Theor Appl Genet (1994) 88:49–55

M. Tersac · P. Blanchard · D. Brunel · P. Vincourt

Relations between heterosis and enzymatic polymorphism in populations of cultivated sunflowers (*Helianthus annuus* L.)

Received: 2 March 1993 / Accepted: 23 July 1993

Abstract Nine polymorphic isoenzymatic systems were studied in 39 cultivated sunflower populations originating from ten countries. Analysis of combining abilities with four tester lines was also performed on these populations for seed yield, seed moisture and seed oil content. The MDH, PGI, PGD and GOT systems appeared to provide the best discrimination of specific combining ability effects with the four testers. The MDH and GOT systems provided a between-population structure that was consistent with the country of origin.

Key words Enzymatic systems · Heterosis · Markers · Sunflower

Abbreviations MDH, Malate dehydrogenase · PGD, phosphogluconate dehydrogenase · PGI, phosphoglucoisomerase · PGM, phosphoglucomutase · ACO, aconitase hydratase · ADH2, alcohol dehydrogenase · GOT, glutamate oxaloacetate transaminase · LAP, leucine amino peptidase · EST, esterases

Introduction

In order to develop new elite commercial hybrids, sunflower breeders have to cross many genotypes and test the offsprings in trials. Field evaluation is quite time consuming and costly. The existence of a relationship between the polymorphism of a polygenic character such as yield and molecular polymorphisms, which are easier to measure, may lead to predictors for high yield and combining abilities. Most of the studies conducted on enzymatic polymorphisms in the *Helianthus* genus have been used for varietal identification or the elaboration of phylogeny from genetic distances (Wain 1983; Kahler and Lay 1985; Anisinova 1987; Rieseberg et al. 1988; Quillet et al. 1992). Only one publication evaluates the relationships between phenotypic and enzymatic polymorphisms (Bazan et al. 1988). These authors studied the relationships between polymorphisms of nine enzymatic systems and the agronomic values of offspring resulting from crosses of 21 cultivated sunflower populations originating from three countries with four homozygous inbred testers.

In maize (*Zea mays* L.) a genetic map is available, and more informations has been published on this subject (Hunter and Kannenberg 1971; Heidrich-Sobrinho and Cordeiro 1975; Hadjinov et al. 1982; Brunel 1985; Price et al. 1986; Lamkey et al. 1987; Lee et al. 1989; Leonardi et al. 1991; Charcosset et al. 1991; Bernardo 1992). Charcosset et al. (1991) studied the linear correlation between a genetic distance index between two parent lines (based on marker loci information) and the heterosis observed in the F_1 hybrid from the two lines for a quantitative character [determined by several loci or by quantitative trait loci QTL)]. They concluded that the prediction of F_1 hybrid heterosis based on marker loci would be more efficient if these markers were selected for their relationships to alleles implicated in the heterotic traits considered. They called these markers "efficient" markers. Bernardo (1992) has used a computer simulation to investigate a genetic model involving incomplete coverage of QTL by molecular markers. He concluded that for the effective prediction of hybrid performance it is necessary that at least 30–50% of the molecular markers be linked to QTL.

With respect to cultivated sunflower where no genetic map currently exists, our research on heterosis predictors led us to study the enzymatic polymorphisms of 39 sunflower populations and to evaluate the relationships between these polymorphisms and heterosis. We chose to characterize enzymatic systems because it is a cheap and fast first approach to the study of molecular variation.

Communicated by G. Wenzel

M. Tersac (✉)
Institut National de la Recherche Agronomique, Station de Génétique et Amélioration des Plantes, 34130 Mauguio, France

P. Blanchard · D. Brunel
SARL LOCUS, Le Sextant rue Perruche, 17000 La Rochelle, France

P. Vincourt
Rustica Semences Domaine de Sandreau Mondonville, 31700 Blagnac, France

Table 1 Allele frequencies for the nine enzymatic systems found in 39 cultivated sunflower populations

Code	Origin	MDH		PGD		PGI		PGM			ACO			ADH2			GOT			LAP			EST		
		a	b	a	b	a	b	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Af 1	Africa	0.33	0.68	0.27	0.73	0.66	0.34	0.13	0.87	0.00	0.00	0.37	0.63	0.46	0.54	0.00	0.17	0.83	0.00	0.00	1.00	0.00	0.01	0.56	0.43
Af 2	Africa	0.36	0.64	0.19	0.81	1.00	0.00	0.33	0.68	0.00	0.00	0.16	0.84	0.41	0.59	0.00	0.22	0.78	0.00	0.05	0.95	0.00	0.24	0.22	0.54
Ar 3	Argentina	0.00	1.00	0.10	0.91	1.00	0.00	0.14	0.86	0.00	0.00	0.16	0.84	0.68	0.32	0.00	0.34	0.66	0.00	0.00	1.00	0.00	0.12	0.59	0.28
Ar 4	Argentina	0.00	1.00	0.44	0.56	1.00	0.00	0.11	0.89	0.00	0.00	0.87	0.13	0.61	0.39	0.00	0.43	0.57	0.00	0.00	1.00	0.00	0.04	0.26	0.70
E 5	Egypt	0.10	0.90	0.70	0.30	0.19	0.81	0.04	0.96	0.00	0.01	0.40	0.59	0.91	0.09	0.00	0.64	0.35	0.01	0.00	0.84	0.16	0.01	0.12	0.86
F 6	France	0.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.62	0.38	0.79	0.11	0.10	0.40	0.58	0.03	0.00	1.00	0.00	0.04	0.80	0.16
F 7	France	0.23	0.77	0.23	0.77	0.94	0.06	0.00	1.00	0.00	0.00	0.07	0.93	0.96	0.04	0.00	0.24	0.76	0.00	0.30	0.69	0.00	0.08	0.66	0.26
F 8	France	0.19	0.81	0.20	0.80	0.75	0.25	0.00	1.00	0.00	0.00	0.20	0.80	0.95	0.05	0.00	0.03	0.97	0.00	0.42	0.58	0.00	0.16	0.84	0.00
F 9	France	0.12	0.88	0.12	0.89	0.61	0.39	0.08	0.92	0.00	0.00	0.72	0.28	0.99	0.01	0.00	0.27	0.72	0.01	0.00	0.75	0.25	0.36	0.44	0.20
F10	France	0.01	0.99	0.00	1.00	0.99	0.01	0.40	0.60	0.00	0.00	0.57	0.43	0.50	0.50	0.00	0.00	0.95	0.05	0.26	0.74	0.00	0.00	0.62	0.38
F11	France	0.09	0.91	0.74	0.26	0.98	0.02	0.00	1.00	0.00	0.00	0.27	0.73	0.80	0.20	0.00	0.35	0.55	0.10	0.00	0.95	0.04	0.12	0.50	0.38
$F12_1$	France	0.00	1.00	0.68	0.33	0.49	0.51	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.08	0.85	0.07	0.00	0.84	0.16	0.00	1.00	0.00
$F12_2$	France	0.00	1.00	0.68	0.32	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.27	0.71	0.02	0.00	0.87	0.13	0.00	1.00	0.00
$H13_1$	Hungary	0.03	0.97	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.01	0.98	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00
$H13_2$	Hungary	0.00	1.00	0.01	0.99	0.99	0.01	0.00	1.00	0.00	0.00	0.01	0.99	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.01	0.99
In14	India	0.28	0.72	0.07	0.93	0.82	0.18	0.04	0.94	0.03	0.00	0.79	0.21	0.98	0.02	0.00	0.00	0.98	0.02	0.00	1.00	0.00	0.00	0.52	0.48
It15	Italy	0.14	0.86	0.36	0.64	0.58	0.42	0.00	1.00	0.00	0.00	0.49	0.51	0.74	0.26	0.00	0.00	0.85	0.15	0.05	0.95	0.00	0.29	0.46	0.25
It16	Italy	0.43	0.56	0.30	0.70	0.92	0.08	0.00	1.00	0.00	0.00	0.22	0.78	0.83	0.17	0.00	0.03	0.97	0.00	0.02	0.98	0.00	0.00	0.98	0.02
M17	Morocco	0.03	0.97	0.01	0.99	1.00	0.00	0.00	1.00	0.00	0.00	0.22	0.78	0.91	0.09	0.00	0.43	0.57	0.00	0.00	1.00	0.00	0.20	0.45	0.35
$M18_1$	Morocco	0.00	1.00	0.16	0.84	0.61	0.39	0.00	1.00	0.00	0.00	0.00	1.00	0.95	0.05	0.00	0.07	0.89	0.04	0.00	1.00	0.00	0.23	0.77	0.00
$M18_2$	Morocco	0.01	0.99	0.16	0.84	0.60	0.40	0.00	1.00	0.00	0.00	0.06	0.94	1.00	0.00	0.00	0.18	0.82	0.00	0.00	1.00	0.00	0.39	0.61	0.00
M19	Morocco	0.00	1.00	0.51	0.49	0.80	0.20	0.14	0.86	0.00	0.00	0.26	0.74	0.69	0.31	0.00	0.36	0.64	0.00	0.00	1.00	0.00	0.00	0.55	0.46
M20	Morocco	0.00	1.00	0.64	0.36	0.50	0.50	0.30	0.70	0.00	0.00	0.04	0.96	0.80	0.20	0.00	0.45	0.55	0.00	0.00	1.00	0.00	0.00	0.54	0.46
M21	Morocco	0.11	0.89	0.81	0.19	0.99	0.01	0.01	0.99	0.00	0.00	0.05	0.95	0.98	0.02	0.00	0.03	0.94	0.03	0.00	0.96	0.04	0.46	0.51	0.03
M22	Morocco	0.07	0.93	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.96	0.04	0.00	0.56	0.44	0.00	0.00	1.00	0.00	1.00	0.00	0.00
M23	Morocco	0.17	0.83	0.10	0.90	0.50	0.50	0.20	0.80	0.00	0.00	0.69	0.31	0.00	0.19	0.81	0.17	0.73	0.10	0.00	1.00	0.00	0.00	0.71	0.29
M24	Morocco	0.00	1.00	0.47	0.53	0.19	0.81	0.00	1.00	0.00	0.00	0.30	0.70	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.86	0.14
R25	Russia	0.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.05	0.95	0.72	0.28	0.00	0.10	0.90	0.00	0.00	1.00	0.00	0.00	0.82	0.18
R26	Russia	0.00	1.00	0.03	0.97	1.00	0.00	0.00	1.00	0.00	0.00	0.34	0.66	0.70	0.30	0.00	0.04	0.62	0.34	0.40	0.60	0.00	0.00	0.67	0.33
R27	Russia	0.00	1.00	0.38	0.62	0.86	0.14	0.00	1.00	0.00	0.00	0.16	0.84	0.80	0.20	0.00	0.00	0.95	0.05	0.04	0.96	0.00	0.33	0.38	0.29
$R28_1$	Russia	0.53	0.47	0.01	0.99	0.99	0.01	0.00	1.00	0.00	0.00	0.54	0.46	0.76	0.24	0.00	0.04	0.93	0.03	0.03	0.97	0.00	0.11	0.39	0.50
$R28_2$	Russia	0.33	0.67	0.09	0.91	0.89	0.11	0.02	0.98	0.00	0.00	0.59	0.41	0.81	0.19	0.00	0.08	0.88	0.04	0.00	1.00	0.00	0.14	0.42	0.44
R29	Russia	0.16	0.84	0.31	0.69	0.62	0.38	0.00	1.00	0.00	0.00	0.60	0.40	0.84	0.16	0.00	0.08	0.92	0.00	0.15	0.85	0.00	0.11	0.71	0.18
R30	Russia	0.33	0.67	0.50	0.50	0.93	0.07	0.00	1.00	0.00	0.00	0.77	0.23	0.96	0.04	0.00	0.00	0.96	0.04	0.03	0.97	0.00	0.00	0.76	0.24
R31	Russia	0.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00
R32	Russia	0.61	0.39	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.88	0.12	0.00	0.00	0.79	0.21	0.00	1.00	0.00	0.07	0.93	0.00
R33	Russia	0.77	0.23	0.53	0.47	0.86	0.14	0.21	0.79	0.00	0.00	0.43	0.57	0.74	0.26	0.00	0.19	0.81	0.00	0.05	0.95	0.00	0.00	0.00	1.00
R34	Russia	0.59	0.41	0.76	0.24	0.51	0.49	0.00	1.00	0.00	0.00	0.76	0.24	0.84	0.16	0.00	0.00	0.98	0.02	0.52	0.48	0.00	0.42	0.28	0.31
R35	Russia	0.85	0.15	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.43	0.57	0.04	0.96	0.00	0.15	0.83	0.02	0.04	0.96	0.00	0.00	1.00	0.00
R36	Russia	0.61	0.39	0.00	1.00	0.53	0.47	0.00	1.00	0.00	0.00	0.35	0.65	0.70	0.30	0.00	0.00	0.36	0.64	0.96	0.04	0.00	0.00	0.44	0.56
T37	Turkey	0.43	0.57	0.14	0.86	1.00	0.00	0.00	1.00	0.00	0.00	0.59	0.41	0.83	0.17	0.00	0.05	0.95	0.00	0.10	0.90	0.00	0.12	0.38	0.50
T38	Turkey	0.24	0.76	0.64	0.36	1.00	0.00	0.00	0.95	0.05	0.00	0.48	0.52	0.94	0.06	0.00	0.50	0.48	0.02	0.02	0.98	0.00	0.19	0.68	0.14
T39	Turkey	0.05	0.95	0.00	1.00	0.64	0.36	0.00	1.00	0.00	0.00	1.00	0.00	0.52	0.48	0.00	0.05	0.95	0.00	0.00	1.00	0.00	0.76	0.24	0.00

Materials and methods

Agronomic polymorphism

We have previously computed and studied the combining values of 39 sunflower populations originating from ten countries with four tester lines (Tersac et al. 1993). We now highlight a relationship between country of origin and specific combining abilities (SCA). Combining ability values and Mandel analyses are used.

Enzymatic polymorphism

Thirteen enzymatic systems were analyzed in the 39 sunflower populations. These populations are listed Table 1, and details can be found in Tersac et al. (1993). Nine of these enzymatic systems were polymorphic: MDH (malate dehydrogenase), PGD (phosphogluconate dehydrogenase), PGI (phosphoglucoisomerase), PGM (phosphoglucomutase), ACO (aconitase hydratase), ADH2 (alcohol dehydrogenase), GOT (glutamate oxaloacetate transaminase), LAP (leucine amino peptidase) and EST (esterases). Analyses were made on 40 seeds of each population. Seeds were allowed to germinate for 24 h at 25 °C in the darkness, then individually crushed in pH 7.4 extraction buffer. After centrifugation, the extract was absorbed onto rectangles of Whatman paper, then loaded onto starch electrophoretic gels. The electrophoreses were carried out using an acid buffer for the PGM, PGD, PGI, ADH, MDH and ACO systems and an alkaline buffer for the LAP, GOT and EST systems. Populations F12, M18 and R28 were analyzed twice to evaluate a 40-seed sample representative of each population. In the H13 population we used two samples of seeds separated by two generations of panmixia to evaluate a possible change in allelic frequencies by panmixia.

Results and discussion

Direct study of allelic frequencies

Examination of allelic frequencies shows that various levels of polymorphisms are correlated to each enzymatic system (Tables 1–3). Most of the populations have a major allele, such as *Mdh-b*, *Pgi-a*, *Pgm-b*, *Adh2-a*, *Got-b* and *Lap-b*, with a mean frequency in all the populations that is at least 0.75. The ACO and EST systems are the most polymorphic (Table 3). Some systems showed rare alleles like *Pgm-c* (In 14, T 38), *Aco-a* (E5 and Spanish populations, data not shown for Spainish populations), *Adh2-c* (F6, M 23), *Got-c* (Egyptian, French, Indian, Italian, Moroccan, Russian and Turkish populations) and *Lap-c* (Egyptian, French and Moroccan populations). When several populations showed one of these rare alleles we found that they have different countries of origin. Therefore, we can suppose that crosses had occurred between these populations or, more likely, that they have common ancestors. We noticed that only 2 populations carry allele *Adh2-c*: the F6 population displays a low frequency of 0.1 while the M23 population has a frequency of 0.8 for this allele. Therefore, the first population may be derived from the second.

Study of the Nei distances

Russian breeding work to convert sunflower from a low-oil content crop to a high-oil content one has shown that most of cultivated oil-type sunflower populations have originated from Russian populations. Therefore, we attempted to measure a genetic distance based on the most common ancestor, and from among the various kinds of distances, we selected the Nei distance (Nei 1972). The dendrogram of the Nei distances is a binary type tree, and this visualization may bend reality (Fig. 1). Nevertheless, we make the following observations. (1) If one considers the control populations from which two samples of seeds were analyzed, the distance is always smaller between these two samples than between two samples coming from 2 different populations (except of R28 where one sample is nearer T 37 than the other sample, but the 2 populations are nevertheless very close). (2) Concerning the H13 population, where two samples of seeds separated by two generations of panmixia were analyzed, the distance between these two samples is 0.001. The allele frequencies do not seem to be changed after two generations of panmixia (3) Our knowledge of the genetic origins of populations is very limited, however we do know that French populations F7, F8 and F10 have common ancestors and that the distance between F7 and F8 has to be closer than between F7 (or F8) and F10. The Nei distances agree with this knowledge. (4) No linkage related to country of origin appears in this dendrogram.

Study of relationships between heterosis and enzymatic polymorphisms

The data concerning combining values and Mandel analyses have been extended from those presented in the previous article of Tersac et al. (1993). To highlight the relationship between enzymatic and phenotypic variability we used three methods: (1) the correlations between phenotypic and enzymatic distances, commonly used in such cases; (2) direct correlations between combining values and allelic frequencies, because we theorize that some enzymatic systems may have

Table 2 Allele frequencies for the nine enzymatic systems found in the four testers

Code	MDH	PGD	PGI	PGM		ACO		ADH2		GOT	LAP	EST		
	a	a	a	a	b	b	c	a	b	b	b	b	c	Nul
T1	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	0.00
T2	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00
T3	1.00	1.00	1.00	0.00	1.00	0.00	1.00	0.50	0.50	1.00	1.00	0.00	1.00	0.00
T4	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00

Table 3 Means by country of origin of alleles frequencies for the nine enzymatic systems found in the 39 cultivated sunflower populations

Origin	Number of population	MDH		PGD		PGI		PGM		ACO			ADH2			GOT			LAP			EST		
		a	b	a	b	a	b	a	b	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Africa	2	0.34	0.66	0.23	0.77	0.83	0.17	0.23	0.78	0.00	0.00	0.27	0.73	0.43	0.56	0.00	0.20	0.81	0.00	0.03	0.97	0.00	0.12	0.39	0.48
Argentina	2	0.00	1.00	0.27	0.73	1.00	0.00	0.12	0.88	0.00	0.00	0.52	0.48	0.65	0.35	0.00	0.39	0.61	0.00	0.00	1.00	0.00	0.08	0.42	0.49
Egypt	1	0.10	0.90	0.70	0.30	0.19	0.81	0.04	0.96	0.00	0.01	0.40	0.59	0.91	0.09	0.00	0.64	0.35	0.01	0.00	0.84	0.16	0.01	0.12	0.86
France	8	0.08	0.92	0.33	0.67	0.78	0.22	0.06	0.94	0.00	0.00	0.56	0.44	0.87	0.11	0.01	0.20	0.76	0.04	0.12	0.80	0.07	0.10	0.73	0.17
Hungary	2	0.01	0.98	0.01	0.99	0.99	0.01	0.00	1.00	0.00	0.00	0.01	0.98	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.01	0.99
India	1	0.28	0.72	0.07	0.93	0.82	0.18	0.04	0.94	0.03	0.00	0.79	0.21	0.98	0.02	0.00	0.00	0.98	0.02	0.00	1.00	0.00	0.00	0.52	0.48
Italy	2	0.28	0.71	0.33	0.67	0.75	0.25	0.00	1.00	0.00	0.00	0.35	0.65	0.79	0.21	0.00	0.01	0.91	0.07	0.04	0.96	0.00	0.14	0.72	0.14
Morocco	9	0.04	0.96	0.32	0.68	0.69	0.31	0.07	0.93	0.00	0.00	0.18	0.82	0.81	0.10	0.09	0.36	0.62	0.02	0.00	1.00	0.00	0.25	0.56	0.19
Russia	13	0.37	0.63	0.20	0.80	0.86	0.14	0.02	0.98	0.00	0.00	0.46	0.54	0.75	0.25	0.00	0.05	0.84	0.11	0.17	0.83	0.00	0.09	0.52	0.39
Turkey	3	0.24	0.76	0.26	0.74	0.88	0.12	0.00	0.98	0.02	0.00	0.69	0.31	0.76	0.24	0.00	0.20	0.79	0.01	0.04	0.96	0.00	0.36	0.43	0.21
** Means**	43	0.25	0.75	0.34	0.66	0.80	0.20	0.08	0.92	0.00	0.00	0.41	0.59	0.78	0.21	0.01	0.19	0.79	0.03	0.04	0.94	0.02	0.10	0.42	0.45

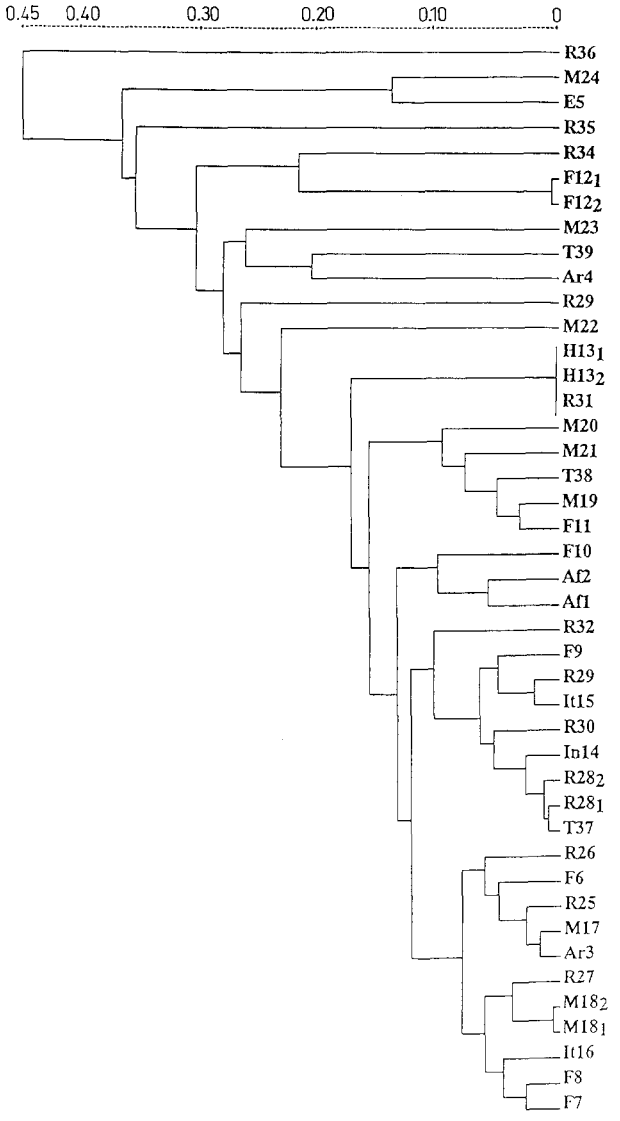

Fig. 1 Dendrogram of Nei distances with the UPGMA method for the nine enzymatic systems

better correlations with agronomic variability than others, and moreover, method 1 fails to take this information in consideration; (3) principal component analysis (PCA) to highlight a possible structure of enzymatic polymorphisms.

Correlations between combining values and Nei distances

Correlations between Euclidian distances were computed on the basis of general GCA and specific combining abilities with four testers and Nei distances. Correlations between Nei distances and GCA are significant at the 0.05 level for the three agronomic characters tested (seed yield, seed moisture content and seed oil content), but correlation coefficients are too low to be used as predictors or general combining abilities (0.24, 0.12 and 0.08, respectively, for the three agronomic characters). Nei distances are not correlated with SCA effects.

Correlations between combining values and allelic frequencies

We theorize that some enzymatic systems may have better correlations with agronomic variability than others, therefore, we computed direct correlations between combining values and allelic frequencies at each locus. An analysis where one or few points had a major weight were excluded. From the significant correlations, shown in Tables 4–6, we conclude that:

1) The alleles *Est-b* and *Est-c* are correlated with the seed yield GCA, while allele *Pgi-b* is correlated with seed moisture content GCA (Table 4).

2) We deduced concordant conclusions both in Tables 5 and Table 6. (1) For seed yield, we observed a positive correlation between *Mdh-a* and tester T4 and a negative correlation between *Mdh-a* and tester T1 (Table 5). Table 6 indicates a correlation between *Mdh-a* and Mandel component 2 that opposes tester T4 to testers T1 and T2. A correlation also appears between the PGI system and tester T2. Table 6 indicates a correlation between the PGI system and Mandel component 2 that opposes tester T4 to testers T2 and T1. (2) For seed moisture content, Table 5 diplays a positive correlation between *Mdh-a* and tester T3 and a negative correlation between *Mdh-a* and tester T2. Table 6 indicates a correlation between *Mdh-a* and Mandel component 2 that opposes tester T3 to tester T2. (3) For seed oil content, Table 5 shows a positive correlation between *Pgi-a* and tester T1 and a negative correlation between *Pgi-a* and tester T2. Table 6 indicates a correlation between *Pgi-a* and Mandel component 1 that opposes tester T1 to tester T2. There also appears to be a correlation between the MDH and GOT systems with tester T1.

3) Only 4 enzymatic systems are correlated to specific combining abilities: MDH, PGI, PGD and GOT. These are, therefore possible "efficient" markers for breeders.

Table 4 Correlations between allele frequencies and general combining abilities of sunflowers populations with the four testers (*CC* Correlation coefficient, *R* probability of *R*, *S* size of population sample)

Allele	Agronomic character	CC	R	S
Est-b	Yield GCA	−0.47	0.006	33
Est-c	Yield GCA	0.44	0.018	29
Pgi-b	Seed moisture content GCA	0.52	0.008	25

Table 5 Correlations between allele frequencies and specific combining abilities of sunflowers populations with the four testers (*CC* Correlation coefficient, *R* probability of *R*, *S* size of population sample)

Allele	SCA population testers	CC	R	S
Mdh-a	T1 (yield)	−0.38	0.046	28
Mdh-a	T4 (yield)	0.48	0.009	28
Pgi-a	T2 (yield)	0.43	0.030	25
Mdh-a	T3 (seed moisture content)	0.56	0.002	28
Mdh-a	T2 (seed moisture content)	−0.60	0.008	28
Pgd-a	T1 (seed moisture content)	−0.37	0.050	29
Mdh-a	T1 (seed oil content)	0.48	0.010	28
Pgi-a	T1 (seed oil content)	0.48	0.016	25
Pgi-a	T2 (seed oil content)	−0.47	0.017	25
Got-b	T1 (seed oil content)	0.41	0.012	36

Table 6 Correlations between allele frequencies and components 1 and 2 of Mandel analysis (*CC* Correlation coefficient, *R* probability of *R*, *S* size of population sample)

Allele	Mandel components	CC	R	S
Mdh-a	C2 (yield)	−0.40	0.036	28
Pgi-a	C2 (yield)	−0.43	0.031	25
Mdh-a	C2 (seed moisture content)	−0.60	0.008	28
Pgi-a	C1 (seed oil content)	−0.44	0.027	25

Principal component analysis

We computed a principal component analysis (PCA) on the allelic frequencies of the nine enzymatic systems to check if a structure exists in these data. Allelic frequencies were used as variables and combining abilities as complementary values. Sunflower populations are the individuals of the PCA. The explained cumulative percentage of the variation for the five principal components are 18.7%, 34.9%, 49.5%, 60.5% and 68.8%.

A study of the variables shows that the first axis is chiefly determined by the MDH system with *Mdh-b* opposite to *Mdh-a*, with a lower contribution by the LAP (*Lap-b* opposite to *Lap-a*) and GOT systems (*Got-b* opposite to *Got-c*) (Fig. 2). The second axis is determined by the PGI system with *Pgi-a* opposite to *Pgi-b* and the PGD system with *Pgd-b* opposite to *Pgd-a*. The study of adding values, that are combining ability values, shows that axes 1 and 2 give the best discrimination of these adding values. Axis 1 states that (1) tester T3 is opposite to tester T4 for seed yield SCA (a lower opposition exists between testers T1 and T2); (2) tester T2 is opposite to tester T3 for seed moisture content SCA; (3) tester T2 and T4 are opposite to tester T1 for seed oil content SCA. Axis 2 states that (1) tester T1 is opposite to tester T2 for seed yield and seed oil content SCA; (2) tester T2 is opposite to tester T1 for seed moisture content SCA. These results are in agreement with oppositions of testers already observed in Mandel analyses (Tersac et al. 1993).

The study of the individuals of the PCA shows a structure correlated to the countries of origin of the populations (Fig. 3). Axis 1 positively separates Moroccan, French and Russian populations, except for the M 23, R 25 and R 31 populations. This clustering is determined by the MDH, GOT and LAP systems. Bazan et al. (1987) found a structure correlated to countries of origin for 21 sunflower populations that is highlighted by the *Got-a* allele of the GOT system and by the SCA of number of leaves. Quillet et al. (1992) found that the MDH system gives the best discrimination between 52 sunflower inbreds. Our work is consistent with these results.

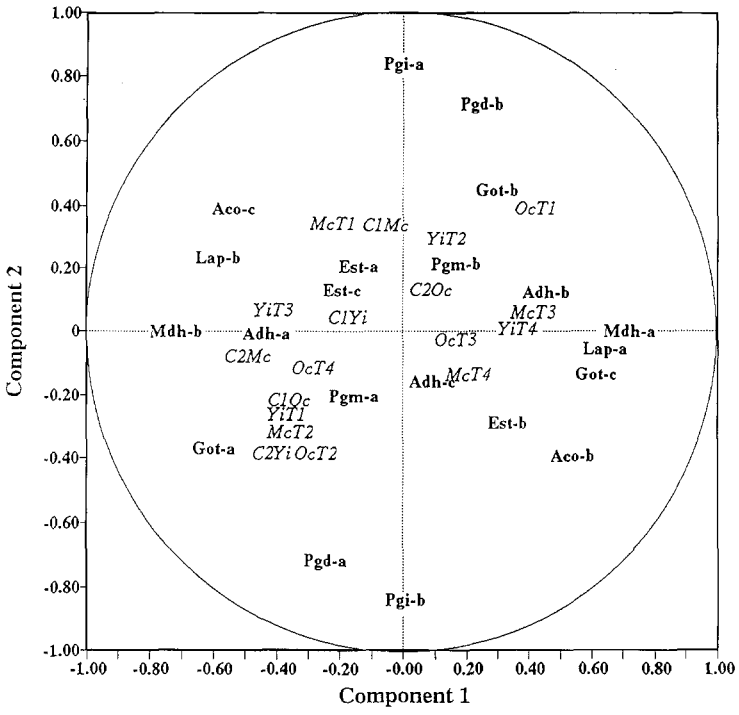

Fig. 2 Correlation circle of the principal component analysis. Allele frequencies are variables (*bold letters*), and combining abilities of sunflower populations are complementary values of the PCA (*italic letters*). The symbols of combining abilities values are: *C1Yi-C2Yi* Mandel component 1 and 2 for seed yield, *C1Mc-C2Mc* Mandel component 1 and 2 for seed moisture content, *C1Oc-C2Oc* Mandel component 1 and 2 for seed oil content, *YiT1-YiT4* SCA of seed yield for testers T1 and T4, *McT1-McT4* SCA of seed moisture content for testers T1 and T4, *OcT1-OcT4* SCA of seed oil content for testers T1 and T4

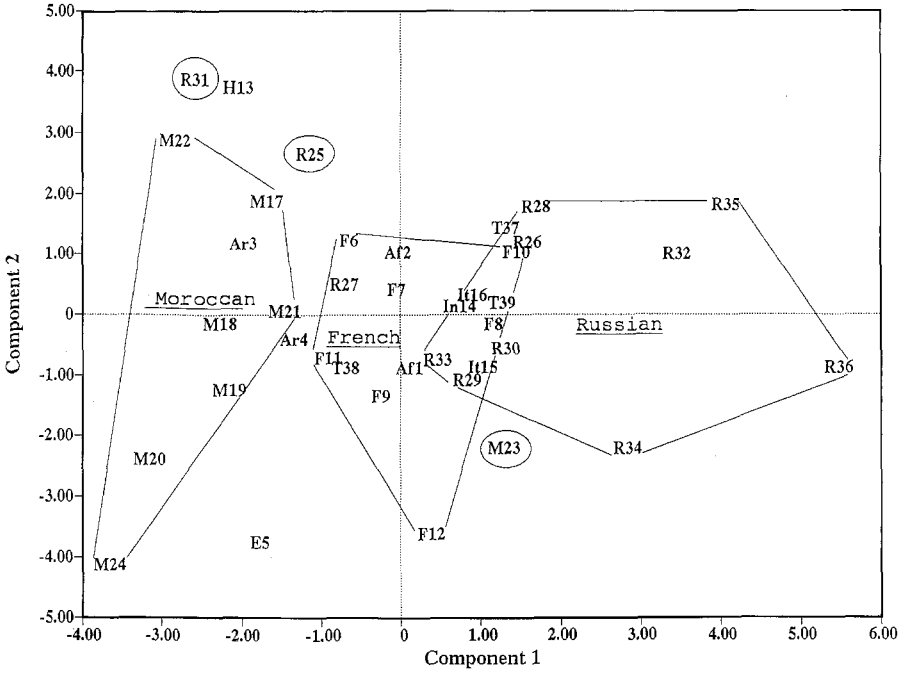

Fig. 3 Principal component analysis: sunflower populations, which are the individuals of the PCA, are clustered by country of origins

Conclusion

From these three approaches of relationships between heterosis and enzymatic polymorphism, we conclude that axis 1 of the PCA highlights a between-population structure consistent with combining groups defined by Mandel analyses (Tersac et al. 1993). This structure is also consistent with the countries of origin of the sunflower populations. Axis 1 of the PCA is determined essentially by the MDH and GOT systems. These systems are more efficient than others in revealing a structure consistent with heterosis and in predicting if a population belongs to one combining group or to another. This result agrees with the conclusions of Charcosset et al. (1991) about "efficient" markers to predict heterosis. In Nei distances, the nine molecular markers have the same weight, and therefore

such a structure cannot be highlighted by a correlation between Nei distance and heterosis because the contributions of efficient markers are diluted by the contributions of other markers.

Acknowledgements We wish to thank A. Bervillé for critical reading of the manuscript. This research was supported by member companies of PROTOURNESOL.

References

Anisinova IN (1987) Identification of sunflower varieties, lines and hybrids by composition of polypeptides of helianthinin. Genet Sel 114:114–126

Bazan O, Blanchard P, Brunel D, Vincourt P (1988) Genetic distances between sunflower populations In: Proc 12th Int Sunflower Conf. Novi Sad, Yugoslavia, pp 478–483

Bernardo R (1992) Relationship between single-cross performance and molecular marker heterozygosity. Theor Appl Genet 83:628–634

Brunel D (1985) Distances génétiques et hétérosis. Utilisation des marqueurs moléculaires. In: de Vienne D, Lefort M (eds) Les distances génétiques. INRA, Paris, pp 159–168

Charcosset A, Lefort-Buson M, Gallais A (1991) Relationship between heterosis and heterozygosity at marker loci: a theoretical computation. Theor Appl Genet 81:571–575

Hadjinov MI, Scherbak WS, Benko NI, Gusen VP, Sukhorzhevskaya TP, Vorona LP (1982) Interrelationships between isozymic diversity and combining ability in maize lines. Maydica 27:135–149

Heidrich-Sobrinho E, Cordeiro AR (1975) Codominant isoenzymic alleles as markers of genetic diversity correlated with heterosis in maize (*Zea mays*). Theor Appl Genet 46:197–199

Hunter RB, Kannenberg LW (1971) Isozyme characterization of corn (*Zea mays*) inbreds and its relationship to single-cross hybrid performance. Can J Genet Cytol 13:649–655

Kahler AL, Lay CL (1985) Genetics of electrophoretic variant in the annual sunflower. J Hered 76:335–340

Lamkey KR, Hallauer AR, Kahler AL (1987) Allelic differences at enzyme loci and hybrid performance in maize. J Hered 78:231—234

Lee M, Godshalk EB, Lamkey KR, Woodman WW (1989) Association of restriction fragment length polymorphisms among maize inbred lines with agronomic performance of their crosses. Crop Sci 29:1067–1071

Leonardi A, Damerval C, Hébert Y, Gallis A, de Vienne D (1991) Association of protein amount polymorphism (PAP) among maize lines with performances of their hybrids. Theor Appl Genet 82: 552–560

Nei M (1972) Genetic distance between populations. Am Nat 106: 283–292

Price SC, Kahler AL, Hallauer AR, Chermley P, Giegel DA (1986) Relationship between performance and multilocus heterozygosity at enzyme loci in single-cross hybrids of maize. J Hered 77:341–344

Quiller MC, Vear F, Branlard G (1992) The use of isoenzyme polymorphism for identification of sunflower inbred lines. J Genet Breed 46:295–304

Rieseberg LH, Soltis DE, Palmer JD (1988) A molecular reexamination of introgression between *Helianthus annuus* and *H. bolanderi*. Evolution 42:227–238

Tersac M, Vares D, Vincourt P (1993) Combining groups in cultivated sunflower populations (*Helianthus annuus* L.) and their relationships with the country of origin. Theor Appl Genet 87:603–608

Wain RP (1983) Genetic differentiation during speciation in the *Helianthus debilis* complex. Evolution 37:1119–1127