RECIPROCAL CROSS AND CYTOPLASMIC EFFECTS ON AGRONOMIC TRAITS MEASURED ON ALLOPLASMIC HYBRIDS OF SUNFLOWER (*H. annuus* L.).

Hervé Serieys[1]✉, Yves Griveau[1], François Kaan[2] and André Bervillé[2].
([1]) INRA UR-GAP. Domaine de Melgueil, Chemin de Mezouls. 34130 Mauguio, France.
✉ Fax: + 33 04 67 29 39 90; e-mail : serieys@ensam.inra.fr
([2]) INRA UR-GAP. 2, Place P. Viala F-34060 Montpellier Cedex 1, France.

Résumé

Certains caractères agronomiques chez les hybrides de tournesol sont à la fois sous contrôle des composantes nucléaires et cytoplasmiques. Dans cette étude, les effets réciproques et cytoplasmiques ont été analysés chez des hybrides de tournesol pour le rendement en grains, la teneur en huile, la taille et la date de floraison. Ces caractéristiques ont été évalués dans des essais conduits sur trois combinaisons hybrides nucléaires [WG, RHA265], [WG, HA89] et [HA89, RHA274]. Chaque hybride fut produit (1) en combinaison réciproque des deux lignées parentales et (2) sur des séries de cytoplasmes parmi lesquels: CMS-ANN1, ANN2, ANN3, ANN4, PEF1, ANL2, PET1, PET2 et GIG1. L'ensemble de ces hybrides a été testé en deux lieux (Montpellier et Toulouse), dans un dispositif en blocs aléatoires avec trois ou quatre répétitions.

L'analyse de variance montre l'existence d'effets cytoplasmiques significatifs chez CMS-ANN2, pour le rendement en grains, la teneur en huile et la date de floraison au sein des combinaisons hybrides [WG x RHA26] et [WG x HA89]. Les résultats font apparaître de fréquents effets réciproques significatifs pour la date de floraison, la teneur en huile et le rendement, à l'exception de la taille des plantes. Le bénéfice moyen attribué aux effets réciproques pour le rendement est de l'ordre de 10.7 % pour les combinaisons hybrides [WG, RHA265] et [WG, HA89]. La précocité de floraison est améliorée de 2 à 2.6 jours lorsque la lignée WG est utilisé comme parent femelle respectivement dans les croisements [WG, RHA265] et [WG, HA89], et de 1.2 jours lorsque RHA274 intervient comme parent femelle dans l'hybride [RHA274, HA89]. De même, la teneur en huile augmente significativement de 2.8 % lorsque le lignée WG contribue au croisement [WG, RHA265] en tant que parent femelle. De fréquents effets significatifs d'interaction croisement réciproque par cytoplasme sont également observés. Les effets dus à la composante cytoplasmique pour le rendement, la teneur en huile et la date de floraison apparaissent toujours plus élevés que les effets de croisement réciproque.

Bien que des études complémentaires soient nécessaires, l'information obtenue suggère que la composante cytoplasmique et l'effet de la direction du croisement, peuvent affecter significativement la performance agronomique liée à la valeur du génotype nucléaire de l'hybride.

Summary

Some agronomic traits of sunflower hybrids are mainly under both control of nuclear and cytoplasmic components. In this study, the reciprocal and cytoplasmic effects were analysed in sunflower hybrids for seed yield, oil content, plant height and flowering time. These characteristics were evaluated in trials performed on three nuclear hybrid combinations [WG, RHA265], [WG, HA89] and [HA89, RHA274]. Each hybrid was produced (1) in reciprocal combination of the two parental inbred lines and (2) a series of different cytoplasms among the following: CMS-ANN1, ANN2, ANN3, ANN4, PEF1, ANL2, PET1, PET2 and GIG1. All hybrids were tested in two locations (Montpellier and Toulouse), in a randomised bloc design, with 3 to 4 repetitions.

Variance analysis indicates that significant cytoplasmic effects, related to CMS-ANN2, occurred for seed yield, oil content, earliness for [WG, RHA265] and [WG , HA89] hybrids. Results showed that significant reciprocal effects arose frequently for the following traits: flowering time, oil content and seed yield, but were not significant for plant height. The average benefit for seed yield attributed to reciprocal cross effects in [WG, RHA265] and [WG, HA89] hybrids was 10.7 %. The flowering date was significantly increased by 2.0 and 2.6 days when the inbred line WG was used as female, respectively in the crosses [WG, RHA265] and [WG, HA89] and by 1.2 days, when RHA274 was used as female parent in the [RHA274, HA89] hybrid combination. Similarly, oil content was significantly enhanced by 2.8 %, when WG inbred line was used as female in the [WG, RHA265] cross. Frequent reciprocal cross by cytoplasm interaction effects were also registered. For seed yield, plant height, oil content and flowering date, the effect due to the cytoplasmic component appeared always higher than related reciprocal cross effect.

Although further study is required on the effects of cytoplasmic source and reciprocal crosses, the information obtained would suggest that evaluation of the cytoplasmic component or direction of the cross, may affect significantly the agronomic performance related to the nuclear genotypic value of the hybrid.

INTRODUCTION

Bibliographical reports on different cultivated crops indicated that nuclear genotype mainly controls the level of expression of most phenotypic traits. Another genetic information carried by the cytoplasmic organelles (mitochondria, chloroplast...) interacts with the nuclear genetic information to give the phenotype. The best known cytoplasmic effects are the control of the male fertility in over 140 species (Laser and Lersten, 1972). Considering quantitative traits, Edwards *et al* (1996) reported cytoplasmic effect in maize. It has been shown that in sunflower the level of important agronomic quantitative traits is also governed both by nuclear and cytoplasmic genetic components (Serieys, 1992; Petrov, 1992, Marinkovic *et al*, 1996). Another aspect, is the cross direction effect, independently of the nuclear genotype or cytoplasmic background. For the breeder, it is important to know the potential level of both CMS and reciprocal effects on the agronomic traits.

The purpose of this paper was to quantify the level of cytoplasmic and reciprocal cross effects facing to important agronomic traits such as flowering period, plant height, seed moisture, oil content and seed yield.

MATERIAL AND METHODS

In this study, hybrid combinations were investigated in a multilocal design to estimate on some agronomic traits, the effects of the cross direction and of the cytoplasmic background. We used three hybrid combinations [RHA265, WG], [HA89, WG] and [HA89, RHA274] generated from the inbred lines HA89, RHA265, RHA274 or from the WG INRA inbred line.

The studied cytoplasms originated from the following CMS sources: PET1 the most used cytoplasm (Leclercq, 1969), PET2 (Whelan and Dedio, 1980) and PEF1 (Serieys et Vincourt, 1987) isolated in *H. petiolaris*; GIG1 (Whelan, 1981) found in *H. giganteus*, ANN1, ANN2, ANN3, ANN4 (Serieys et Vincourt, 1987) and ANL2 (Heiser, 1982) obtained from wild *H. annuus* sunflowers.

These cytoplasms were compared across a series of alloplasmic hybrids. Isogenic alloplasmic female lines were developed by successive backcrosses (not less than six generations of backcrossing, providing theoretically an homozygoty level higher than 98.5 %.) Both parental lines of a given hybrid existed under male sterile form, so that creation of reciprocal hybrids was possible as well as hybrid purity secured. The production of alloplasmic hybrids was realised after crossing female isogenic lines, in isolated plots.

So, a total of nine cytoplasmic male sterile sources were compared onto three hybrid combinations, as indicated hereafter.

	Hybrid combination		
Cytoplasm	*RHA265, WG*	*HA89, WG*	*HA89, RHA274*
ANL2	X	X	
ANN1	X		X
ANN2	X	X	X
ANN3		X	X
ANN4	X		X
GIG1	X		
PET1		X	
PET2	X		
PEF1			X

(X) "Cytoplasm x hybrid" combinations (and their reciprocals) tested.

F1 hybrids with ANN1, ANN2, ANN3 and ANN4 cytoplasms did not display any male-fertility restoration, whereas some male-fertile plants were found in F1 hybrids with ANL2. So, forty five and twenty two percent restored plants were observed, respectively, in the hybrids [HA89, WG] and [WG, RHA265]. Similarly, the hybrid [WG, RHA265] expressed 13.5 % and 9.4 % of male-fertility restoration, on GIG1 and PET2 cytoplasms respectively.

The experiments, using complete balanced-block design, were performed at Montpellier and Toulouse. The field trials consisted of 4 replications (plots of 3 rows and 20 plants / row) in the first location and 3 replications (plots of 4 rows and 20 plants / row), in the second one. Flowering date (expressed as difference to July 1st), plant height, seed moisture, seed yield and oil content were evaluated in the two locations. Location, cytoplasm, direction of the cross, and interaction effects, were analysed using the GLM procedure (SAS Institute Inc.). Data related to each of the three hybrid combinations were analysed separately.

RESULTS

Location effects were observed for most characters, except seed yield (Table 1).

1) Cytoplasmic effects

Comparison of alloplasmic hybrids pointed out significant cytoplasmic effects for flowering period, seed yield, and oil content for each hybrid (Table1).

- **Flowering time**

In all three hybrid combinations, the flowering period was significantly affected by the nature of the cytoplasm. According to the considered hybrids, the largest differences in flowering time varied from 2.4 to 2.7 days due to the different cytoplasmic backgrounds. ANN2 induced either significant greater lateness (+ 2.6 and +2.7 days) than ANN3, in [RHA274, HA89] and [WG, HA89] hybrid combinations, respectively; whereas the same CMS source induced the earliest flowering date in the [RHA265, WG] hybrid. These results suggest that flowering time is strongly interacting with the nucleocytoplasmic structure.

- **Plant height**

Significant cytoplasmic effects were observed in the [WG, HA89] hybrid. The highest plants developed when hybrid nucleus was associated to the ANN2 cytoplasm.

- **Oil content**

Significant cytoplasmic effects were observed only in the [HA89, WG] hybrid. The largest range reached 4.6 %, the highest value being found on ANN2 and the lowest on ANL2 cytoplasm (Table 3).

- **Seed yield**

Two hybrid combinations [WG , RHA265] and [WG, HA89] expressed cytoplasmic effects for seed yield. Largest differences in seed yield reached 4.7q. ha^{-1} and 6.1 q.ha^{-1} (related to 13 % and 18% yield increase) respectively, when the ANN2 cytoplasmic background was used (Table 2 & table 3).

2) Reciprocal cross effects

The reciprocal cross effects were determined taking into account the direction of the cross, in the hybrid combinations with similar CMS backgrounds.

- **Flowering date**

The direction of the cross has significant effect on the sowing-flowering duration, in all three F1 hybrids. The duration of the sowing-flowering phase was increased by of 2.1 and 2.6 days when WG was used as female parent in the crosses [WG, RHA265] and [WG, HA89], respectively; and by 1.2 days when the RHA274 inbred line was used as female in the [RHA274, HA89] hybrid.

- **Seed moisture**

This trait was also affected by the direction of the cross, since the utilisation of RHA274 inbred line as female parent in the cross [RHA274, HA89] increased seed moisture by 1.6 %.

- **Oil content**

Seed oil content was significantly affected in the cross [WG, RHA265], where reciprocal crosses produced 1.1 % change in the level of this trait. The WG inbred line induced significant increase in oil content, when used as female parent.

- **Seed yield**

Similarly, the direction of the cross has significant effect on seed yield, in two combinations. The seed yield was increased by 3.2 and 3.0 $q.ha^{-1}$ (squaring with 10.2 % increase), respectively when WG was used as female parent in the crosses [WG, RHA265] and [WG, HA89].

DISCUSSION

The agronomic traits in alloplasmic hybrids frequently appeared modified either by their cytoplamic background or the direction of the cross. In our experiments, significant cytoplasmic effects were registered for flowering period, plant height, seed moisture, oil content and seed yield. In the three hybrid combinations the largest effects due to cytoplasm varied from 13.5 % to 18.2 % for seed yield. Considering oil content, this effect can reach 10 % (between ANN2 and ANL2 cytoplasms). The examination of restoration status indicates that all three hybrids built on ANN2, ANN1, ANN3, ANN4 , PET1 and PEF1 where completely male sterile, while ANL2 cytoplasm induced partial restoration in [HA89, WG] and [WG, RHA265] hybrid combinations. Since ANL2 presented both lower seed yield or oil content and partial male-fertility we suggest that these characteristics could be due to an allocation resource effect related to male-sterility advantage (Vear, 1984). Nevertheless, existence of nucleo-cytoplasmic interactions make difficult the prediction of beneficial effects linked to a specific CMS background (Serieys, 1992).

On an other hand, reciprocal effects were clearly identified for important agronomic traits. The most striking effects are linked to specific female parent used in the cross, for flowering date and seed yield. So, days to flowering varied in the range of 1.2 to 2.6 days, seed yield in the range 3.0 -3.6 $q.ha^{-1}$ and oil content in the range 0.4 to 1.3 to %. In the crosses [HA89, WG], [WG, RHA265], the seed density (thousand seed weight) of the parental inbred lines WG, HA89 and RHA265 was respectively 72.1, 46.2 and 41.2 g. We observe in the two former hybrids, that when WG was used as female the seed yield was significantly increased. These data suggest some possible vigour effects linked to seed size. Oil content of the hybrids was significantly increased by 1.1 % when WG was used as female in [WG, RHA265], but decreased by 1.3 % in the [HA89, WG] hybrid.

These results underline the importance of CMS background and direction of the cross in the performance of sunflower hybrids. These parameters should be taken in consideration in the breeding programmes.

REFERENCES

Edwards J.W., Allen J.O. and Coors J., 1996. Teosinte Cytoplasmic genomes: I. Performance of Maize inbreds with Teosinte Cytoplasms. Crop Sci. 36:1088-1091.

Heiser C B, 1982. Registration of Indiana1 CMS sunflower germplasm (reg Nø GP6). Crop Sci. 22: 1089.

Laser K.D., Lersten N.R.,1972. Anatomy and microsporogenesis in cytoplasmic male sterile angiosperms. Bot. Rev. 38, 38 425-454.

Leclercq P, 1969. Une stérilité cytoplasmique chez le tournesol. Ann. Amélior. plant. 19, 99-106
Serieys H, Vincourt P, 1987. Caractérisation de nouvelles sources de stérilité mâle chez le tournesol. Les colloques de l'INRA. 45: 53-64. INRA, Paris.
Serieys H., 1992. Cytoplasmic effects on some agronomical characters in sunflower. Proc of the 13th Intern. Sufl. Conference. 1245-1250. 7-11 September 1992. Pisa (Italy).
Petrov P., 1992. effects of various cytoplasmic male sterile sources on some sunflower qualities. Proc of the 13th Intern. Sufl. Conference. 1211-1215. 7-11 September 1992. Pisa (Italy).
Marinkovic R., Dozet B., Joksimovic J. 1996. Interdependance among a genotype Cms source and some other charaters of sunflower (*Helianthus annuus* L.). Proc. of the 14th Intern. Sunfl. Conference. 162-167 12-20 June 1996 Beijing (China).
Vear F., 1984. The effect of male sterility on oil content and seed yield in sunflowers (*Helianthus annuus* L.). Agronomie, 4 (9) 901-904.
Whelan E D P, Dedio E, 1980. Registration of sunflower germplasm composite crosses CMG-1, CMG-2 and CMG-3. Crop Sci. 20: 832.
Whelan E D P., 1981. Cytoplasmic sterility in *Helianthus giganteus* L. x *H. annuus* L. interspecific hybrids. Crop Science 21, 855-858.

Table 1. Variance analysis and effects measured on agronomic traits, in three hybrids combinations.

Hybrid	Effects					
	Trait	Location	Cytoplasm	Bloc	Cross direction	Cyto * Cross dir.
RHA265 + WG	Height	***	NS	NS	NS	*
	Flowering	***	***	NS	***	***
	Moisture	***	NS	**	NS	*
	Yield	NS	***	***	***	**
	Oil	***	NS	***	**	NS
HA89 + WG	Height	***	**	**	NS	***
	Flowering	NS	***	NS	***	***
	Moisture	***	NS	***	NS	***
	Yield	NS	***	***	**	***
	Oil	***	***	**	NS	NS
HA89 + RHA274	Height	***	NS	**	NS	NS
	Flowering	**	***	NS	***	***
	Moisture	***	NS	NS	***	NS
	Yield	NS	NS	***	NS	NS
	Oil	***	NS	**	NS	NS

F. test: Significant at $P < 0.001$ (***), $P < 0.01$ (**) or $P < 0.05$ (*). Not significant (NS).

Table 2. Alloplasmic hybrid combinations between WG and RHA265 inbred lines.

a) Cytoplasmic effects.

Cytoplasm	Yield (q/ha)	Height (cm)	Flowering (days)	Seed moisture (%)	Oil content (%)
ANL2	30.44 b*	159.75	7.23 a	5.48	40.70
ANN1	30.54 b	170.83	7.09 a	5.33	40.32
ANN2	34.99 a	166.54	4.80 d	5.26	39.72
ANN4	32.49 b	163.33	5.16 d	5.08	40.48
PET2	30.28 b	164.40	5.87 c	5.13	39.88
GIG1	31.75 b	170.47	6.45 b	5.52	39.77
Significance (Pr > F)	**0.002**	0.070	**0.001**	0.070	0.510

(*) Newman & Keuls test (0.05 level)

b) Reciprocal cross effects.

Female parent	Male parent	Yield (q/ha)	Height (cm)	Flowering (days)	Seed moisture (%)	Oil content (%)
RHA265	WG	30.13	167.25	7.11	5.34	39.58
WG	RHA265	33.37	164.52	5.09	5.26	40.71
Significance (Pr > F)		**0.000**	0.260	**0.000**	0.450	**0.000**

Table 3. Alloplasmic hybrid combinations between WG and HA89 inbred lines.

a) Cytoplasmic effects.

Cytoplasm	Yield (q/ha)	Height (cm)	Flowering (days)	Seed moisture (%)	Oil content (%)
ANL2	26.89 c*	133.87 b	4.13 b	6.28	41.52 b
ANN2	33.01 a	142.44 a	5.70 a	5.71	46.12 a
ANN3	27.71 bc	135.65 b	2.99 c	5.81	44.30 c
PET1	30.53 ab	135.65 b	5.27 a	5.58	45.84 a
Significance (Pr > F)	**0.001**	**0.008**	**0.001**	0.290	**0.002**

(*) Newman & Keuls test (0.05 level)

b) Reciprocal cross effects

Female parent	Male parent	Yield (q/ha)	Height (cm)	Flowering (days)	Seed moisture (%)	Oil content (%)
HA89	WG	28.03	137.79	5.81	5.85	45.12
WG	HA89	31.04	136.01	3.24	5.85	43.78
Significance (Pr > F)		**0.004**	0.320	**0.001**	0.980	0.080

Table 4. Alloplasmic hybrid combinations between RHA274 and HA89 inbred lines.

a) Cytoplasmic effects.

Cytoplasm	Yield (q/ha)	Height (cm)	Flowering (days)	Seed moisture (%)	Oil content (%)
ANN1	32.30	143.13	5.62 a*	6.67	43.52
ANN2	30.66	144.91	6.20 a	6.43	43.60
ANN3	35.15	145.27	3.62 b	5.61	43.43
ANN4	33.99	142.77	3.91 b	6.85	44.50
PEF1	33.84	150.24	6.14 a	6.41	44.24
Significance (Pr > F)	0.061	0.230	**0.001**	0.150	0.450

(*) Newman & Keuls test (0.05 level)

b) Reciprocal cross effects.

Female parent	Male parent	Yield (q/ha)	Height (cm)	Flowering (days)	Seed moisture (%)	Oil content (%)
HA89	RHA274	32.54	145.06	4.51	5.59	44.03
RHA274	HA89	33.84	145.47	5.69	7.21	43.69
Significance (Pr > F)		0.190	0.850	**0.001**	**0.001**	0.440