Theor Appl Genet (1997) 95:932–941

J. L. David · M. Zivy · M. L. Cardin · P. Brabant

Protein evolution in dynamically managed populations of wheat: adaptive responses to macro-environmental conditions

Received: 10 February 1997 / Accepted: 18 April 1997

Abstract Dynamic management of genetic resources aims to conserve genetic variability between different populations evolving in contrasting environments. It is thus of importance to determine whether differences appearing between populations are stochastic or if they come about from adaptation. Two-dimensional electrophoresis (2DE) was used to study genetic differentiation of 11 wheat populations evolving since 1984 in a multi-site network covering the major cultivation area of wheat in France. Gels were scanned and protein-spot intensities were measured through image analysis. As it was not possible to assay each individual, populations were characterized using pooled extracts from several plants. In the first step, two parents among the 16 parental lines involved in the initial wheat composite-cross population were exhaustively studied to identify a set of polymorphic spots against which the entire set of evolved populations could be compared. This analysis confirmed the efficiency of gel image-processing to determine the composition of pooled extracts. Of the 48 spots used to investigate population differentiation, 15 showed significant differences at the $P < 0.05$ level. Populations that evolved independently at the same location showed similar differentiation, even when their cultivation methods were different. These results suggest that natural selection acted strongly on the evolution of the populations, and that responses to selection were determined primarily by macro-environmental conditions.

Communicated by P. M. A. Tigerstedt

J. L. David (✉) · M. Zivy[1] · M. L. Cardin[1] · P. Brabant[1,2]
INRA, Station de Génétique et d'Amélioration des Plantes, Domaine de Melgueil, F-34130 Mauguio, France

Present addresses:
[1] INRA-UPS-INAPG-CNRS URA 1492, Station de Génétique Végétale, Ferme du Moulon, F-91190 Gif sur Yvette, France
[2] INA-PG, Institut National Agronomique, 16 rue Claude Bernard F-75005 Paris, France

Key words Genetic resources · Dynamic conservation · Two-dimensional electrophoresis · Artificial population · *Triticum aestivum*

Introduction

The conservation of the genetic diversity of cultivated plant species has been traditionally accomplished by storing seeds (or other propagules) of several thousand accessions per species at various agricultural institutions around the world. Such 'static' conservation has allowed for the preservation of landraces that have now become endangered by the adoption of modern elite varieties (Frankel et al. 1995). But the advantage of preserving intact the genetic integrity of individuals or populations may become a disadvantage if continuous adaptation is required for growth under new environmental conditions like those required by a climatic global change (Simmonds 1962; Henry et al. 1991). While genetic resources stored in a static manner could in theory permit breeders to select new varieties for future conditions (Marshall 1990), their use will require burdensome evaluation, involving crossing and selection programmes. New complementary methods have been proposed to ensure that crop genetic diversity will fit the future needs of plant breeding. These strategies, termed 'dynamic conservation', allow adaptive processes to continue, e.g., in situ cultivation of land races in their conserved traditional agro-systems (Brush 1995), genetic reserves for wild species (Dinoor 1976), and mass reservoirs (Simmonds 1962). Mass reservoirs are highly heterogeneous populations derived from crosses between a large number of parental types and maintained for a number of generations. Though this method contains certain components of dynamic management, its utility in the conservation of genetic diversity has been questioned since morphological variability declined in barley composite crosses

multiplied at certain sites (Jana and Khangura 1986). But no loss of neutral diversity (allozymes) was observed in the same study. Moreover, similar experimental populations remained genetically variable even after 50 generations of propagation at a single site (Allard 1988), increased their diversity for disease resistance (Allard 1990), and were shown to quickly react to fluctuations in local selective pressures (Allard 1988).

While genetic diversity may decrease in local populations, the propagation of mass reservoirs over a number of different environmental conditions may lead to the structuring of the collection as a whole, and thus preserve a large part of variability for immediate use in improving plant genetic resources. This extension of dynamic conservation has been referred to as 'dynamic management' (Henry et al. 1991). Such a strategy was aimed at the management of diversity, including the choice of the best sites for multiplication, controlling the outcrossing rate, the monitoring of gene flow between local populations, and the use of 'adapted human selective pressure', when necessary, to avoid unfavourable evolution brought about by natural selection (Goldringer et al. 1994). It parallels the metapopulation concept (Olivieri et al. 1990) which has become increasingly accepted as a model in population biology (Harrison and Hastings 1996).

Dynamic management has recently been applied in France to winter wheat (*Triticum aestivum* L.) in a pilot experiment started in 1984 (Henry et al. 1991). Fifty two populations, derived from three highly polymorphic composite pools, were distributed over several locations in France, then sown and harvested at each location, but were otherwise left free to evolve. After several years, differentiation had already been detected among populations for many characteristics including morphological traits (David et al. 1992), powdery mildew resistance (Leboulc'h et al. 1994), pollen production (David and Pham 1993) and seed storage protiens (Pontis 1992). The greater part of the differentiation observed between the populations has been interpreted as adaptative responses to local environmental conditions (Leboulc'h et al. 1994). To be useful in dynamic management, these local differentiations must be at least partly predictible, i.e. the selective pressures at work have to be stable over a number of generation, so that two independent populations propagated under the same conditions will differentiate identically. If this can be demonstrated, it would mean that diversity may be managed in a dynamical manner within a network of rather small populations, provided that most of the environmental conditions encountered by the crop are represented in the network.

Our previous results showed that population differentiation was important, but we have no idea about the number and the type of genes that have been selected. It could be that only a few genes of major effect are involved. Or, conversely, many genes may underline the differentiation observed. To investigate this question at the biochemical level, we applied two-dimensional elctrophoresis of proteins (2DE). Two-dimensional electrophoresis of proteins allows the products of hundreds of genes to be observed on a single gel through the measure of spot intensities by automatic image analysis. According to the genotypes, spot intensities can vary from null to intense. Qualitative variation involves the presence or absence of spots according to the genotype and is often due to allelic variation [different isoelectric points or apparent molecular masses (De Vienne et al. 1996)]. Quantitative variation involves continuous variation in spot intensity, reflecting differences in the relative abundance of the protein, whose expression can be controlled by several loci (Damerval et al. 1994). Since individual-by-individual analysis of 2DE patterns is a lengthy process, we studied between-population differentiation using pooled extracts of several plants per population.

In the present paper we report results from such a study on a set of wheat populations descended after 8 years from a composite cross and dynamically managed in six sites. Two experiments were carried out. In the first of these, we verified the ability of 2DE gels to estimate the genotypic composition of a protein extract obtained by pooling known proportions of extracts of two well-assayed parental lines of the composite. 2DE is shown to be a reliable method for detecting the composition of pooled extracts. This resulted in a list of reproducible spots which were polymorphic in the initial composite. In the second experiment, these polymorphic spots were used to study the differentiation between the dynamically managed populations, under the assumption that variation in genotypic proportions results in differences in population mean-spot intensities. The results confirmed the rapid action of natural selection over the evolution of the populations, and suggested that local adaptation played a major role in their differentiation.

Material and methods

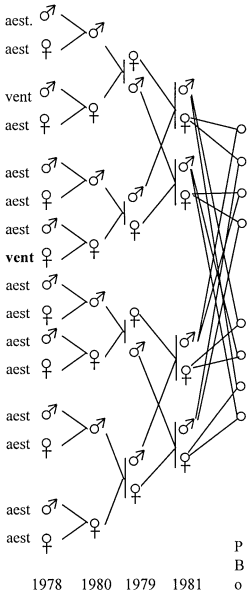
Populations

The 11 studied populations originated from a common ancestral composite population, referred to as PB_0 in the INRA programme of dynamic management of genetic resources (Henry et al. 1991). PB_0 was obtained by the pyramidal crossing of 16 parental lines (Table 1), followed by 3 years of bulk. PB_0 was polymorphic for both nuclear and cytoplasmic genomes. According to the crossing design (Table 1), the expected frequency of *Aegilops ventricosa* cytoplasm is 0.25 in PB_0, and is 0.75 for *T. aestivum* cytoplasm. Subsequently, the 11 original populations were created in 1984 by random sampling from PB_0.

They were then grown for 8 years in six different locations: Gif sur Yvette (49°N, −0.5°E), Le Chesnoy (48°N, 0.5°E), Rennes (48°N, −4°E), Venours (46.5°N, −2°E), Toulouse (43.5°N, −1°E) and Vervins (49.5°N 1.5°E). In all sites except Vervins, two isolated populations were multiplied: one under "intensive" cultivation conditions (i.e. with a high level of nitrogen fertiliser and fungal disease

Table 1 The 16 parental lines of the composite cross B, their genealogy, their cytoplasm (aest: *T. aestivum*, vent: *A. ventricosa*) and the pyramidal cross used to create population PB_0 (after Picard 1984). Of the two parents carrying the *Ae. ventricosa* cytoplasm only V1 7-5-PV-5-3 was used as a female parent in the first cross in 1978. The expected frequency of this cytoplasm is expected to be approximately 0.25 and conversely the expected frequency of the *T. aestivum* cytoplasm is 0.75

Parental line	Genealogy	Cytoplasm
C45-6-3	Artois/2-6-11//2-6-11/A18	aest.
C3275-1-2	FAO(61)69/US(67)136	aest
DC²-30	*Ae.ventricosa/T.dicoccum* // Nainari 60/3/2 Capelle	vent
Mironovskaïa 808	Sélection Artemovka (CEI)	aest
Clément	Hope/Timstein/3xHeinesVII/Riebesel57-41 /2xHeinesVII/4/Cleo (Netherland)	aest
C61-5-6-2	US(60)43/Prieur	aest
Top	TF354/Capelle	aest
V1 7-5-PV-5-3	(VPM x Capelle)	**vent**
Rivoli	Reso/Champlein	aest
C1-6-5	Martonvasari/2-6-11 (Courtot sib-line)	aest
Kavkaz	Lutescens314 H147/Bezotaya1 (CEI)	aest
TJB636	?	aest
D48	Martin/K3/Hohenheimer/3/Oro/4/Etoile de Choisy/5/Capelle	aest
Oxley	Penjamo 62/4 x Gabos 56//TPP/Nainari 60 /4/Lerma Rojo//Norin 10/Brevor/4/3 x Andes (Australia)	aest
Loros29	Loros/3/Capitole/VPM/Moisson	aest
Weihenstephan 1007/53	Heines Bart/Rye 4X//? x *T. aestivum* (Germany)	aest



control), and the other in "extensive" cultivation conditions (1/3 of the nitrogen dose used in the intensive treatment, no fungal disease control). These treatments at each site are hereafter referred to as Int and Ext respectively. At Vervins, only the Int population was cultivated. The populations were always propagated under the same cultivation method at the same site, and were isolated from one another by at least 30 m. This distance was sufficient to avoid cross-pollination between populations because wheat is mainly a self-pollinator and its pollen does into disperse very far (only a few m).

Two-dimensional electrophoresis

Proteins of 7-day-old dark-germinated seedlings were extracted as described by Damerval et al. (1986). 2DE was performed according to Bahrman and Thiellement (1987). Isoelectrofocusing (IEF) was run in 24 cm × 1.5 mm-diameter rod gels submitted to 38 000 Vh. The pH gradient was established with a 3 : 1 mixture of Pharmalytes pH 5–8 and Pharmalytes pH 5–6. Seventy five microliters of protein extract were loaded at the basic end of the gels. SDS electrophoresis was run in 24 × 26 × 0.15-cm slab gels, with an 11% concentration of acrylamide. The 2DE gels were stained with colloidal Coomassie Blue (G250). This staining was modified from Neuhoff et al. (1988). After fixation for 2 h in 2% phosphoric acid and 50% ethanol (Anderson et al. 1991), the gels were rinsed with double-distilled water (10 min) followed by 2% phosphoric acid (30 min). They were soaked in 17% ethanol, 15% ammonium sulphate, 2% phosphoric acid, and 0.1% Coomassie Blue G250 was directly sprinkled in the solution under agitation. The stain developed under shaking for 72 h at room temperature.

Images of the 2 DE gels were digitized using an Eikonix 7899, with a spatial resolution of 1 pixel per 0.1 mm, and an optical density range of 0–0.75. Automatic analysis (image processing, spot detection and quantification, matching of spots across the gels) was done using the KEPLER-2D package from the Large Scale Biology Corporation (Maryland, USA). Spot detection followed a procedure developed by Zivy (unpublished).

Preliminary bi-parental analysis

According to 2DE data from an earlier study (Points 1992), two genotypes of the 16 parental lines, DC²30 (DC) and Weihenstephan 1007/53 (W), appeared to be the most divergent for protein pattern. These genotypes also showed large differences in many morphological traits (Picard 1984) and have different cytoplasmic genomes: DC has the *Ae. ventricosa* cytoplasm while W has the *T. aestivum* cytoplasm (Table 1). To detect the polymorphic spots between these two lines, we performed a co-electrophoresis experiment, referred to as the 'DC-W' experiment. For each of these two lines, the protein extract of reference was obtained by pooling different extracts of several seedlings, in order to reduce the influence on spot intensity of possible non-controlled variations in environmental growing conditions. In the co-electrophoresis experiment, 11 different extracts obtained by controlled pooling of the reference extracts of DC and W were checked, i.e. the relative contribution of each parent (p_W and p_{DC}) in the protein extracts was varied from 0 to 1 by a 0.1 step (assuming that $p_W + p_{DC} = 1$), and spanning a range from a complete W extract ($p_W = 1$, $p_{DC} = 0$) to a null W extract ($p_W = 0$, $p_{DC} = 1$). Each composition was repeated five times (five gels) in a complete balanced block design. A block comprised a replicate of each of the 11 concentration modalities plus an additional replicate for ($p_W = 1$, $p_{DC} = 0$) and ($p_W = 1$, $p_{DC} = 0$) patterns, run in the same batch of IEF, SDS-PAGE and stained together. As artefactual variations in staining or protein loading are known to influence the staining intensity among gels (Burstin et al. 1993), the intensities of all spots in gel were scaled relative to a calibration parameter equal to the sum of the intensities of a large number of spots detected in all gels (Burstin et al. 1993).

For each spot, observed intensities were averaged over the five replicates for each level of parental concentrations and denoted as Q_{PW}. If Q_1 is the mean spot intensity observed for the W line (i.e. $p_W = 1$) and Q_0 is the mean spot intensity observed for the DC line (i.e. $p_W = 0$), the expected mean intensity QE_{PW} for any value of p_W is $QE_{PW} = p_W (Q_1 - Q_0) + Q_0$. Regression between Q_{PW} and QE_{PW} was then identical to the regression between Q_{PW} and p_W. When, for a given spot, the regression coefficient between the 11 values of Q_{PW} and p_W differed from 0 at the $P < 0.05$ level, the spot was recorded as polymorphic between the DC and W lines. Spot exhibiting many missing data points for p_W varying in the (0.3–0.7) interval were discarded, i.e. we considered that any valuable spot had to be reproducibly detected at least in intermediate parental concentrations. This procedure gave a list of reproducible polymorphic spots between DC and W. The quality of the regressions observed between p_W and the spots intensities permitted us to determine the reliability of the method of pooling for estimating the genotypic composition of a composite population.

Differentiation experiment

Material

Detecting differences in genotypic composition among the 11 evolved populations followed the procedure shown in Fig. 1. Four independent protein extracts were assembled for each population by pooling 30 individuals per extract, i.e. the population means for spot intensities were averaged over 120 individuals. Since seeds of PB_0

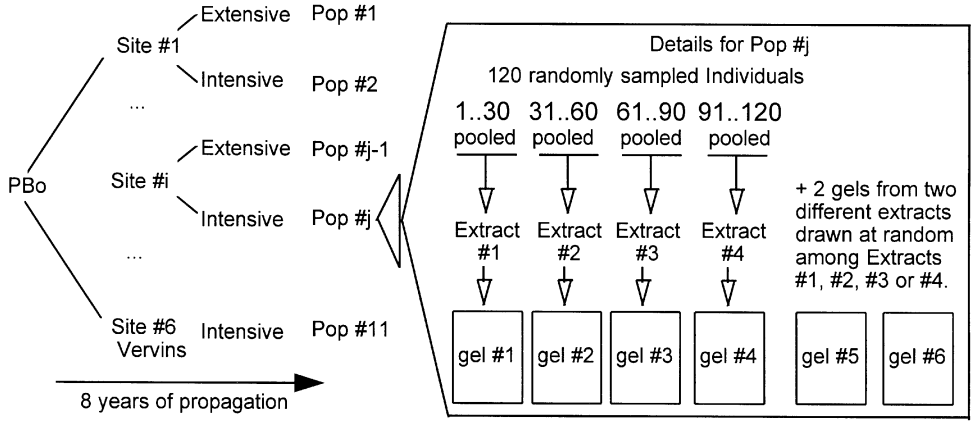


Fig. 1 Experimental design used in the survey of the 11 dynamically managed populations. Four independent protein extracts were assembled for each population by pooling 30 individuals per extract. The genotypic composition of each population was assayed with six gels. Four gels were run with the four different extracts, and the last two gels were run with two different extracts drawn at random among the four extracts

were not available, the reference population was represented by a co-electrophoresis of a balanced pooled extract of the 16 parental lines. This reference sample was thereafter treated in the same way as the samples from the other 11 populations. The genotypic composition of each population was assayed with six replicates (gels) distributed in five blocks. Four gels were run with the four different extracts, and the last two gels were run with two different extracts drawn at random among the four extracts (Fig. 1). In each block, each population was represented by at least one gel. As a block could contain 18 gels (SDS-Page batch size), six gels remained per block which were used in an incomplete but balanced block design to finally ensure the six replicates per population.

Statistical analysis

Analyses of variance were used to compare populations on the basis of variation in mean spot intensities, with populations and blocks as crossed factors. To eliminate non-reproducible spots, only those present in at least four out of the six replicates for each population were considered. Spots whose average intensities were found to significantly differ between populations were compared with those previously denoted as polymorphic in the DC-W experiment.

To study the respective contribution of the sites of multiplication and the cultivation methods to the differentiation of the populations, we partitioned the between-population variation into three variance components alloted to sites, var(Site), cultivation methods, var(Cult), and their interactions, var(Site × Cult). For example, if n is the number of sites, and S_j the effect of site j, then according to Scheffe (1959):

$$\text{var(Site)} = \frac{1}{(n-1)} \sum_{j=1}^{n} (S_j)^2.$$

These variances were estimated by the appropriate expectations of the mean squares of the ANOVA (Scheffe 1959) considering both site and cultivation effects as fixed. When any estimate of variance was negative, it was set to 0. The relative contributions of the factors to the differentiation of populations, i.e. CD_{Site} CD_{Cult} and $CD_{Site \times Cult}$, were then calculated as the ratios of their respective variance to the sum of three variances, e.g.

$$CD_{Site} = \text{var(Site)}/[\text{var(Site)} + \text{var(Cult)} + \text{var(Site} \times \text{Cult)}].$$

We calculated these parameters on spots showing significant differences within the appropriate set of ten populations (five sites with two cultivation methods), i.e. in all populations other than the parental reference and Vervins.

A multivariate analysis of variance was run using the same model as that used in the individual spot study. This analysis is equivalent to a factorial discriminant analysis that takes into account the balanced block design and allows the determination of independent axes useful in differentiating the populations. To reduce the risk of building discriminant axes due to artefactual effects of non-reproducible spots, we used only those spots that had previously exhibited significant intensity differences between populations at the $P < 0.01$ level. Missing data were replaced for each spot by its general mean. The coordinates of each gel and the barycenters of populations were recorded and plotted for the three first discriminant axes. All statistical analyses used the SAS (1988) package.

Results

Polymorphic spots in the DC-W experiment

Of the spots detected, 117 showed a significant regression at the $P < 0.05$ level between the known proportion of W, p_W and their mean intensity in the 11 co-electrophoreses. Many of these spots were later discarded, however due to many missing data points (more than 25%) in the intermediate concentrations [p_W included in the (0.3–0.7) interval]. These were generally light spots for which detection often failed. We retained 63 spots as reproducible and polymorphic between the DC and W lines. The mean R^2 (determination coefficient of regression) between average spot intensities and p_W for these 63 spots was 0.62, varying from 0.33 to 0.99. Figure 2 plots the regression on means ($R^2 = 0.99$) observed for spot S004, which revealed polymorphism between *Ae. ventricosa* and *T. aestivum* cytoplasm (Bahrman et al. 1987). Among these 63 spots, ten were qualitatively polymorphic, i.e. present in one line and absent in the other.

Between-population differentiation

Polymorphism

We first verified that the effect of protein loading, estimated by the calibration parameter, was not

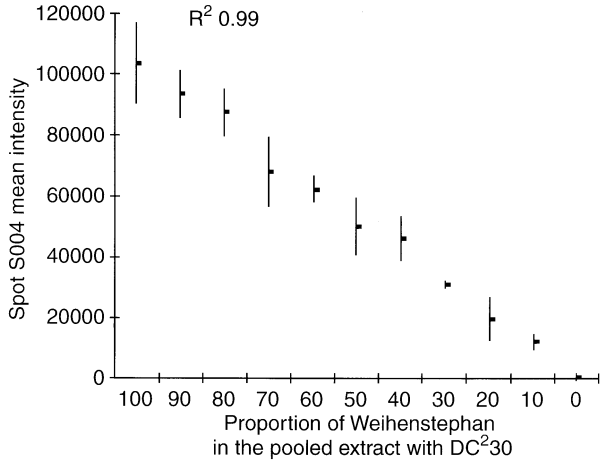


Fig. 2 Regression between the intensity of the cytoplasmic spot S004 and the proportion of Weihestephan 1007/53 (W) in pooled extracts with $DC^2 30$ in a co-electrophoresis experiment, with the proportion of W varying from 0 to 1 by a 0.1 step. Mean values are given with their standard deviation

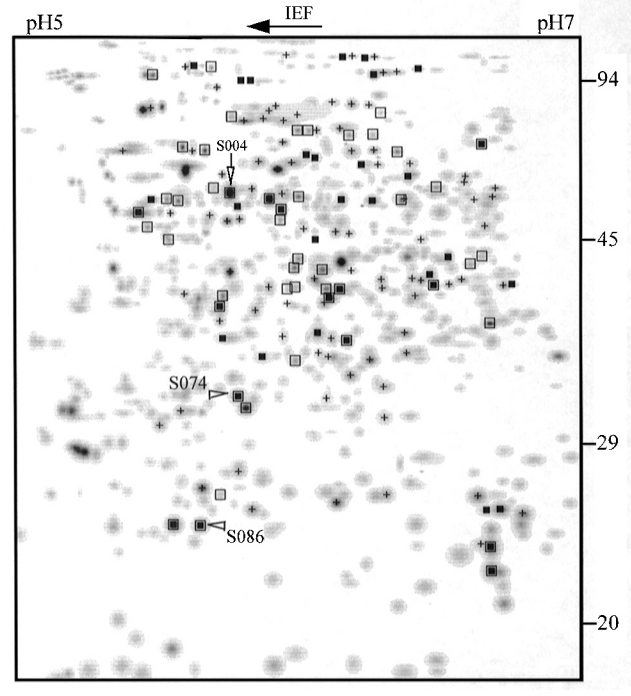


Fig. 3 Processed image of a 2DE gel of the studied populations. *Spots with no symbol* were not included in analyses (more than two missing data out of the six replicates for each population). *Spots with* (+) were not polymorphic either between DC-W or between the studied populations. *Spots with an empty square* are polymorphic between DC-W but not between populations. *Spots with a plain dark square* only have been found polymorphic between populations but not between DC-W. *Spots with both plain dark and empty squares* were found polymorphic both between DC-W and between the populations. The nomenclature of spots is not given here except for three spots (see text) but is available from the authors on request

confounded with the population effect ($P = 0.35$, n.s.), so we could consider that populations had the same calibration parameter mean and that differences observed between populations could be interpreted as differences in genotypic composition. A strong block effect was clearly present ($P < 10^{-3}$) as already observed by Burstin et al. (1993). A total of 393 spots were detected of which 162 fitted the criteria of reproducibility mentioned above (Fig. 3). Out of the 63 spots previously defined in the DC-W experiment, 15 were not included in the latter 162 population list. Among these 15 spots, 11 were light spots, not detected frequently enough in the populations to be used for comparison. The remaining four spots had been found qualitatively polymorphic between DC and W lines, but had medium intensity values when present in DC or W lines. The frequency of their present allele was probably low in the populations, making them undetectable in more than two gels per population. This left 48 spots among the 63 reproducible DC-W spots for use in the study of between-population differentiation.

Spot differentiation

Among the 48 common spots in the DC-W experiment, 33 did not show significant differentiation between populations, while the remaining 15 spots did (Table 2-A). Twenty four spots that were not in the DC-W set showed significant differences between populations (Table 2-B) and were probably polymorphic in the 14 remaining parental lines. Finally 39 spots out of 162 (24%) proved to be polymorphic between populations and underwent differentiation during the 8 years of propagation.

For spots with a presence/absence polymorphism in the DC-W experiment, spot intensities were considered to be strongly related to allelic frequencies, assuming that individual genotypic variation in the intensity of the present spot was low. To contrast the dynamically managed populations, only three of these spots were of interest (Table 3) since the other qualitatively varying spots in the DC-W experiment either showed no significant variation between populations (three spots) or were not present among the 162 reproducible spots (four spots). For the cytoplasmic spot S004, we corrected the reference value since the parental extract did not directly give the intensity value expected in PB_0 for this spot. The *T. aestivum* cytoplasm was present in 14 parental lines out of 16, so that the intensity observed in the co-electrophoresis of the parents ($I_{16\,parents}$) should represent 0.87 of the intensity of spot S004 in a *T. aestivum* cytoplasm. As the expected intensity value in PB_0 should be 0.75 of the intensity in *T. aestivum* cytoplasm (see above), we estimated the expected intensity of spot S004 in PB_0 as $0.75 * I_{16\,parents} / 0.87 = 75\,376$ (Table 3). According to this value, this

Table 2 Differentiation observed for protein spots revealed by 2-dimensional electrophoresis of pooled extracts between two parental lines of composite PB, lines DC²30 (DC) and Weihenstephan 1007/53 (W) and the 8 year-evolved populations of winter wheat derived form PB. A: spots polymorphic between DC and W, whose intensities significantly differed between populations. B Spots monomorphic between DC and W, whose intensities significantly differed between populations

Spots A	Polymorphic between DC and W	Significantly different between populations	Spots B	Significantly different between populations
	R square	Significance level		Significance level
S074	0.95****	****	S098	****
S010	0.74***	***		
S279	0.65**	**	S015, S027, S033	**
S046	0.79***	**	S040, S054, S110	**
S086	0.97****	**	S162, S253, S449	**
S075	0.55**	**		
S133	0.45*	**	S072, S082, S121	*
S145	0.51*	**	S142, S218, S220	*
S062	0.33*	**	S293, S378, S508	*
S344	0.58**	**	S535, S746, S748	*
S089	0.68***	*	S595, S141	*
S004	0.99****	*		
S044	0.59**	*		
S041	0.58**	*		
S006	0.42*	*		

****$P < 0.0001$; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

Table 3 Differentiation observed between wheat populations in mean intensities (six replicates) of three qualitatively polymorphic spots. Populations sharing the same letter were not significantly different at the $P < 0.05$ level with the Ryan-Einot-Gabriel-Welsch Q test (SAS 1988)

Site	Cult.	Spot S004 intensity	Spot 074 intensity	Spot 086 intensity
Chesnoy	Ext	97 687 ab	16 909 ab	18 472 ab
Chesnoy	Int	134 577 a	17 946 ab	16 969 ab
Gif	Ext	98 151 ab	20 915 a	18 290 ab
Gif	Int	100 555 ab	18 731 ab	19 183 ab
Rennes	Ext	91 723 ab	13 952 bc	17 412 ab
Rennes	Int	83 844 b	10 919 c	12 458 b
Toulouse	Ext	101 775 ab	15 937 abc	22 860 a
Toulouse	Int	103 619 ab	18 349 ab	15 508 ab
Venours	Ext	102 423 ab	21 973 a	23 934 a
Venours	Int	118 208 ab	20 933 a	23 763 a
Vervins	Int	99 595 ab	19 537 ab	13 938 ab
16 Parents		87 436 ab	10 195 c	18 013 ab
PB_0		75 376 c	10 195 c	18 013 ab
W[a]		95 529	19 946	30 787
DC		0	0	0

[a] Intensity values obtained in the DC²30-Weihenstephan 1007/53 experiment are given as a complement. They should not be compared directly to the populations means since they were not obtained in the same 2DE experiment

should correspond to a strong increase in the frequency of *T. aestivum* cytoplasm in all populations; the two populations from Rennes showing the highest percentage of the *Ae. ventricosa* cytoplasm and Le Chesnoy Int the lowest. Note that the Le Chesnoy Int population exhibited a significantly higher intensity than W. For the two other spots, S086 and S074, the reference parental value was included in the means observed for the dynamically managed populations, in the middle for S086 and on the lowest part for S074 (not significantly different from the two populations of Rennes and from Toulouse Ext).

Effect of sites and cultivation methods

The site effect was the most important contributor to the observed differentiation (Table 4). Out of the 28 spots whose intensities differed among the restricted set of ten populations (5 sites × 2 methods), 23 differed between sites, four between cultivation methods and five between site × cultivation combinations. The average value of CD_{Site} suggested that approximately 60% of the differences observed between populations could be attributed to the differences between the sites of multiplication. When the cultivation method contributed to population differentiation, it did so through an interaction with site ($CD_{Site \times Cult} = 0.29$) having only little effect by itself ($CD_{Cult} = 0.13$).

Multivariate representation

Five of the eleven discriminant axes were significant at the $P < 0.01$ level and the sixth was significant at the $P < 0.03$ level (data not shown). The first three axes accounted for 71% of the entire variation between populations; the first axis accounting for 40%, the second and the third accounting for approximately 16% each. Figures 4 and 5 show that the first axis discriminated between the co-migration of the 16 parental lines and the evolved populations group, while axes 2 and 3 discriminated between the populations.

Table 4 Contribution to the population differentation of the site of multiplication, the cultivation method and their interaction. The variation observed involves spot mean intensities on a set of ten wheat populations (5 sites × 2 cult. methods). Significant differences observed between site, cult. method and site × cult. method are reported from ANOVA

Spot	Population differentiation	CD_{Site}	$CD_{Cult.}$	$CD_{Site \times Cult.}$
S004	*	0.38	0.25	0.37
S006	*	0.77*	0.00	0.23
S010	***	0.75***	0.13	0.11
S015	*	0.71*	0.00	0.29
S027	*	0.79*	0.21	0.00
S033	*	0.33*	0.00	0.67
S040	*	1.00**	0.00	0.00
S054	**	0.29*	0.00	0.71*
S074	***	0.93***	0.03	0.04
S075	**	0.38*	0.26*	0.35
S082	*	0.66*	0.00	0.34
S086	*	0.56*	0.24	0.20
S098	**	0.44**	0.15	0.41
S110	**	0.07	0.49**	0.44*
S133	*	1.00**	0.00	0.00
S142	*	0.76*	0.00	0.24
S145	*	0.25	0.74**	0.01
S162	*	0.43*	0.08	0.49
S218	*	1.00*	0.00	0.00
S220	**	0.78**	0.06	0.16
S253	**	0.66**	0.10	0.23
S279	**	0.58*	0.15	0.28
S293	*	0.31	0.55*	0.14
S344	**	0.19*	0.00	0.81**
S378	*	0.21	0.04	0.75*
S449	*	0.46*	0.00	0.54
S595	*	0.70**	0.00	0.30
S746	*	1.00**	0.00	0.00
Mean		0.59	0.13	0.29
SD		0.05	0.04	0.05

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

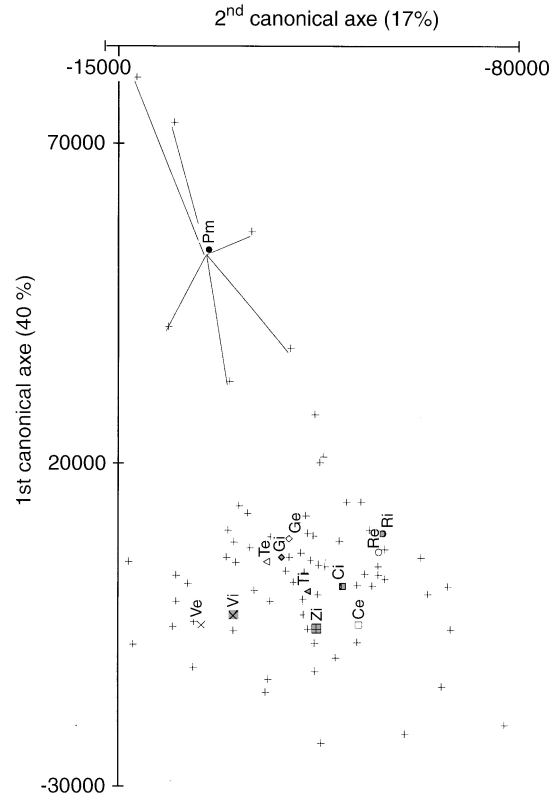


Fig. 4 Discrimination of the populations on the 1st and 2nd "between-populations" canonical axes of a multivariate analysis of variance. Barycenters of commonn sites are indicated with a common symbol (o for Rennes for example). Intensively grown populations are *grey-shaded*, while extensive ones are *white-shaded*. Legend × First character: *R* Rennes; *C* Le Chesnoy, *T* Toulouse; *G* Gif sur Yvette; *V* Venours; *Z* Vervins. Second character: *i* intensity grown; *e* extensively grown. *Pm* co-migration of the 16 parental lines

Individual values were located close to their respective population barycenter (except for one gel from Venours Int), indicating good discrimination with the 2DE data. On the second and third axes, populations originating from the same site were frequently closer to each other than to any other random chosen population, confirming that sites had the largest effect on the differentiation of populations.

Discussion

The DC-W experiment demonstrates that the use of pooled protein extracts is possible for determining the genotypic composition of heterogeneous populations. Among the spots defined with DC-W, many were useful for the larger survey of materials from the network of dynamically managed populations. But our results pointed out that the proposed method can not be used to monitor rare genes or rare spot variants in populations since a minimum threshold value exists below which detection fails. The assumption of equal intensity among genotypes carrying the present allele of a qualitatively varying spot could also not be reliable, as demonstrated for spot S004 (Chesnoy Int > W). Consequently, interpreting differences in spot intensities as differences in allele frequencies should be done carefully for these spots, either because reference intensities had been under-estimated or because a quantitative genotypic variation existed among genotypes for which the spot had been detected (Zivy et al. 1984). Finally, as enough polymorphic spots remained after discarding the low or poorly represented ones, we consider that sufficient information was available to obtain a picture of the differentiation of our populations by treating quantitatively varying spots as quantitative characters. With hexaploid species such as *T. aestivum*, however, there is no clear-cut difference between quantitative and qualitative variations. Some spot intensities can

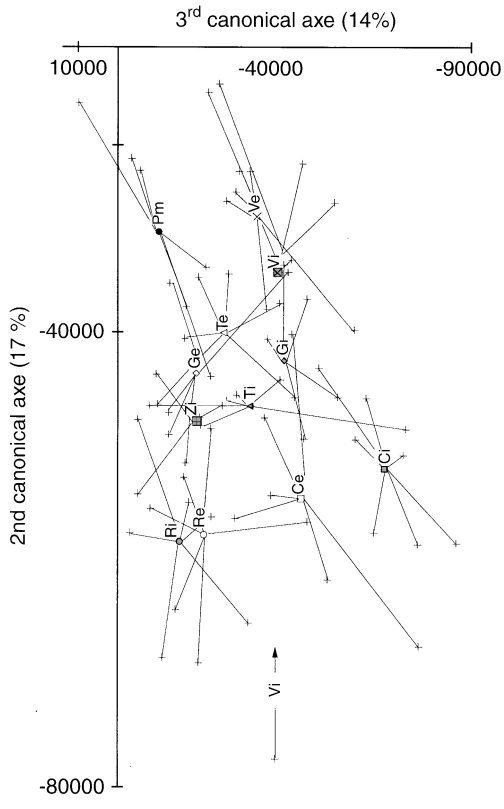


Fig. 5 Discrimination of the populations on the 2nd and 3rd "between-populations" canonical axes of a multivariate analysis of variance. Population barycenters are linked to their individual data (+), common sites are indicated with a common symbol (o for Rennes for example). Intensively grown population are *grey-shaded* while extensive ones are *white-shaded*. Legend × First character: *R* Rennes; *C* Le Chesnoy, *T* Toulouse; *G* Gif sur Yvette: *V* Venours; *Z* Vervins. Second character: *i* intensively grown; *e* extensively grown. *Pm*: co-migration of the 16 parental lines

vary from 0 to 6 doses depending on the homozygosity of each of the three genomes. In wheat homozygous lines, approximately 80% of spots are four- or six-dose spots (Thiellement et al. 1989).

Our results suggested that initial polymorphism was large in the composite population PB, since DC and W supplied a set of 48 reproducible polymorphic spots that were also detected in all studied populations. Approximately 30% of them (15/48) underwent differentiation, leading to significant variation between populations ($P < 0.05$). Furthermore, for the entire set of 162 reproducible spots available among populations, 39 differed significantly in intensity between populations at $P < 0.05$ (20 at $P < 0.01$). Except for the few qualitatively varying spots, it is difficult to consider the variation in intensity of each spot as being due to the frequency of one single independent gene. Spot intensities often depend on complex regulatory systems, involving several genes and exhibiting strong epistatic interplays (Damerval et al. 1994). On one hand, varying frequencies of a single regulatory gene can modify several spot intensities, but absence of differentiation does not necessarily mean that regulation were not modified through selection. Strong selection forces may have acted to maintain the expression level of some proteins, as has been described for morphological traits (Lande and Arnold 1983). Nevertheless, the proportion of genes that underwent a frequency differentiation is suspected to be reasonably important.

Differentiation between dynamically managed populations was accompanied by a global differentiation of the whole group of populations from the parental reference value (approximately 40% of the whole differentiation observed). In previous papers it has been demonstrated that competition between individuals within populations is one of the strongest evolutionary forces acting on these wheat populations. It led to an increase in adult plant height in all studied populations (Leboul'ch et al. 1994). An identical process acting at the biochemical level may explain the distance found between the parental reference value and the evolved populations. If so, competition between individuals within populations will be confirmed as an important force of natural evolution in heterogeneous composites. Nevertheless, the real distance between the dynamically managed population and the PB_0 reference value may be less than that observed with the parental value as a reference value, e.g. PB_0 resulted from crosses between very diverse parental lines: i.e. from Mexican to Russian wheats, with interspecific (with *Ae. ventricosa* and *T. dicoccum*) and integeneric hybridisations, carrying for example the 1B/1R translocation of a chromosomal segment of rye. Recombination between such distant backgrounds may have dramatically disrupted previously co-adapted metabolic regulation chains. New regulatory systems may have been built-up, making the 2DE protein pattern of PB_0 different from the 2DE co-electrophoresis pattern of the 16 parental lines. Pontis (1992) showed that PB_0 was intermediate between populations and parental lines, confirming the reliability of this hypothesis.

Our results suggest that selection for local adaptation acted to differentiate populations in as few as eight generations. Populations managed using intensive cultivation method were often found biochemically close to their extensively grown counterparts at the same site. This is seen in the case of the two populations from Rennes (Venours and Le Chesnoy) and to a lesser extent for the populations from Toulouse and Gif. Even if genetic drift explains some of the observed differentiation, this similar differentiation in paired but independent populations grown at the same site suggests that natural selection for local adaptation is an important evolutionary force responsible for the observed differentiation. Local adaptation could be explained by a response to common climatic conditions [as already suggested for heading date in these populations (David et al. 1992)], to pedological

characteristics, or other selective pressures such as pathogens whose genotypic composition and prevalence are geographically structured (Leboulc'h et al. 1994). In natural populations of wild or primitive cereals, adaptation in response to environmental conditions has frequently been invoked to account for geographic differentiation [seed lysine content in *T. dicoccoides* (Nevo and Beiles 1992), vernalisation genes and heading characters in wheat cultivars (Kato et al. 1988; Kato and Yokoyama 1992)]. Our results suggest that these adapation phenomena occur rapidly. In our experiment, selection gradients among locations embodied the main selective pressures and were sufficiently strong and stable over a 8-year period to cause a parallel differentiation of the 2DE protein patterns of two independent populations grown at the same site. On the other hand, differences in cultivation method within a location had little impact.

Because PB populations are preferential selfers, a combination of genetic drift and selection may have contributed to differentiation of neutral traits between populations at the same geographical sites, through hitch-hiking with selected traits. We do not know if the proteins that underwent a differentiation process have any selective value. We also lack information about the biological role of the proteins observed on 2DE gels and about the relation of variations in 2DE protein patterns of 7-day-old seedlings with the variation in fitness of adult plants in the field. Only cytoplasmic spot S004 could be interpreted in terms of selection, i.e. *Ae. ventricosa* has been used to transfer eyespot (*Pseudocercosporella herpotrichoides* Fron) resistance genes into bred wheat (Dosba et al. 1978). Several genes are implied in the expression of eyespot resistance and the efficiency of some of them seem to be enhanced when expressed in the *Ae. ventricosa* cytoplasm (Dosba and Doussinault 1981). Our experiment suggests that all populations experienced counter-selection of the *Ae. ventricosa* cytoplasm, due surely to a lack of adaptation to a *T. aestivum* nuclear genetic background. Nevertheless at the $P < 3\%$ level, the two populations of Rennes exhibited the highest percentage of *Ae. ventricosa* cytoplasm suggesting that *Ae. ventricosa* cytoplasm had been less counter-selected in Rennes compared with other populations, e.g. eyespot is frequent in the area of Rennes and may have served to maintain the *Ae. ventricosa* cytoplasm. If true, this explanation indicates that geographically structured pathogen pressures can be used to manage the variability of resistance genes in a network of populations.

Conclusion

Demonstrating that populations could rapidly adapt to changes in local selective pressures was a keystone for justifying the dynamic management of genetic resources. The complex differentiation image obtained in our wheat populations (six canonical independent axes) suggests that evolutionary pressures independently and rapidly modified several components of fitness. A second point was to demonstrate that if the observed differentiation was a response to local selective pressure, then the knowledge of the selection gradients at work in the network could help to define strategies of dynamic management. For the results presented here, a portion of these gradients appears to be repeatable and consequently usable for structuring genetic diversity. It then becomes possible to determine if the differentiation of the populations among the network is properly balanced with regard to the genetic diversity initially present, and if gene flow is required to permit the populations to enhance their local adaptation. From the users viewpoint, the research for adapted genetic resources in these populations will be assisted by a knowledge of the characteristics of the local selection forces.

Each of our wheat populations involve approximately 15 000 individuals (N) on a area of 100 m^2. The Ne/N ratio (Ne is the effective size) in wild populations of plants is frequently found to be at least equal to, or larger than, 0.1, rarely lower (Frankham 1995). If such a ratio can be applied to wheat mass reservoirs, small areas will be sufficient at each site to permit natural selection to apply without being much disturbed by genetic drift. By multiplying composite populations in varying environmental conditions, we think that the diversity of crop species or of endangered species for which gene flow is required (Hauser et al. 1994) could be efficiently managed. Controlling population size, selective gradients in a well-balanced network coupled with gene flow in order to achieve local adaptation without disturbing the already adapted regulation mechanisms, may provide an interesting complementary method to static conservation.

Acknowledgements Many thanks to Daniel J. Schoen who provided invaluable comments and help on several earlier drafts of this paper. This work was supported by the French Institut National de la Recherche Agronomique through the PRODIGE funding programme.

References

Allard RW (1988) Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. J Hered 79 : 225–228

Allard RW (1990) The genetics of host-pathogen co-evolution. Implication for genetic resources conservation. J. Hered 81 : 1–6

Anderson NL, Esquer-Balsco R, Hofmann JP, Anderson NG (1991) A two-dimensional gel database of rat liver proteins useful in gene regulation and drug effect studies. Electrophoresis 12 : 907–930

Bahrman N, Thiellemant H (1987) Parental genome expression in synthetic wheats (*Triticum turgidum* sp. *T. tauschii* sp.) revealed by two-dimensional electrophoresis of seedling proteins. Theor Appl Genet 74 : 218–223

Bahrman N, Cardin M-L, Séguin M, Zivy M, Thiellement H (1987) Variability of three cytoplasmically encoded proteins in the *Triticum* genus. Heredity 60 : 87–90

Brush SB (1995) In situ conservation of landraces in centers of crop diversity. Crop Sci 35 : 346–354

Burstin J, Zivy M, de Vienne D, Damerval C (1993) Analysis of scaling methods to minimize the experimental variations in two-dimensional electrophoresis quantitative data. Application to the comparison of maize inbred lines. Electrophoresis 14 : 1067–1073

Damerval C, Maurice A, Josse J-M, de Vienne D (1994) Quantitative trait loci underlying gene product variation – a novel perspective for analyzing regulation of genome expression. Genetics 137 : 289–301

Damerval C, De Vienne D, Zivy M, Thiellement H (1986) Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis 7 : 52–54

David JL, Pham J-L (1993) Rapid changes in pollen production in experimental populations of winter wheat (*Triticum aestivum* L.). J Evol Biol 6 : 659–676

David JL, Savy Y, Trottet M, Pichon M (1992) Méthode de gestion dynamique de la variabilité génétique en milieu natural. Exemple de populations composites de blé. Colloque international en Hommage à Jean Pernès, 8–10 Janvier 1992, Paris, France, pp 337–350

De Vienne D, Burstin J, Gerber S, Leonardi A, Le Guilloux M, Murigneux A, Beckert M, Bahrman N, Damerval C, Zivy M (1996) Two-dimensional electrophoresis of proteins as a source of monogenic and cod-dominant markers for population genetics and mapping the expressed genome. Heredity 76 : 166–177

Dinoor A (1976) Germplasm and the phytopathologist. Plant Gene Res 32 : 36–8

Dosba F, Doussinault G (1981) Les lignées d'addition blé – *Aegilops ventricosa*. I. Etude du comportement vis-à-vis du piétin-verse des différentes lignées obtenues. Agronomie 6 : 503–511

Dosba F, Doussinault G, Rivoal R (1978) Extraction identification and utilization of the addition lines *T. aestivum* and *Ae. ventricosa*. In: Proc 5th Int Wheat Genet Symp, New Delhi, India, pp 332–337

Frankel OH, Brown AHD, Burdon JJ (1995) The conservation of plant biodiversity. Cambridge University Press, UK

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. Genet Res 66 : 95–107

Goldringer I, Pham J-L, David JL, Brabant P, Gallais A (1994) Is dynamic management of genetic resources a way of prebreeding? In: Eucarpia Genetic Resources Section Meeting, Evaluation and Exploitation of Genetic Resources-Prebreeding. 15–18 March 1994, Clermont-Ferrand, France, pp 167–170

Harrison S, Hastings A (1996) Genetic and evolutionary consequences of metapopulation structure. Tree 11 : 180–183

Hauser TP, Damagaard C, Loeschcke V (1994) Effects of inbreeding in small populations: expectations and implications for conservation. In: Loeschcke V, Tomiuk J, Jain SK (eds) Conservation genetics. Birkhaüser Verlag, Basel, Swizerland, pp 115–129

Henry JP, Pontis C, David JL, Gouyon PH (1991) An experiment on dynamic conservation of genetic resources with metapopulations. In: Seitz A, Loeschcke V (eds) Species conservation: a population-biological approach. Birkhäuser Verlag, Basel, pp 185–198

Jana S, Khangura BS (1986) Conservation of diversity in bulk populations of barley (*Hordeum vulgare* L.) Euphytica 35 : 761–776

Kato K, Yokoyama H (1992) Geographical variation in heading characters among wheat landraces, *Triticum aestivum* L., and its implication for their adaptability. Theor Appl Genet 84 : 259–265

Kato K, Ikoma H, Hayashi K (1988) Geographical distribution of the genes for vernalization response and its implication for the adaptability of wheat. In: 7th Int Wheat Gen Symp vol. 1. Cambridge, UK, pp 533–539

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37 : 1210–1226

Le Boulc'h V, David JL, Brabant P, de Vallavieille-Pope C (1994) Dynamic conservation of variability: responses of wheat populations to different selective pressures including powdery mildew. Genet Sel Evol 26, suppl. 1 : 221s–240s

Marshall DR (1990) Crop genetic resources: current and emerging issues. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding and genetic resources. Sinauer Associates Inc Publishers, Sunderland, Massachussets, pp 367–388

Neuhoff V, Arold N, Taube N, Ehrhardt W (1988) Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brillant Blue G-250 and R-250. Electrophoresis 9 : 255–262

Nevo E, Beiles A (1992) Amino-acid resources in the wild progenitors of wheats, *Triticum dicoccoides*, in Israel. Polymorphism and predictability by ecology and isozymes. Plant Breed 108 : 190–201

Olivieri I, Couvet D, Gouyon PH (1990) The genetics of transient populations: research at the metapopulation level. Tree 5 : 207–210

Picard E (1984) Contribution à l'étude de l'hérédité et de l'utilisation en sélection de l'haplodiploïdisation par androgénèse in vitro chez une céréale autogame *Triticum aestivum* L. Thèse d'état n°2902, Université Paris Sud, Paris, France

Pontis C (1992) Utilisation de marqueurs génétiques pour le suivvi de la variabilité de 3 composite de blé tendre d'hiver (*Triticum aestivum* L.) menés en gestion dynamique. Thèse de docteur de l'Institut National Agronomique Paris-Grignon, Paris, France

SAS (1988) Stat user's guide. Release 6.03 Edition. SAS Institute Inc, Cary, North Carolina, USA

Scheffe H (1959) The analysis of variance. John Wiley, New York

Simmonds NW (1962) Variability in crop plants, its use and conservation. Biol Rev 37 : 442–465

Thiellement H, Seguin M, Bahrman N, Zivy M (1989) Homoeology and phylogeny of the A, S, and D genomes of the Triticinae. J Mol Evol 29 : 89–94

Zivy M, Thiellement H, de Vienne D, Hofmann JP (1984) Study on nuclear and cytoplasmic genome expression in wheat by two-dimensional electrophoresis. II. Genetic differences between two lines and two groups of cytoplasms at five developmental stages or organs. Theor Appl Genet 68 : 335–345