMW) glutenin (Nevo and Payne 1987), or ribosomal DNA (Flavell et al. 1986).

In the present paper one Turkish and 24 Israeli wild emmer wheat populations were analysed using a different approach. The genetic structure and differentiation of Israel's natural populations of *T. dicoccoides* is detected here at the molecular level, by using a molecular DNA marker of the polymerase chain reaction (PCR) with specific primers as possible tools to maximize the germplasm utilization program.

The relations between the PCR results and the ecological characteristics have been correlated to underline the evolutionary potential of, and the ecological factors affecting, the genetic variability and the population structure in *T. dicoccoides* (Nevo et al. 1995). The present results are compared with previous studies conducted on the same material by other methods.

The use of a molecular marker, such as the PCR, may lead to a substantial improvement of selection efficiency in breeding for both qualitative and quantitative traits. Major advantages of molecular, over traditional, markers are: (1) they are unlimited in number; (2) they do

not affect the phenotype but may detect genetic variation efficiently (Helentjaris et al. 1985; Beckmann and Soller 1986, 1988; Tanksley et al. 1989). A further advantage is the possibility of using genomic DNA extracted at any plant stage and from any tissue. Consequently, analysis can be shifted to a very early stage of plant growth, saving time and resources.

Polymerase chain reaction (PCR) amplification using specific primers (Saiki et al. 1985; Mullis and Faloona 1987) is a fast and relatively simple technique which is able to detect polymorphisms directly on the gel.

The aim of the present work is to define the genetic variability and population structure of HMW and low-molecular-weight (LMW) glutenins in *T. dicoccoides*. Linkage or association of genetic markers to quantitative traits of agronomic importance can substantially simplify the genetic analysis of complex quantitative traits.

Materials and methods

Germplasm characteristics

Field collections were made between 1979 and 1987 (Nevo and Beiles 1989) and seeds have been stored at the Institute of Evolution, University of Haifa, Israel, until their analysis. Twenty-four populations of wild emmer wheat from Israel and one from Turkey (*T. dicoccoides*) were chosen from ecologically contrasting environments. For each population ten random genotypes were analyzed totalling 249 genotypes. Population No. 27 (Nesher) was analyzed only for HMW glutenins. The locations of all the Israeli populations appear in Fig. 1 and their ecological background is reported in Table 1 of Nevo and Beiles (1989).

DNA extraction

Twenty seeds per genotpye were surface-sterilised by sodium hypochlorite (final concentration 1% in water) and germinated in Petri-dishes. Genomic DNA was extracted from week-old etiolated seedlings following the Dellaporta method (Dellaporta et al. 1983), and re-suspended in 100 µl of TE (10 mM Tris HC1, 1 mM EDTA).

PCR conditions

In the PCR oligonucleotides synthesized on the basis of published sequences of *Triticum* genes coding for seed storage proteins were used (Halford et al. 1987; Colot et al. 1989; D'Ovidio et al. 1990). Primer sequences were chosen in order to give maximal amplification of gene tracts, without the possibility of incomplete amplification (see below).

In the PCR of LMW glutenin, the following two primers were used: 5′ ATG AAG ACC TTC CTC GTC TT 3′ and 5′ C AAC GCC GAA TGG CAC ACT A 3′ (Colot et al. 1989). These two primers amplify all the Glu-A3 and Glu-B3 genes of *T. dicoccoides*.

For the HMW glutenin PCR, primers were chosen in order to amplify the repeated domain and the N-terminal tract. Previous amplifications with different primers amplifying a longer tract have not worked properly since they have produced too many non-specific bands due to large gene length (Innis et al. 1988; Schwarz et al. 1990). The sequences of the two oligonucleotides primers used to amplify the HMW glutenin in this paper, were: 5′ ATG GCT AAG CGG TTG GTC CT 3′ and 5′ CTG TGT TAA CAT GGT ATG GGT TGT C 3′ and they were extracted from a sequence of the y-type Glu-B1 gene (Halford et al. 1987) which also amplifies the y-type Glu-A1 gene (D'Ovidio et al. 1993).

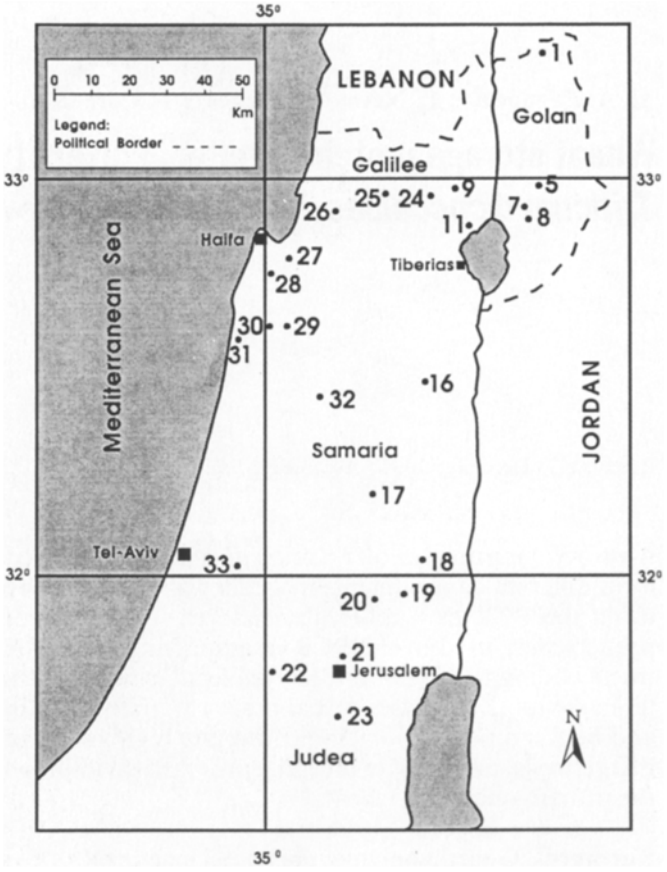

Fig. 1 Geographic distribution of the 24 Israeli populations of wild emmer wheat, *T. dicoccoides*, analyzed in this study for PCR diversity of DNA of glutenins. The populations consist of: (1) central (nos. 5–11); xeric marginal: (2) cold northern population (Mt. Hermon, no. 1) and (3) warm, eastern and southern populations (16–23); (4) mesic west marginal (24–33). For population names, see Table 2

The PCR mixture per genotype consisted, in both cases (i.e. LMW and HMW glutenin), of 250 ng of genomic DNA, 250 ng of each of the two primers (prepared by Dr. J. Wunderlich, University of Georgia), 300 mM each of dATP, dGTP, dCTP, dTTP (Pharmacia), 2.5 units of *Taq* polymerase (Boehringer) made up to 100 µl in water. The mixture was covered with two drops of mineral oil (Perkin Elmer). For DNA amplification of LMW glutenin, a Perkin Elmer Cetus thermal cycler was programed for 30 cycles at 94 °C for 1 min (denaturing), 55 °C for 2 min (annealing), and 72 °C for 2 min (extension) (D'Ovidio et al. 1992). For amplification of HMW glutenin, the conditions were changed because with an annealing of 55 °C the amplified product showed a smear. As a result, after a series of checks, the same machine was programed for a start of 2 min at 94 °C followed by 35 cycles each of 94 °C for 1 min, 60 °C for 2 min, and 72 °C for 2 min and 30 s; the 35 cycles were then followed by an extension of 7 min at 72 °C. After the amplification cycles were then followed by an extension of 7 min at 72 °C. After the amplification cycles were completed, 15 µl of amplified product per sample was run in 1 × TBE buffer on a 1% agarose mini gel containing 0.5 µg/ml of ethidium bromide at 80 V for about 2 h. The gels were photographed under UV light with Polaroid film 667.

Statistical analysis

The dimensions of the bands found in the PCR analyses were measured using the lambda marker as a reference. In order to have more precision in band-size measurements, and avoid variation of

differences in gel-running, gels were re-run loading different combinations of PCR products. The results were considered as a matrix of 0 and 1, where 0 indicates absence and 1 the presence of a 'possible band'. The matrices so obtained were used to perform a statistical analysis using local computer programs, Biosys-1 (Swofford and Selander 1989), and SPSS (1990) statistical packages. The analyses were run considering both HMW and LMW matrices. Two analyses have been conducted: (1) by assuming that each band represents a locus with two alleles (present or absent); (2) by regarding each LMW and HMW band pattern as a different multilocus profile.

Results

Variability and polymorphism

The PCR of the 25 populations identified 29 characteristic patterns for the HMW glutenin and the PCR of 24 populations identified 27 characteristic patterns for the LMW glutenin (Table 1). The patterns (the type of multi-band profile of a LMW and HMW genotype) have very different frequencies and distributions (Table 2): some of them were very rare, as in the case of patterns nos. 9, 11, 12, 15, 17, 24, 25, 26 for the LMW glutenin and nos. 5, 9, 12, 13, 17, 19, 23, 25, 27 for the HMW glutenin, which were present in only one genotype over the 249 genotypes analyzed. Some others were widely distributed among populations, as was the case for patterns nos. 1, 2 and 7 of the LMW glutenin (present in seven and eight different populations, respectively) and nos. 3 and 6 of the HMW glutenin (present in seven different populations). Moreover, some populations (Mt. Gerizim and Kabara for the LMW, Qazrin, Daliyya and Yabad for the HMW glutenins) showed great uniformity having no polymorphism, whereas others have a predominant pattern but with a small proportion of a second pattern (i.e. Nahef and Daliyya for the LMW, and Mt. Gilboa and Ahihud for the HMW glutenins). On the other hand, other populations (i.e. Gitit and Amirim for the LMW, Rosh-Pinna, Sanhedriyya and Bat-Shelomo for the HMW glutenins) have five or six different and almost equally represented patterns.

Table 1 PCR patterns found in 25 populations of *T. dicoccoides* amplified with primers for the low-molecular-weight glutenin (LMW) and primers for the high-molecular-weight glutenin (HMW)

Pattern number	LMW pattern	HMW pattern
1	0010100	010001000010
2	0010110	000001000010
3	1101100	000010000010
4	0011100	010010000010
5	1010100	010010010010
6	1010001	010010000000
7	1011100	010000000000
8	1011010	000100000010
9	1111010	010010000001
10	1011110	000010000001
11	0101010	000000000000
12	1000110	010001000001
13	0110100	000100000001
14	1010101	000001000001
15	1110110	010001000000
16	1011000	010000100010
17	0010111	000000100000
18	1110100	000000100010
19	0010000	011000000010
20	0010010	000001000000
21	1110000	000000010000
22	0011010	010010001000
23	1010110	010010001010
24	1111100	010100000010
25	0000100	010010000101
26	0011110	010000001000
27	0001000	000000100001
28		000010000100
29		100010000100

Patterns can be interpreted as multilocus structures. Here we analyzed the data considering each potential band as a locus with two alleles: present or absent. This data interpretation can be analyzed by standard programs of genetic analysis. We used Biosys-1 (Swofford and Selander 1989) and local programs. The results appear as genetic indices (Table 3); genetic diversity within and between populations (Gst) following Nei (1973), and genetic distances (Nei 1978). The detailed Gst analysis and the tables of genetic distances can be obtained from the second author upon request.

The polymorphism at each population including both LMW and HMW glutenins ranged from 5% at Daliyya and Yabad to 58% at Rosh-Pinna, with an average of 28.8% (Table 3); also the genetic diversity (H_e) was quite different among the populations ranging from 0.010 to 0.170. The mean polymorphism obtained by analyzing 24 populations using PCR, together with the mean number of alleles per locus and the genetic diversity (H_e), are comparable with the values of the same parameters found by Nevo and Beiles (1989) obtained by analyzing 37 populations for different isozyme polymorphisms.

Genetic differentiation within and between populations (Gst analysis)

The genetic diversity between populations averaged 64% of the total diversity of 0.253 of the 19 glutenin bands. This is quite impressive compared to the variability within populations (36%). These results are comparable with allozyme differentiation (Nevo and Beiles 1989) where interpopulation allozymic diversity was 60% (of the total diversity of 0.165) compared to 40% within populations.

The table of the absolute (Dst) and relative (Gst) contribution of single bands to the glutenin differentiation between populations can be obtained from the second author upon request. For example, in the LMW group higher interpopulation absolute diversity (Dst) was present in bands L1180, L1054 and L830 (40, 32 and 23%, respectively), while bands L1180 and L650 had higher Gst relative to the genetic diversity between populations (80 and 72%, respectively). These bands are

Table 2 Glutenin DNA PCR pattern frequencies and distribution in 25 populations of *T. dicoccodies* in Israel and Turkey. Pattern number in parenthesis

Locality	Pattern frequencies	
	LMW glutenin	HMW glutenin
1. Mt. Hermon	(1) 0.200; (2) 0.500; (13) 0.300	(1) 0.200; (2) 0.800
5. Qazrin	(3) 0.800; (4) 0.100; (25) 0.100	(3) 1.000
7. Yehudiyya	(5) 0.700; (6) 0.300	(4) 0.300; (5) 0.100; (6) 0.400; (7) 0.200
8. Gamla	(7) 0.200; (8) 0.500; (9) 0.100; (10) 0.200	(8) 0.100; (9) 0.100; (10) 0.700; (11) 0.100
9. Rosh-Pinna	(1) 0.800; (11) 0.100; (12) 0.100	(1) 0.100; (10) 0.300; (12) 0.100; (14) 0.400
11. Tabigha	(6) 0.100; (14) 0.900	(1) 0.500; (15) 0.500
16. Mt. Gilboa	(15) 0.100; (16) 0.500; (17) 0.100; (18) 0.300	(16) 0.900; (17) 0.100
17. Mt. Gerizim	(1) 1.000	(3) 0.100; (6) 0.100; (18) 0.800
18. Gitit	(1) 0.100; (2) 0.200; (4) 0.300; (7) 0.300; (16) 0.100	(3) 0.100; (4) 0.700; (7) 0.100; (19) 0.100
19. Kokhav-Hashahar	(1) 0.556; (7) 0.111; (10) 0.222; (16) 0.111	(2) 0.111; (7) 0.222; (20) 0.667
20. Taiyiba	(4) 0.400; (19) 0.200; (20) 0.400	(7) 0.500; (21) 0.500
21. Sanhedriyya	(2) 0.700; (4) 0.100; (21) 0.100; (22) 0.100	(2) 0.100; (3) 0.300; (4) 0.300; (7) 0.100; (21) 0.100; (22) 0.100
22. Bet Meir	(7) 0.300; (8) 0.200; (16) 0.500	(2) 0.800; (6) 0.200
23. Jaba	(1) 0.300; (2) 0.300; (19) 0.400	(2) 0.500; (4) 0.300; (6) 0.200
24. Amirim	(2) 0.100; (5) 0.200; (7) 0.500; (10) 0.100; (14) 0.100; (23) 0.100	(2) 0.100; (3) 0.600; (4) 0.200; (24) 0.100
25. Nahef	(10) 0.900; (24) 0.100	(3) 0.600; (4) 0.300; (6) 0.100
26. Ahihud	(5) 0.636; (23) 0.364	(6) 0.900; (25) 0.100
27. Nesher	Not recorded.	(8) 0.700; (16) 0.300
28. Beit Oren	(7) 0.800; (21) 0.200	(22) 0.800; (26) 0.200
29. Daliyya	(22) 0.900; (26) 0.100	(4) 1.000
30. Bat-Shelomo	(5) 0.100; (7) 0.300; (20) 0.600	(3) 0.600; (4) 0.100; (6) 0.100; (10) 0.100; (22) 0.100
31. Kabara	(7) 1.000	(11) 0.100; (16) 0.100; (18) 0.800
32. Yabad	(2) 0.600; (20) 0.400	(24) 1.000
33. Givat-Koach	(1) 0.111; (2) 0.778; (20) 0.111	(4) 0.778; (27) 0.111; (28) 0.111
37. Diyarbakir (Turkey)	(1) 0.100; (2) 0.600; (27) 0.300	(28) 0.400; (29) 0.600

Table 3 Genetic variation based on bands regarded as 19 loci in 23 populations in Israel and one Turkish population of *T. dicoccoides* of low- and high-molecular-weight glutenin detected by PCR

Locality	Sample size (N)	Mean no. of alleles per locus (A)	Polymorphism of population (P-5%)	Genetic diversity (He)	(SE)
1. Mt. Hermon	10	1.158	0.158	0.069	0.038
5. Qazrin	10	1.211	0.211	0.055	0.027
7. Yehudiyya	10	1.263	0.263	0.101	0.042
8. Gama	10	1.421	0.421	0.120	0.037
9. Rosh-Pinna	10	1.579	0.579	0.143	0.034
11. Tabigha	10	1.105	0.105	0.038	0.029
16. Mt. Gilboa	10	1.421	0.421	0.140	0.045
17. Mt. Gerizim	10	1.158	0.158	0.045	0.026
18. Gitit	10	1.421	0.421	0.125	0.039
19. Kokhav-Hashahar	9	1.368	0.368	0.135	0.045
20. Taiyiba	10	1.263	0.263	0.135	0.053
21. Sanhedriyya	10	1.526	0.526	0.156	0.039
22. Bet-Meir	10	1.316	0.316	0.112	0.039
23. Jaba	10	1.316	0.316	0.151	0.053
24. Amirim	10	1.421	0.421	0.131	0.041
25. Nahef	10	1.211	0.211	0.057	0.030
26. Ahihud	11	1.158	0.158	0.045	0.028
28. Beit-Oren	10	1.211	0.211	0.071	0.032
29. Daliyya	10	1.053	0.053	0.010	0.010
30. Bat-Shelomo	10	1.421	0.421	0.170	0.051
31. Kabara	10	1.158	0.158	0.030	0.016
32. Yabad	10	1.053	0.053	0.027	0.027
33. Givat-Koach	9	1.421	0.421	0.105	0.031
37. Diyarbakir (Turkey)	10	1.263	0.263	0.123	0.049
Mean	239	1.287	0.287	0.096	0.010

better descriptors of populations. In the HMW group the bands which displayed higher diversity, and which could be population specific, were: H2010, H1740, H1780 and H2030 with 86, 84, 80 and 79% interpopulation genetic diversity, respectively. Again, these are optimal bands for characterizing populations.

Genetic and geographic distance

Considering the band distribution of the PCR-amplified products of LMW and HMW glutenins it was possible to draw the unbiased genetic distance, *D*, between populations (Nei 1978) (the detailed table can be obtained from the second author, upon request). D ranges in Israel from 0.006 between Sanhedriyya and Givat-Koach to 0.487 between Qazrin and Yabad. All Israeli-Turkish genetic distances were less than those between Qazrin and Yabad, except that of Tabigha, $D = 0.522$. The genetic distance clearly did not match the geographic distance. There are geographically close populations as in the case of Qazrin, 10 km apart from Yehudiyya and 50 km apart from Rosh-Pinna, with a genetic distance of 0.217 and 0.409, respectively. On the other hand, there were populations far apart as in the case of Qazrin from Gitit, or Amirim from Jaba, which are on the opposite positions of the wild emmer range in Israel, and which had a genetic distance of only 0.141 and 0.099, respectively. Moreover, the *D* between Diyarbakir (Turkey) and Taiyiba was only 0.127. A disagreement between geographic and genetic distance had previously been detected by Nevo and Payne (1987) and by Nevo and Beiles (1989).

The range of wild emmer wheat in Israel can be subdivided into central populations and several types of marginal populations: (1) North, cold and dry environment (Mt. Hermon); (2) West, humid (nine populations); (3) East-south, warm and dry (eight populations) and (4) one population from Turkey, warm and semi-dry (Nevo and Beiles 1989). The glutenin genetic distance between different types of marginal regions in Israel was lower than the *D* between the five central populations. Likewise, the within-marginal-region distances were similar to the between-regional ones (Table 4). These results emphasize again that isolation by distance (Wright 1943) is not the main factor determining glutenin differentiation. Clearly, microgeographic differentiation, either edaphic (Nevo et al. 1988 a) or climatic (Nevo et al. 1988 b), plays an important role in the genetic differentiation of wild emmer, including also glutenin differentiation (Nevo and Payne 1987; Nevo et al. 1995).

Table 4 Matrix of Nei's unbiased genetic distance coefficients (*D*), averaged by marginality

Marginality	Number of populations	1	2	3	4	5
1. North	1	–				
2. West	9	0.249	0.185			
3. East-South	8	0.147	0.171	0.142		
4. Turkey	1	0.318	0.274	0.250	–	
5. Central	5	0.255	0.240	0.211	0.329	0.280

Discussion and conclusion

In the present paper, each possible band was considered as a different locus with two alternative alleles (presence or absence). This is not strictly correct since a locus could actually be represented by different 'possible bands'. Presence of one band or another could represent alternative forms of the same locus differentiated by deletion or mutation of the DNA tract within the two primers. In any event, even if our analysis is not strictly correct in terms of loci and alleles, it gives an estimate of the variability present within and between populations.

The DNA analysis by the PCR used in the present work corroborates the evidence of genetic diversity and divergence found in natural populations of *T. dicoccoides* across Israel (Nevo et al. 1982; Nevo and Payne 1987; Nevo and Beiles 1989; Carver and Nevo 1990). The populations of *T. dicoccodies* showed great variability both between and, more surprisingly, within populations, emphasizing the importance of this wild wheat relative in future breeding programs and confirming the proposal that it may be an important source for wheat improvement (Nevo et al. 1995).

In the present study we found an impressive high glutenin diversity between and within populations. Population divergence does not always follow the isolation by distance model of Wright (1943). Quite often it is easier to find a greater genetic difference between close populations than among populations which are far apart. This confirms the island population genetic model of wild emmer (Nevo and Beiles 1989). Thus, the genetic structure of wild emmer wheat populations in Israel is mosaic.

This patchy genetic distribution appears to reflect the underlying ecological heterogeneity (Nevo et al. 1995). The high polymorphism and genetic variation found within and between populations could be explained by spatio-temporal selection. Moreover, the micro-environmental variation, coupled with the limited migration of *T. dicoccoides*, can explain the within-population variation.

The present work, together with previous studies of Israeli populations of wild emmer wheat, reveals the great potential of this wild wheat for introducing useful genes into cultivated wheat varieties.

Considering the high variability found within populations, germplasm collectors should also consider micro-environmental factors. Likewise, considering that the large germplasm collection needs to be reduced into "core collections" (Frankel 1984; Brown 1989), the accurate ecological data of collection sites provides a most powerful guideline for this process of germplasm reduction.

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