

THE USE OF POPULATION GENETICS IN SUNFLOWER RESOURCE MANAGEMENT

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SUMMARY

We have established a methodology of dynamic conservation of genetic resources using population genetic tools. In the present experiment, we created artificial populations composed of wild, interspecific and cultivated genotypes, with natural pollination and natural selection acting at each generation. The present paper shows a) the optimal strategy followed for this allogamous plant, and b) the characterization of the parental populations upon seven quantitative traits. This will allow to follow their evolution during several generations, with a long term objective of increasing genetic resources of Sunflower.

INTRODUCTION

Population genetics, which can be defined as the study of the evolution of gene frequencies in natural populations of a species, can provide powerful tools for plant breeding. Indeed, the natural evolutionary forces (natural selection, drift, gene flow ...) have interesting similarities with plant breeding tools (mass selection, sampling effect, introgression ...). Important investigations have been done on the effect of microevolution on the diversity of natural populations (e.g. Anthonovics *et al.*, 1971; Thurston *et al.*, 1976; Ayafrou & Bell, 1981). One of the research areas of plant breeding which can benefit from population genetics is genetic resource management. Indeed, a common question of both fields is "how genetic diversity can be maintained and/or increased?". Can we use the natural evolutionary forces, which are providing the diversity of the wild, in plant resource management in order 1) to maintain or to increase diversity in crop species 2) to direct diversity evolution toward specific patterns of traits?

This question was deeply analysed by Allard *et al.* who illustrate microevolution dynamics in artificial population experiments on Barley (see Allard, 1988 for a review of 20 papers). Starting from a synthetic population of 28 Barley varieties mixed in 1929 (Harlan & Martini, 1929), more than 50 generations were followed under natural evolutionary forces. Other similar experiments were carried out on Lima Bean (Allard & Workman, 1962), Ray grass (Snaydown, 1970), and Wheat (David, 1992).

We designed a methodology of dynamic resource management for Sunflower with a long term purpose of providing germplasm adapted to drought tolerance. A construction of artificial populations including wild, interspecific and cultivated genotypes, was carried out in four Mediterranean locations, where different types of hydric stress occur. The purposes of this experimentation are to follow the genetic evolution of those artificial populations, and to detect genotypes adapted to Mediterranean types of drought.

MATERIAL AND METHODS

Experimental design

The design consisted in 22 artificial populations, 11 sites and 4 locations. Three locations were in France: Montpellier (4 sites), Valence (2 sites), Toulouse (2 sites), and one in Spain in Cordoba (3 sites) in collaboration with Professor Fernandez-Martinez.

Each site consisted in two populations with different treatments: a control (with irrigation, amendment, fungicide...) and a non-control treatment (no irrigation, no-amendment, no tilling...). Each control population is under an insect proof cage with a beehive inside. The non-control population was isolated by distance to other Sunflower and another beehive was maintained close to it.

Fifteen genotypes were chosen (Fig. 1): five wild species (*H. annuus*, *argophyllus*, *debilis cucumerifolius*, *niveus canescens*, *petiolaris fallax*) five interspecific hybrids (with the same wild species

Figure 1: Plant material for artificial populations

- 5 wild ecotypes :
- *H. annuus sauvage* (376)
 - *H. argophyllus* (585)
 - *H. debilis cucumerifolius* (218)
 - *H. niveus canescens* (197)
 - *H. petiolaris fallax* (200)
- interest
interfertility
drought tolerance
Phomopsis resistance
combining ability
drought tolerance
male-steril cytoplasm
- 5 interspecific hybrids :
- *H. argophyllus* X WG (11)
 - *H. debilis* X MH (13)
 - *H. neglectus* X MH (12)
 - *H. petiolaris petio.* X RHA274 (14)
 - *H. praecox praecox* X WG (15)
- good fertility
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- 5 inbred lines :
- 89B1
 - 89HR3
 - AL5.4
 - HA89
 - RHA265
- good genotype
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TABLE 1 : ANALYSIS OF VARIANCE OF SEVEN MORPHOLOGICAL TRAITS OF PARENTAL POPULATIONS.

Fixed model: $P_{ijkl} = \mu + S_i + G_j + T_k + (L^*G)_{ij} + (L^*T)_{ik} + (G^*T)_{jk} + (L^*G^*T)_{ijk} + E_{ijkl}$

where μ is the grand mean, S_i ($i=1,2,\dots,4$) the site effect of the i^{th} site, G_j ($j=1,2,\dots,5$) the genotypic effect of the j^{th} genotype, T_k ($k=1,2$) the treatment effect of the k^{th} treatment, E_{ijkl} ($i=1,2,\dots,5$) the residual variation, and $(L^*G)_{ij}$, $(L^*T)_{ik}$, $(G^*T)_{jk}$, $(L^*G^*T)_{ijk}$ the interactions between effects.

* P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001; NS: not significant.

	WILD GENOTYPES							model
	EFFECTS							
	S	G	T	S*G	S*T	T*G	S*G*T	
height at maturity	****	****	****	***	****	NS	NS	****
mean head diameter	NS	****	****	NS	NS	*	NS	****
head number	NS	****	***	NS	NS	NS	NS	****
aken number	**	****	NS	****	NS	NS	NS	****
aken weight	*	****	NS	****	NS	NS	NS	****
flowering duration	**	****	NS	**	NS	NS	*	****
blooming date	NS	****	NS	****	NS	NS	NS	****

	INTERSPECIFIC GENOTYPES							model
	EFFECTS							
	S	G	T	S*G	S*T	T*G	S*G*T	
height at maturity	****	****	***	NS	**	NS	NS	****
mean head diameter	NS	****	NS	NS	NS	NS	NS	****
head number	NS	****	*	NS	NS	**	NS	****
aken number	*	*	NS	NS	NS	NS	NS	****
aken weight	***	****	NS	NS	**	NS	*	****
flowering duration	*	**	*	NS	NS	NS	NS	****
blooming date	*	****	NS	NS	NS	NS	NS	****

	LINES							model
	EFFECTS							
	S	G	T	S*G	S*T	T*G	S*G*T	
height at maturity	****	****	****	NS	*	NS	NS	****
mean head diameter	*	**	**	NS	NS	NS	NS	****
head number	////	////	////	////	////	////	////	////
aken number	NS	NS	NS	NS	*	NS	NS	****
aken weight	NS	NS	*	NS	*	NS	NS	****
flowering duration	****	*	NS	****	****	NS	NS	****
blooming date	NS	NS	NS	NS	NS	NS	NS	****

plus *H. praecox praecox*) and five inbred lines. All the genotypes are diploid, annual, interfertile with the Sunflower and are on non-sterilising cytoplasms. The choice of those material was based on specific characteristics of each genotypes (drought tolerance abilities, disease resistance, combining abilities... see Fig. 1). Five plants of each genotypes were taken, which results in seventy five plants per population.

In order to optimize the methodology concerning the multiplication of the populations over several generations, we have followed four of the height populations of Montpellier location during two generations. In those "test populations", general behaviour was followed (phenotypic variability, number, type and vigour of offsprings).

Characterization of parental populations

Quantitative traits were measured on each individual of the artificial populations. We will present here the study done on 8 populations of the 4 sites of Montpellier. Seven traits were scored: height at maturity, mean head diameter, head number, aken number and weight, flowering duration, blooming date.

Analysis of variances were carried out on the compiled data for the 15 genotypes (general model) and on each of the three classes of genotypes (wild, interspecific and lines; grouped model). The fixed model used was: $P_{ijkl} = \mu + S_i + G_j + T_k + (S^*G)_{ij} + (S^*T)_{ik} + (G^*T)_{kj} + (S^*G^*T)_{ijk} + E_{ijkl}$, where μ is the grand mean, S_i ($i=1$ to 4) the site effect of the i^{th} site, G_j ($j=1$ to 15 for the general model and $j=1$ to 5 for the grouped model) the genotype effect of the j^{th} genotype, T_k ($k=1$ to 2) the treatment effect of the k^{th} treatment, E_{ijkl} ($l=1$ to 5) the residual variation, $(S^*G)_{ij}$, $(S^*T)_{ik}$, $(G^*T)_{kj}$, $(S^*G^*T)_{ijk}$, E_{ijkl} the interactions between effects.

Because of high genetic variability of the material used factorial discriminant analysis were carried out on those variables in order to calculate the Mahalanobis distances, which allows to calculate distance between classes while taking into account the among class variations.

A principal component analysis was carried out on one controlled population in order to visualize the structuration of the individuals inside the population for the seven traits.

RESULTS AND DISCUSSION

Optimal methodology

The individuals of artificial populations of Sunflower, while exposed to almost natural pollination conditions, were able to produced seeds in sufficient quantities in order to reproduce itself. The crucial problem of the perpetuation of the populations for both treatments was solved as follow. For the non-controlled populations, the number of heads per individual was scored and half of them were harvested; this will allow to build one pool per population per generation which will be used as a reserve of seeds for eventual new populations and for genetic diversity analysis. The other half of seeds were disseminated naturally, and the population were perpetuated by spontaneous seedlings. Dormancy being important for wild genotypes, overlapping of generations will occur. We observed on the two test non-controlled populations such seedlings (about 30 and 70 plants for each population) appearing in the next generation. Those plants were frequently issued from interspecific crosses (especially *H. annuus* x *H. argophyllus*, Sunflower x *H. annuus*, *H. praecox* x sp.) as can be inferred with morphological traits.

Concerning controlled populations, all the seeds were harvested and counted for each plant. Fifty five offsprings have to be planted for the next generation since the initial row and plant spacing are maintained. The simplest way of sampling would have been to take a number of offsprings per genotype, estimated linearly from the seeds produced by this genotype. In that case, the offsprings would have came only from two or three genotypes which were higher producer (such as wild *Helianthus annuus*). In order to avoid such bias, we use a parabolic curve where the number of offsprings to be plantes (O) is a logarithmic function of the seeds produced (S):

$$O = 75 * \text{Log}(S) / \sum \text{Log}(S)$$

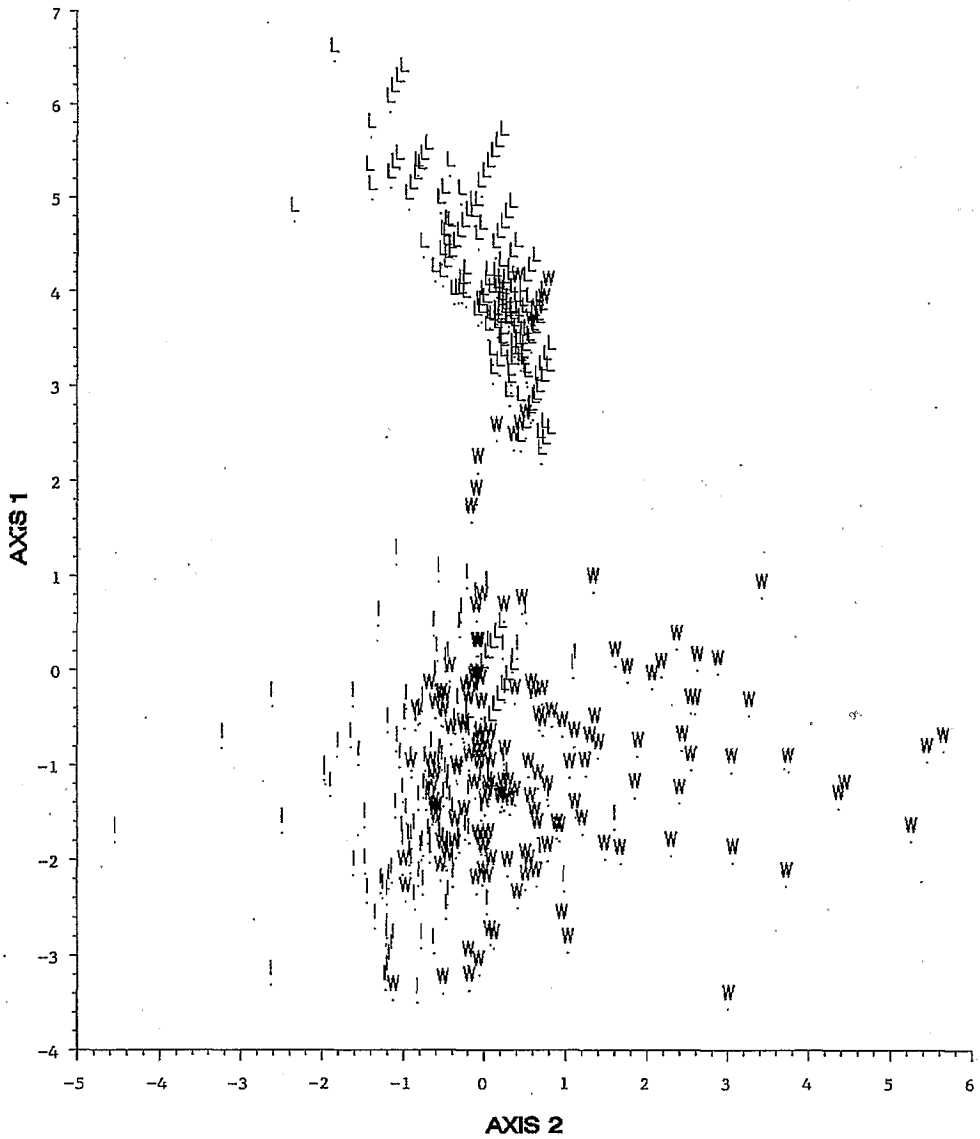


Figure 2: Factorial discriminant analysis of 8 artificial populations for 7 quantitative traits.
 W: Wild genotypes, I: Interspecific genotypes, L: Lines.
 Mahalanobis distance: $d(\text{wild}, \text{interspe.}) = 1.85$; $d(\text{wild}, \text{lines}) = 26.89$; $d(\text{interspe.}, \text{lines}) = 25.32$

In that way, genotypes which were producing a few number of seeds, had some chance to be represented at the next generation.

Characterization of parental populations

The analysis of variance on the seven traits scored was highly significant for the general model. However, for the general model, the homogeneity and normality of variances were not respected for all variable unless height at maturity. Thus, discussion will refer only to the grouped model where genotypes are shared into three classes (Table 1). The model explained significantly the data for all variables for wild and interspecific genotypes, while for the lines, only height at maturity and flowering duration were correctly interpreted. This could be explained by a late planting date of the lines in the 1990 experimentation. Interestingly, those two last traits were always significantly dependant of site and genotype, which mean that as expected, they were simultaneously influence by environment and genetic background. The treatment was significant for three classes simultaneously only for the height at maturity, and did never influence the aken number. The treatment which was supposed to reveal disease resistances, drought tolerance and competition abilities, was in fact mostly different in the starting generation experiment for the last component, because of low level of pathogenic attacks and drought in 1990. The effect of genotype was significant in wild and interspecific classes for all traits, suggesting the high variability of the populations.

This variability was visualized and quantified by the factorial discriminant analysis (Fig. 2). Lines were, as expected, well separated from interspecific and wild genotypes: the Mahalanobis distances (which allows to calculate distance between classes while taking into account the among class variations) were respectively equal to 25.3 and 26.9. However, interspecific and wild classes were much closed ($D^2=1.8$). This should infer some dominance effects of wild genotypes for the quantitative trait scored.

The variability inside a single population was very important as illustrated by the principal component analysis (Fig. 3). While lines were quite grouped for the yield component axis and distributed along half of the flowering and head diameter axis, wild genotypes were dispatched upon the remaining space of the projection. Important variability inside single wild genotypes was revealed. Interspecific class was situated at an intermediary position since it partly overlapped both other classes.

Discriminant analysis of canonical correlations between variables in this controlled population can reveal redundances and allow reduction of the traits to be scored. The variable were formed into the following hierarchy: blooming date, flowering duration, height, head diameter, aken number, head number, aken weight.

CONCLUSION AND PERSPECTIVES

The methodology of dynamic management of genetic resources of Sunflower was defined by the construction of artificial populations where natural evolutionary forces acted. Precise strategies were elaborated in order to perpetuate the artificial population upon generations. The characterisation of the starting generation revealed the high level of variability inside and among populations. Cross pollinations seemed to occur between species and will be confirmed with RFLP and isozymes markers. After several generations of experiments, we will sample some of the best genotypes concerning agronomical traits (such as drought tolerance) and include them in pools, in a long term purpose of Sunflower breeding.

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