TRIACYLGLYCERIDE COMPOSITION IN F1 SEEDS USING FACTORIAL AND DIALLEL CROSSES BETWEEN SUNFLOWER LINES.

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Summary

We produced and studied F1 seed between 10 parent inbred lines, which were simultaneously selfed in the same field experiment. Seven inbred lines were oleic, 3 were linoleic. All oleic lines were issued directly or not from Pervenets population. The traits studied were 1000 seed weight, kernel oil content, and oleic kernel content. Three experimental designs were worked out. In the factorial design, we analysed all the hybrid heads of 47 F1 without the parents. Significant paternal and maternal effects for oil content and oleic content were found. Maternal effect was the major component for oil content. Only significant maternal effects were found for 1000 seed weight. Reciprocal parental effect for oleic content was analysed in a distinct design of 21 F1 hybrids. We found this effect near a limit of signification (p=0.047). The third design was a diallel on seven parent inbred lines with a sample of 2 repetitions (heads) by F1 hybrid and by selfed parent. We confounded reciprocal and residual effects. We found General Combining Ability significant and specific combining ability not significant. These results suggest essentially additive effects. If we adjust these results with the average effect of 1 or 2 rearrangements affecting Δ12-desaturase genomic region, General Combining Ability stays significant. This indicates a genetic variability independent from this genomic rearrangement could be worked out.

Key words: Sunflower, fatty acids, oleic acid, kernel weight, oil content, factorial cross, diallel cross.

Résumé

VARIATION DE LA COMPOSITION EN TRIGLYCERIDES DE SEMENCE F1 DANS DES CROISEMENTS FACTORIELS ET DIALLELES ENTRE LIGNEES DE TOURNESOL.

Nous avons réalisé et étudié les semences d'hybrides F1 et des 10 lignées parentales correspondantes autofécondées dans une expérience de croisement au champ. 7 lignées étaient oléiques et 3 linoléiques. Toutes les lignées oléiques étaient issues de la population Pervenets Les caractères mesurés étaient le poids de 1000 grains, la teneur en huile du grain, la teneur en acide oléique du grain. 3 plans d'expérience ont été analysés. Le plan factoriel comprenait tous les capitules de 47 F1 sans les parents. Des effets paternels et maternels significatifs sont observés pour la teneur en huile et en acide oléique. Par contre seul un effet maternel significatif est observé pour le poids de 1000 grains. Pour la teneur en huile du grain, l'effet maternel est prépondérant. Le plan des effets réciproques pour la teneur en acide oléique était réalisé sur 21 hybrides F1. Cet effet est à la limite de la signification (p=0.047). Le plan diallèle sur la teneur oléique portait sur 7 parents avec un échantillon de 2 descendances par hybride et par parent, nous avons confondu les effets réciproques avec la variance résiduelle. La variance d'aptitude générale à la combinaison est significative contrairement à l'aptitude spécifique à la combinaison. Ces résultats suggèrent des effets additifs prépondérants. Si l'on ajuste les résultats du diallèle par l'effet moyen d'un ou 2 réarrangements affectant la Δ12-désaturase, la variance d'aptitude générale à la combinaison reste significative, ce qui indique qu'une variabilité génétique indépendante de ce réarrangement peut être mise en œuvre.

Introduction

Mutation breeding and recurrent selection for increased oleic acid Triglyceride (TG) content (Soldatov, 1976) then classical breeding yielded impressive results and commercial high oleic hybrids over 80 % oleic content. Xenia i.e. alien pollen effect is commonly observed and isolation zones are to be organised in order to maintain high oleic standards. Recently, it was found that all the high oleic material studied and issued from Pervenets did not accumulate Δ12-desaturase transcript in seeds (Kabbaj *et al.*, 1996; Lacombe and Bervillé, 2000). Moreover it was characterised by a specific RFLP pattern revealed by Δ-12 desaturase cDNA used as a probe indicating an important rearrangement neighbouring or containing a Δ12-desaturase seed specific gene (Lacombe *et al*. 2000). Urie (1985) found a single gene determining high oleic inheritance. However other classic genetic approach results does not agree with a simple inheritance way, and environmental effects on oleic content are well known (Lagravère *et al*., 2000). Miller *et al*. (1987) found a digenic inheritance of high oleic content, Fernandez Martinez *et al*. (1989) a trigenic inheritance. Demurin and Skoric (1996) found an unstable expression of high oleic trait.

In this study the direct analysis of F1 seed issued from a factorial cross design was used as a mode of investigation of some aspects of the heredity of quality related traits. A similar approach was published by Thompson *et al*. (1979) on sunflower about seed oil content and seed weight. Our results will be also compared to these investigations for these characters.

Material and methods

Ten parental inbred lines were used. Ha89 (USA-Canada), FT2603 (INRA-Montpellier), 83HR4 (INRA-Montpellier), are standard linoleic lines used also with their male sterile counterparts (PET1 cytoplasm for Ha89 and FT2603, ms2 for 83HR4). All high oleic lines have Pervenets in their pedigree and the Δ12-desaturase rearrangement mentioned before: OA and OC are issued from INRA (Clermont-Ferrand), HaOl9 from Spain (Cordoba), HOC from Monsanto, Ha342, RHA344 and RHA345 from USDA-NDSU. PET1 male sterile counterparts of these lines were also used for crossing except for the restorer lines RHA344 and RHA345.

The crosses and selfs were obtained manually under paper bags in field nursery conditions near Montpellier by July 1999. F1 and self seed were harvested at maturity and dried at 5% moisture.

Seed oil content was analysed through Nuclear Magnetic Resonance apparatus. Oleic content was analysed by refractive method at 25°C on 10 seeds by head (Goss, 1978) calibrated after regression with a sample of Gas Chromatograph data on 30 contrasted samples of this study

I Factorial design.

47 F1 combinations (118 heads) were studied between the 8 females and 10 male inbred parents with about four crosses by combination. General Linear Model (GLM using Statistica software) was used due to unbalanced parent and repetition effect.

II Analysis of reciprocal effects for oleic content in 20 F1 combinations.

However, we decided to test previously for reciprocal cross effect 20 F1 combinations from 81 heads issued from direct and reciprocal crosses. In order to define a reciprocal effect we defined logically that the cross of higher oleic content female by lower oleic male was direct cross and the inverse the reciprocal cross. General Linear Model (GLM using Statistica software) was used due to unbalanced repetition effect.

III 7*7Diallel cross for oleic content

A subset random sample of data from 2 repetitions (58 heads) of the crosses between 7 lines and the respective selfed parent lines was analysed. In order to minimise the eventual reciprocal effects (see later), we preferably sampled each repetition reciprocal to the other. We decided to confound reciprocal effect and error. We analysed the data with Griffing (1957) 7*7 diallel model I (fixed effects) type 2 (parent lines and F1 without reciprocals).

Results and discussion

I Factorial cross design analysis of 1000 seed weight, seed oil content seed oil oleic content.

The respective evaluation of female and male contribution to F1 seed traits was obtained through variance analysis excluding the selfed parents.

Female and male effect were found significant for seed oil content, oleic acid content of seed oil (Table 1). 1000 seed weight and seed oil content are strongly related with female (maternal) genotype, (F statistic respectively 60.22 and 58.59) but also significantly to male parent for seed oil content (F statistic 2.48). Only female effect was found significant for 1000 seed weight.

Female and male contribution to seed oil oleic content was more balanced (F statistic of 40.91 for female effect compared to 18.34 for male effect). These observations allowed us to interpret F1 seed oil oleic acid content in a diallel design.

II Analysis of reciprocal effects for oleic content in 20 F1 combinations

In this case also General Linear Model analysis was used (Table 2). We found a reciprocal effect with a limit confidence level F statistic of 4.08 (Probability=0.0479). We concluded that oleic content was essentially dependent of the embryo genotype. This allowed us to confound reciprocal and error effect in the subsequent diallel analysis.

III 7*7Diallel cross for oleic content

We found General Combining Ability Mean squares significant and Specific Combining Ability Mean squares non-significant (Table 3 and 5). This suggests preponderant additive effects against dominance effects.

We attempted to test the diallel data excluding the major effect of genomic rearrangement around the Δ12-desaturase gene. Each oleic acid content value was adjusted to the genotype in an additive manner in relation with the number of rearrangements: no change for linoleic genotypes (lines and hybrids), subtraction of 15.74% for semi oleic hybrids (one rearrangement), subtraction of 31.11% for high oleic genotypes (two rearrangements in lines or hybrids). This was done in accordance with analysis of diallel data means in function of categorical rearrangement situation. Diallel analysis was not modified for specific combining ability and error mean squares. However general combining ability stayed very significant (Table 4).

Conclusions

Seed oil content is commonly considered as a maternal inherited quantitative trait (Thompson *et al,* 1979. In the same publication seed weight appeared mainly determined by the female parent with some influence of male parent. Our findings are at variance with these results, we found F1 seed oil content somewhat dependent of male parent. Moreover the relation between male effect and female effect is not obvious: for example oil content for OA female (Least Square Estimation) mean is 30.88 contrasting with 37.52 for OA male oil content mean. Female effect could be somewhat related to the physiological state of maternal plant. In contrast we found no significant effect of male parent on 1000 seed weight.

Reciprocal effects on oleic acid content were for the first time evidenced at our knowledge. However, they are not comparable in magnitude to embryo genotype effects.

The semi-dominance of high oleic content is not always observed. High oleic acid content was found dominant by Dehmer and Friedt (1998) in a family of F2 plants. The amount of dominance effects could depend on genetic background.

Another conclusion of this work is that some genetic variation for oleic acid content seems independent from Δ12-desaturase genetic rearrangement. This variation could be of utmost importance for breeders who work for very high oleic acid types for industry or food. A genetic variability independent from Δ12-desaturase genetic rearrangement could be used by the breeders.

These results were obtained with a broad genetic basis, but with only one rearrangement of Δ12-desaturase genomic region. However, they were observed only in one season and should be repeated.

Acknowledgements: Griffing diallel software was helpfully given by CIMMYT.
Gas Chromatographic analysis was performed by INRA UR GAP, Centre de Rennes.

Table 1: Analysis of variance of male and female effects for 3 traits on all F1 combinations studied.

Sou-rce	D.F.	1000 seed Weight (g)				Oil content (%)				Oleic content (%)			
		Sum Square	Mean Squ.	F test	Prob	Sum Squ.	Mean Squ.	F	Prob.	Sum Squ.	Mean Squ.	F test	Prob.
Fema-le	7	65422	9346	60.22	.0000	4065	580.7	58.59	.0000	10671	1524.5	40.91	.000
Male	9	2505	278	1.80	.0078	221.6	24.6	2.48	.0132	6150	683.3	18.34	.000
Error	101	15675	155			1001	9.9			3763	37.3		
Total	117	88740				5701				19564			

Table 2: Analysis of variance of genotype and reciprocal effect for oleic content on F1 combinations obtained from reciprocal crosses.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F	Probability
Reciprocal	1	147.6	147.6	4.080	.0479
Genotype	19	10744.1	565.5	15.636	.0000
Error	60	2169.9	36.2		
Total	80	13029.8			

Table 3: Griffing 7*7 diallel analysis model I method 2 for oleic content.

Source	DF	Sum of Squares	Mean Square	F	Probability	Variance component
General Combining Ability	6	4058.55	676.42	50.86	.000	73.68
Specific Combining Ability	21	505.19	24.06	1.81	>.05	10.76
Error	28	372.40	13.30			13.30

Table 4: Griffing 7*7 diallel analysis model I method 2 for oleic content with modified data (without Δ12 rearrangement effect).

Source	DF	Sum of Squares	Mean Square	F	Probability	Variance component
General Combining Ability	6	342.95	57.16	4.30	<.01	4.87
Specific Combining Ability	21	505.50	24.07	1.81	>.05	10.76
Error	28	372.62	13.31			13.31

Table 5 Least square means estimation of diallel data for oleic content (% of oil)

	Ha89	OC	HaOl9	OA	2603	83HR4	RHA 344
Ha89	47.6						
OC	61.6	79.9					
HaOl9	72.3	75.8	80.4				
OA	54.4	76.2	71.8	73.0			
2603	53.7	58.9	65.9	53.9	47.8		
83HR4	42.4	63.2	72.1	50.8	42.4	37.0	
RHA344	52.7	77.7	79.6	77.5	65.0	59.6	70.4

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