THE EFFECT OF TEMPERATURE FROM FLOWERING TO MATURITY ON SEED COMPOSITION OF HIGH OLEIC SUNFLOWER INBREDS AND MID OLEIC HYBRIDS

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Summary:

Two high oleic sunflower lines, one with a stable oleic acid content (80-85%) between years, one showing variation from 60 to 85% according to year, and their hybrids with 2 classic high linoleic lines, were subjected to different temperature regimes from flowering to maturity: (either 20°/14°C day/night or 26°/20°C day/night). Overall analysis of all genotypes and treatments showed that seed oil contents and protein contents were negatively correlated, with a reduction of 1% in oil content and an increase of 0.7% in protein content for a 1°C increase in temperature. The oleic acid content of the stable line changed very little according to temperature (80-85%) whereas that of the unstable line varied by 25%. The hybrids had intermediate oleic acid contents (50-65%), but 3 out of 4 were stable in all temperatures. Only one showed the behaviour of its unstable parent. It is concluded that it is possible to breed stable mid-oleic acid hybrids and that instability is probably controlled by genes which are present in both high oleic and high linoleic genotypes.

Résumé :

Deux lignées de tournesol, une avec plus de 80% d'acide oléique (C18:1) tous les ans, et l'autre qui montre une teneur en C18:1 variant de 60 à 85% selon les années, ainsi que leurs hybrides avec deux lignées classiques linoléiques, ont été soumis à partir de la floraison à différentes températures : températures jour/nuit 20/14°C ou 26/20°C. Sur l'ensemble des expérimentations, teneurs en huile et teneurs en protéines des graines sont inversement corrélées et on peut estimer qu'une augmentation d'1°C de la température à la floraison conduit en moyenne à 1% de diminution de la teneur en huile et inversement 0.7% d'augmentation de la teneur en protéine. On retrouve pour la lignée stable la richesse en acide oléique et la bonne stabilité à la température alors que la lignée instable montre une variation de la teneur en C18:1 selon la température de 25%. Trois des quatre hybrides ont des teneurs intérmédiaires en C18:1 quelles que soient les températures, seul un hybride montre une variation de C18:1 selon la température comme son parent instable. On peut conclure qu'il est possible de sélectionner des hybrides mi-oléiques stables et que les facteurs contrôlant l'instabilité peuvent probablement être originaires aussi bien de lignées classiques, linoléiques, que de lignées riches en acide oléique.

Introduction

It has been known for many years that temperatures above 16°C during flowering reduce oil content in several oil seed crops. Canvin (1965) reported that there was a reduction of 1.2% in oil content for each 1°C rise. Recently, Pritchard et al. (1999) concluded that year and region were far more important factors than cultivar concerning oil and seed protein content of Australian canola crops. Further, oil content varied more with year than with region. On average, oil content fell by 0.38% per 1°C increase in maximum spring temperatures.

Concerning oil composition, Harris et al.(1978) reported that the level of linoleic acid (C18:2) in sunflower crops could fall below 62%, the minimum industry standard for margarine, due to the occurrence of high night temperatures during the seed maturation period. Similarly, Trémolières et al. (1982) showed that a stable low temperature (12°C instead of 27°C) increased oleic acid (C18:1) desaturation, resulting in a higher linoleic content of mature sunflower seeds. A similar temperature response was obtained on low-linolenic flax seeds (Green, 1986), the effect of increased temperature from 15/10°C to 30/25°C was to reduce linoleic acid from 70% to 47% and to increase oleic acid from 17 to 34%. The conversion of stearic acid (C18:0) to oleic acid, showed only slight temperature sensitivity but the oleic acid desaturation step was very sensitive.

Among inbred sunflower lines bred for a high oleic acid content, some have shown stability in the field between years, whereas others have variable oleic acid percentages according to year. It would be useful to be able to distinguish and eliminate the second type of line early in the breeding programmes. Fick (1984) reported a high oleic acid content to be determined by one partially dominant gene, the heterozygotes having intermediate oleic acid levels. Miller et al. (1987) reported that modifier genes were also involved, whereas Fernandez-Martinez et al. (1989) found evidence for several complementary genes. Miller (1992) concluded that part of the explanation for the different genetic controls reported was the fact that the studies were made under different climatic conditions. The aim of this study was to determine whether genotypes with intermediate levels of oleic acid plant always showed instability related to temperature, or whether instability is controlled by genetic factors that could be counter-selected.

Materials and Methods

Sunflower genotypes:

OA (A) and OC (B) are INRA CMS maintainer lines, bred for their high oleic acid content, from crosses made at the F.A.L., Braunshcweig, Germany, between the USDA high oleic pool, NDO1 (from the Russian population Perevenets), and the French hybrid Luciole and the Russian population Salyum respectively. OC produces more than 80% oleic acid in all years, whereas OA has shown an oleic acid percent varying from 60 to 85% according to year. RHA801 (F) and PPR3 (G) are branched restorer lines of the usual high linoleic acid type, bred by USDA and INRA respectively. Hybrids were made using the CMS forms of OA and OC. The two inbred lines were studied in 1997,1998 and 1999, their hybrids with RHA801 (AF and BF) in 1998 and their hybrids with PPR3 (AG and BG) in 1999.

Growing conditions:

The sunflower plants were grown in 2 m² containers under natural environmental conditions with 10 plants/m². At the onset of flowering, the containers were placed into four enclosed tunnels in which CO_2 concentration and hygrometry were controlled and photosynthesis, respiration and evapotranspiration simultaneously and continuously recorded

(Triboi et al., 1996). In 1997, 4 treatments were applied to the plants: 26/20°C (Hot) or 20/14°C (Cold) day-night temperatures with or without water stress (50% of the evapotranspiration of the control), not discussed in this paper. In 1998 and 1999, there was no water stress and 26/20°C or 20/14°C day-night temperatures were applied at the onset of flowering. These temperatures remained constant for two treatments (Hot and Cold) but were switched for the two others. In 1998, the initial temperatures were changed after 330 degree-days (14 days at 26/20°C and 20 days at 20/14°C), but in 1999, this was done after 10 days for both treatments (Hot -Cold and Cold -Hot).

Seed composition analyses:

For each plant, seeds were harvested at physiological maturity (12 replicates for 1997 and 5 replicates in 1998 and 1999) and dried to constant weight at 40°C. Oil content was determined by Nuclear Magnetic Resonance (Brucker Mini-Spec 10). Fatty acid composition was analysed using gas chromatography of methyl esters (Plant Breeding Unit INRA-Rennes). Each fatty acid was expressed as a percent of the total fatty acids. Protein content was estimated by Kjeldahl (N determination x 6.25).

Results

Effects of temperature on oil and protein contents:

Table 1 and Figure 1 present the oil and protein contents of all the seed samples analysed over the three years for the inbred lines, A and B and the hybrids AF, BF, AG and BG, subjected to the treatments Hot, Cold, Hot-Cold and Cold-Hot. An overall analysis for all genotypes and treatments shows a negative correlation between oil content and protein content, with the following equation: Oil % = - 1.11 % Protein + 70.68

The amplitude of variation was about 12 points both for oil content and for protein content. The four points indicated by (*) were not included in the equation as they represent a tunnel with treatment Hot in which there was a climatic incident (halted ventilation, with a large rise in temperature and reduction in hygrometry over 24h). It may be noted that, in this case, there was an exceptional reduction in oil content, but no corresponding increase in protein content. Over the three years and 6 genotypes, the mean oil content with treatment Hot was 43.3% and with treatment Cold, 50.0%. Protein contents were 21.9% and 17.3% respectively. Thus, a reduction of 6°C from the beginning of flowering resulted in an increase in oil content of 6.7% and a reduction in protein content of 4.6%. The mean overall effect was a 1.1% increase in oil content and a 0.7% decrease in protein content for a reduction in temperature of 1°C

The effects of temperature on fatty acid composition:

In the 1997 trials, differences in temperature from flowering to maturity had significant effects on oleic acid content in inbred A but not in B. With 26°C by day and 20°C by night (Hot), A had 78% oleic acid and B 89%; with 20°C by day and 14°C by night (Cold), A had 52% oleic acid and B 88%. In 1998 (Figure 2), the two inbreeds were studied, together with hybrids AF and BF. The inbreeds gave results similar to those of 1997: Hot : 72% oleic acid, Hot-Cold : 64% ; Cold : 45% ; Cold-Hot : 52% . For inbred B, there was very little difference: Hot 81%, Hot-Cold 78%, Cold 81% and Cold-Hot 82 %. In contrast, both hybrids showed intermediate oleic acid contents whatever the temperature conditions: AF: 56%, 50%, 52% and 57% respectively and BF : 57%, 54%, 51% and 61% respectively.

In 1999 trials, the hybrids with the second male parent, AG and BG, were studied with the first temperature period shortened to 10 days from onset of flowering. As for the hybrid BF in 1998, the hybrid BG showed intermediate oleic acid contents whatever the temperature

conditions: 67%, 52%, 61% and 65% respectively, whereas the hybrid AG differed from AF, with a behaviour much closer to its oleic parent A: Hot 72% oleic acid, Hot-Cold 65%; Cold 41%, Cold-Hot 57%.

Discussion

The increase in oil content with low temperatures agrees with the literature. The correlation between this effect and a reduction in protein content is comparable with that reported by Triboi and Renard (1999) on rapeseed, where there was a 5.5% de reduction in oil content and a 6.4% increase in protein content for an increase in temperature of 8°C. In the future, it would be useful to take this effect on seed metabolism into account in studies on seed development related to yield.

Concerning fatty acid content, these experiments in controlled temperature conditions have confirmed field observations of the inbred lines over several years. The intermediate oleic acid contents of the hybrids, which were all heterozygotes for the high oleic acid gene, agrees with semi-dominance proposed by Fick (1984). The results for the two hybrids made with B (OC) and the hybrid AF indicate, however, that instability is not necessarily linked with an intermediate oleic acid content, mid-oleic hybrids can be stable. The result with AF suggested that the instability of the homozygous inbred line OA was recessive, since it did not appear in the hybrid, but the instability of the hybrid AG makes it more likely that even if there is an « instability » gene in OA, the classic type inbreds RHA801 (F) and PPR3 (G) must also have some differences which affect the expression of this gene. Such modifier genes could be the same as those which cause instability in the classic high linoleic hybrids. Thus, it does appear possible to breed stable mid-oleic hybrids if required. Analyses made over several generations in the course of breeding will permit selection of the most stable genotypes, but if an unstable line has highly satisfactory agronomic characteristics, it would appear worthwhile to make a number of hybrids and test whether some of them have stable oil compositions. It would be most useful to conclude as to the existance of « instability » genes in order to select against them for all uses of sunflower oil.

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Table 1. Oil and protein contents of oleic sunflower seeds at maturity.

	Oil %				Protein %			
Temperatures⇨ Genotype/Year ⇩	Hot	Hot-Cold	Cold	Cold-Hot	Hot	Hot-Cold	Cold	Cold-Hot
A 97	46.8 a		47.6 a		23.68 b		20.75a	
A 98	33.6 a*	46.88 b	44.66 b	54.66 b	31.29 c	24.37 b	19.26 a	16.01 a
AF 98	37.82 a*	46.56 b	41.98 ab	47.14 b	24.35 a	20.78 a	23.9 a	20.15 a
AG 99	49.2 a	46.8 a	57.3 b	56.5 b	20.18 b	19.4 b	12.31 a	14.98 a
B 97	46.8 a		50.4 b		22.56 b		18.87 a	
B 98	38.06 a*	48.94 b	47.64 b	54.98 b	22.62 b	18.31 a	15.71 a	15.63 a
BF 98	38.26 a*	53.88 b	52.64 b	55.64 b	16.68 b	16.04 b	14.39 ab	12.27 a
BCG 99	56.2 a	57.2 ab	57.8 b	56.9 ab	13.98 a	13.53 a	13.15 a	13.5 a
Mean	**43.3**		**50.0**		**21.9**		**17.3**	

The values with the same letters are not significantly different ($P<0.05$).

Figure 1. Relationship between Oil and Protein content of oleic sunflower seeds at maturity.

Figure 2. Modification of fatty acids composition : C16:0 + C18:0 saturated acid, C18:1 oleic acid, C18:2 linoleic acid, by temperatures from flowering to maturity: H= "hot" = 26/20°C, H-C= hot then cold, C= "Cold" = 20/14°C, C-H= cold then hot, for 2 inbreds, A and B and their hybrids AF, AG and BF, BG obtained by crossing with 2 male parents F and G. The values (oleic or linoleic acid) with the same letters are not significantly different ($P<0.05$) using the Newman & Keuls test.