# EVALUATION OF INBRED TESTERS IN SUNFLOWER HYBRID BREEDING

**J. DOMINGUEZ GIMENEZ, J. FERNANDEZ MARTINEZ**

Department of Breeding and Agronomy, S.I.A., D.G.I.E.A., Junta de Andalucía, Córdoba (Spain)

## INTRODUCTION

The use of sunflower (*Helianthus annuus* L.) $F_1$ commercial hybrids is currently widespread in Spain with a percentage of 65% of the total sunflower cropped area which surpassed one million hectares in 1986. For that reason the primary objective of most of the sunflower breeding programmes is the development of inbreds with superior hybrid performance. Their successful obtention depends upon the accurate evaluation of inbred genotypes in hybrid combinations. This evaluation requires reliable methods for testing the potential of the new breeding lines, which involves selection of the most adequate testers.

The choice of a tester is always a complex decision in any breeding programme and depends on the ultimate goal of the breeder.

In population improvement, the different schemes of selection based on testcrossing, lead in most of the cases to a change in the population mean independently of the tester used, being difficult to distinguish between general and specific combining ability (g.c.a. and s.c.a.) (Hallauer and Miranda, 1981).

In line evaluation the ideal tester should reveal the maximum genetic differences among the lines under study, discriminating and classifying correctly their relative performances. Moreover, it is important that this information will be useful in predicting the performance of the lines when used in other combinations.

In corn, (*Zea mays* L.), since the number of hybrid combinations increases with increased number of lines, breeders developed a system of initial selection in top cross combinations for g.c.a., using a broad genetic basis tester followed by testing for s.c.a among lines exhibiting high g.c.a. (Jenkins and Brunson, 1932). More recently, the use of inbreds as testers has been studied in this crop (Horner *et al.*, 1973 and Russell *et al.*, 1973). They showed improvement in the g.c.a. as well as in the s.c.a with testcross selection using an inbred tester.

The development and use of broad genetic basis testers in sunflower has never been reported. The fact that sunflower inbred lines are produced on the basis of cytoplasmic malesterility renders difficult the development and the use of those type of testers, mostly when the inbred lines to test are restorer ones, since the tester should be cytoplasmic male-sterile or have been mechanically or chemically malesterilized. The use of inbred lines as testers was reported early in sunflower breeding (Unrau, 1947). He suggested that the best method for testing inbred lines for combining ability was the use of two testers lines with good combining ability. More recently, Miller *et al.* (1980) has also reported the use of outstanding lines as testers for evaluating inbred lines, a practice widely extended in sunflower breeding programmes. An important requirement of this method of testing is the existence of large additive effects, as in the case of corn (Sprague and Eberhart, 1977). In sunflower, additive effects have been reported extensively for oil content (Putt, 1966 ; Fick, 1975 ; Skorič, 1978 and Miller *et al.*, 1980). For seed yield early studies reported that s.c.a was more important than g.c.a. However, Anaschenko (1974) found a high heritability for yield (60—86%) suggesting additive gene actions and Miller *et al.* (1980) found additive effects to be of first importance for yield. A second requirement in inbred line evaluation, using inbred testers, is the accuracy in ranking relative performance of lines, especially when a large number of them are evaluated. Miller *et al.* (1980), used two inbred sunflower restorer lines and their single $F_1$ cross as testers for evaluation of 10 cytoplasmic sterile inbred lines. They concluded that for seed yield the three testers did not classify female lines identically but they did identify the top four lines when the top 40% for yield was selected.

We have carried out since 1974 a sunflower breeding programme for increasing yield under mediterranean conditions using oil content, earliness and resistance to downy mildew as

main criteria of selection (Downes, 1974 and Dominguez *et al.*, 1978). The starting base population was formed recombining germplasm from different origins with a variable degree of earliness and oil content. Base population was subsequently divided into two groups, with and without restorer genes respectively (Downes, 1974). One of the ultimate goals of this programme was to develop, from these populations, female and restorer inbreds to be combined between them or with foreign lines for producing $F_1$ hybrids. The objective of the work described in this paper was to determine the relative effectiveness of two cytoplasmic male sterile lines — one foreign and another derived from the mentioned starting population — for testing inbred restorer lines obtained in our breeding programme and derived from the same starting population.

## MATERIALS AND METHODS

Two female inbred lines (A—1 and HA 89) converted into cytoplasmic male sterility were used as testers in this study. The two tester lines were chosen as lines related and unrelated to the tested ones.

A—1 is a cms line, early flowering under our conditions, with reasonable level of oil content (45—48%), developed by selfing and selecting in a B population formed at the beginning of our breeding programme in 1974. This line has proved to be a fair combiner in preliminary tests.

HA 89 is a cms line, late flowering under our conditions, medium oil content, selected from the Russian open pollinated variety VNIIMK 8 931 and released by the U.S.D.A. This line is widely used in hybrid sunflower production all around the world and has proved to be an excellent combiner.

Twenty one randomly selected restorer inbred lines obtained in our breeding programme from the same base population as the line A—1 were crossed to the two testers in the summer of 1983 by hand pollination of previously bagged tester capitula.

Hybrid seed was planted under rainfed conditions in three locations in Southern Spain fairly representative of the sunflower cropped area. The experimental design for the three locations was a randomized complete block design with three replications. Hybrids were planted in four rows 10 m long, 75 cm between rows, with 9 m of the two central rows harvested. Plantings were made on March 22, 1984 in the three locations. Thinning at four leaves stage was made to leave 40 plants/10 m row. Capitula were harvested by hand and threshed in a Hege microcombine. Single plot yields were recorded and transformed into kg/ha. Analysis of variance for each location was performed as well as the combined ANOVA for the three locations. The following models were used :

For each individual location,

$$T_{ijk} = u + r_i + a_j + t_k + f_{jk} + l_{ijk}$$

where $u$ denotes the mean of all hybrids,

$r_i$ represents the effect of the ith replication,

$a_k$ is the effect of the jth restorer inbred line,

$t_{jk}$ is the effect of the kth sterile tester,

$at_{jk}$ is the interaction effect of the jth restorer with the kth sterile tester,

$l_{ijk}$ is the random error associated with the ith replication of the dth restorer with kth tester.

For the combined experiment,

$$T_{yk} = u + l_i + r(l)_{ij} + s_k + l_{sik} + e_{ijk}$$

where $u$ denotes the mean of all hybrids,

$l_i$ represents the effects of the ith location,

$r(l)_{ij}$ is the effect of the dth replication within the ith location

$s_k$ is the effect of the kth hybrid,

$l_{sik}$ is the interaction of the ith location and the kth hybrid,

$e_{ijk}$ is the random error associated with the ith location and with jth replication of the hybrids.

In the combined experiment the sum of squares (SS) due to hybrid effects was partitioned in SS due to restorer inbred lines, SS due to female tester and SS due to the interaction restorer × testers.

The sum of squares (SS) due to the interaction location × hybrid was also partitioned in SS due to restorer inbred line × location, SS due to female tester × location and SS due to triple interaction : line × tester × location.

## RESULTS AND DISCUSSION

Yields of the individual locations and their mean for each inbred restorer and tester are given in Table 1. It is noticeable that mean yields of the A—1 hybrids were in all cases higher than the ones with HA—89. Seed yields were variable with locations, averaging over testers 971, 1 320 and 2 146 kg/ha. There were significant differences between restorer lines although lines R—45, R—46 and R—56 gave hybrids with consistently higher yields.

Analysis of variance of individual locations showed significant effects of restorer, tester and interaction restorer × tester indicating that the two testers did not rank the inbred restorers identically in each location (Table 2). Combined experiment analysis showed significant effects for restorer lines and testers and not for the interaction of restorer with testers, suggesting that restorer lines are ranked simi-

*Table 1*

**Yield of sunflower hybrids between 21 restorer inbred lines and two testers (A-1 and HA-89) at there different locations**

| Restorer line | Location 1 | | | | Location 2 | | | | Location 3 | | | | Mean over the three locations | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | x A-1 | | x HA 89 | | x A-1 | | x HA 89 | | x A-1 | | x HA 89 | | x A-1 | | x HA 89 | | Average over testers and locations | |
| | Yield kg/ha | Rank | Yield kg/ha | Rank | Yield kg/ha | Rank | Yield kg/ha | Rank | Yield kg/ha | Rank | Yield kg/ha | Rank | Yield kg/ha | Rank | Yield kg/ha | Rank | Yield kg/ha | Rank |
| R-21 | 718 | 20 | 934 | 11 | 1 226 | 18 | 1 380 | 5 | 2 275 | 13 | 1 818 | 19 | 1 406 | 20 | 1 377 | 8 | 1 391 | 18 |
| R-23 | 810 | 18 | 953 | 9 | 1 410 | 15 | 1 470 | 1 | 2 541 | 3 | 2 195 | 4 | 1 587 | 10 | 1 539 | 3 | 1 563 | 4 |
| R-32 | 1 045 | 12 | 968 | 6 | 1 515 | 11 | 1 050 | 13 | 2 031 | 19 | 1 948 | 12 | 1 530 | 14 | 1 322 | 12 | 1 426 | 14 |
| R-33 | 1 276 | 1 | 916 | 12 | 1 665 | 5 | 1 038 | 14 | 2 598 | 2 | 1 833 | 18 | 1 846 | 1 | 1 262 | 17 | 1 554 | 5 |
| R-34 | 968 | 16 | 767 | 19 | 1 098 | 20 | 1 090 | 11 | 2 248 | 15 | 1 916 | 14 | 1 438 | 19 | 1 257 | 18 | 1 347 | 21 |
| R-35 | 1 071 | 9 | 842 | 17 | 1 665 | 4 | 1 166 | 9 | 2 455 | 4 | 1 990 | 11 | 1 730 | 6 | 1 332 | 11 | 1 531 | 8 |
| R-36 | 1 155 | 6 | 915 | 13 | 1 758 | 3 | 896 | 21 | 2 335 | 11 | 2 043 | 7 | 1 749 | 4 | 1 284 | 14 | 1 516 | 9 |
| R-37 | 1 066 | 10 | 978 | 4 | 1 911 | 2 | 911 | 19 | 2 040 | 18 | 1 728 | 21 | 1 672 | 7 | 1 205 | 21 | 1 438 | 13 |
| R-38 | 1 040 | 13 | 940 | 10 | 1 488 | 12 | 1 263 | 7 | 2 195 | 17 | 2 030 | 9 | 1 574 | 11 | 1 411 | 6 | 1 492 | 10 |
| R-39 | 1 185 | 4 | 913 | 14 | 1 650 | 6 | 1 000 | 15 | 2 631 | 1 | 1 918 | 13 | 1 822 | 3 | 1 277 | 16 | 1 549 | 6 |
| R-40 | 988 | 15 | 886 | 16 | 1 408 | 16 | 1 206 | 8 | 2 285 | 12 | 2 017 | 10 | 1 560 | 12 | 1 369 | 9 | 1 464 | 12 |
| R-41 | 1 058 | 11 | 776 | 18 | 1 136 | 19 | 996 | 16 | 2 257 | 14 | 1 876 | 16 | 1 483 | 17 | 1 216 | 20 | 1 349 | 20 |
| R-43 | 1 178 | 5 | 1 038 | 2 | 1 483 | 14 | 1 300 | 6 | 2 342 | 10 | 1 913 | 15 | 1 667 | 9 | 1 417 | 5 | 1 542 | 7 |
| R-45 | 1 266 | 2 | 1 078 | 1 | 1 508 | 12 | 1 460 | 2 | 2 443 | 5 | 2 191 | 5 | 1 739 | 5 | 1 576 | 2 | 1 657 | 1 |
| R-46 | 1 211 | 3 | 904 | 16 | 1 968 | 1 | 988 | 17 | 2 358 | 8 | 2 113 | 6 | 1 845 | 2 | 1 335 | 10 | 1 590 | 3 |
| R-49 | 951 | 17 | 960 | 8 | 1 260 | 17 | 1 088 | 12 | 2 357 | 9 | 1 796 | 20 | 1 522 | 15 | 1 285 | 15 | 1 401 | 16 |
| R-50 | 745 | 19 | 681 | 21 | 1 530 | 10 | 896 | 20 | 2 233 | 16 | 2 290 | 1 | 1 502 | 16 | 1 289 | 13 | 1 395 | 17 |
| R-51 | 1 111 | 7 | 961 | 7 | 1 590 | 7 | 930 | 18 | 1 941 | 20 | 1 873 | 17 | 1 547 | 13 | 1 254 | 19 | 1 414 | 15 |
| R-56 | 1 090 | 8 | 1 029 | 3 | 1 531 | 9 | 1 450 | 3 | 2 383 | 6 | 2 263 | 2 | 1 668 | 8 | 1 580 | 1 | 1 624 | 2 |
| R-57 | 1 036 | 14 | 976 | 5 | 1 565 | 8 | 1 446 | 4 | 1 813 | 21 | 2 031 | 8 | 1 471 | 18 | 1 484 | 4 | 1 477 | 11 |
| R-61 | 678 | 21 | 766 | 20 | 925 | 21 | 1 138 | 10 | 2 380 | 7 | 2 240 | 3 | 1 327 | 21 | 1 381 | 7 | 1 266 | 19 |
| $\bar{x}$ | 1 030 | | 913 | | 1 490 | | 1 150 | | 2 292 | | 2 001 | | 1 604 | | 1 354 | | 1 475 | |
| LSD | 238 | | 238 | | 227 | | 227 | | 205 | | 205 | | 282 | | 282 | | | |
| $\bar{x}$ Over testers | 971 | | | | 1 320 | | | | 2 146 | | | | 1 479 | | | | | |

*Table 2*

**Mean squares of yield (kg/ha) for each individual location as well as for the combined data**

| Source of variation | df | Location 1 | Location 2 | Location 3 | Combined |
|---|---|---|---|---|---|
| Restorer lines | 20 | 98 997 ** | 118 182 * | 115 262 ** | 285 787 ** |
| Testers | 1 | 158 639 ** | 3 630 524 ** | 2 494 480 ** | 5 541 358 ** |
| Restorer x tester | 20 | 126 356 ** | 200 176 ** | 170 365 ** | 41 235 ns |
| Restorer lines location | 40 | — | — | — | 12 364 * |
| Tester x location | 2 | — | — | — | 4 561 ns |
| Restorer x tester x location | 40 | — | — | — | 181 209 ** |

* significant at 0.05 probability level
** significant at 0.001 probability level
ns — non significant.

larly by the testers. In this case yields of the HA 89 hybrids were very similar and the differences between them were in most of the cases nonsignificant. Interaction tester × location was nonsignificant, the A—1 hybrid yields being consistently higher. The triple interaction tester × line × location was highly significant, which might be caused by differences in soil availability moisture and temperature at blooming time in each of the three locations, which could produce differential effects on hybrids of different maturities.

Interpretation of results regarding the choice of the tester is related to the definition of what constitutes a "good" tester. Rawling and Thompson (1962) defined a good tester as the one which classifies correctly the relative performance of lines ranking the crosses in the same order as the appropiate g.c.a. of their tested parents and secondly discriminates efficiently among lines under test. In the present study, since the true values of the relative performance levels of restorers are not previously known it is not possible to surely establish the goodness of the testers in predicting the performance of the lines under test in different combinations. However, A—1 seems to discriminate better than HA—89 when the three different locations are considered. If the mean yield of hybrids over environments and testers is considered as an estimation of inbred line performance, the best six restorer lines would be selected in the three environments, testing with A—1 and only three if cms HA—89 is used. Taking into account only combined data, four out of the seven highest yielding lines, are selected in each case regardless of the tester considered. Moreover, restorer lines were not ranked identically by the two testers and a breeder selecting the highest 30% yielding hybrids would have selected only two restorer lines, R—45 and R—56, utilizing data of both testers at the same time.

The comparison between combined and single location data showed that only with the last data a reasonable number of top restorer lines could be selected. This fact can be important for those breeding programmes where testing in several locations is not always possible.

A second requisite of a good tester is that it must discriminate efficiently among material under test, that is, must give the most precise classification among entries for a given amount of testing. Again A—1 seems to perform better as a tester showing more discriminating sensitivity than HA—89. This is shown in Table 1 where the range of hybrid yields is wider when tester A—1 is considered. This tester also distinguishes larger number of groups of lines with significant differences between them.

It can be concluded from this work that A—1 is a more appropiate tester than HA—89. Besides, A—1 seems to have better g.c.a. than HA—89 as estimated by the average yield over restorers and locations. This fact is not in agreement with the overall results in corn, which are in favor of the theory that low performing and poor combining testers are the most effective (Hallauer and Miranda, 1981). However this lack of agreement could be misleading if other environment is considered, since the above mentioned estimation of g.c.a. of testers was obtained under semiarid conditions where HA—89 hybrids, with longer cycle, could have been at a disadvantage as against A—1 hybrids. In fact, in other experiments under optimal irrigated conditions, in the same environment, hybrids using HA—89 were always superior to A—1 hybrids (Gimenez, 1985).

A final consideration is the connexion between the effectiveness of the tester and its relationship to the tested inbreds. In the present study the most efficient tester, A—1, has a higher degree of relationship to the tested inbred restorers since they come from a common population which was divided later into two (Downes, 1974). In corn, Lonnquist and Castro (1967) reported a slight advantage of the related tester in the evaluation of lines. The use of an unrelated tester would result in the selection of lines having contrasting alleles at overdominant loci (Lonnquist and Lindsey, 1964). In sunflower, additive genetic variance seems to account for the major portion of the genetic variation for seed yield (Miller *et al.*, 1980), what could explain the advantage of the more related tester, A—1, observed in this study. However, as mentioned before, the fact that HA—89 hybrids did not achieve their maximum potential could also have some influence.

The results of this study are further evidence that inbred testers can be used effectively for evaluating unselected inbred lines in sunflower breeding programmes. It is also concluded that the best tester is the one closely related to the lines under test. However, since this tester and lines were selected for the conditions of the experiment, the better adaptation of their hybrids could be the major explanation of the results.

REFERENCES


Anaschenko A. V., 1974, *The initial material for sunflower heterosis breeding*, Proc. 6th Int. Sunflower Conf., Bucharest, Romania, 391—393.

Dominguez J., Fernandez J., Gimeno V., Marquez F., Ortiz J., 1978, *Resultados y evolución de tres anos de selección en girasol en condiciones de clima mediterráneo*, Proc. 8th Int. Sunflower Conf., Minneapolis, U.S.A., 414—417.

Downes R. W., 1974, *Breeding sunflower for mediterranean type climatic conditions*, Proc. 6th Int. Sunfl. Conf. Bucharest, Romania, 395—399.

Fick G. N., 1975. *Heritability of oil content in sunflower.* Crop Sci. 15, 77—78.
Gimenez C., 1985, *Resistencia a la sequia de cultivares de girasol bajo condiciones de campo,* Ph. D. Thesis, University of Córdoba, 190.
Hallauer A. R., Miranda J. B., 1981, *Quantitative genetics in maize breeding,* Iowa State University Press. Ames, I.A.
Horner E. S., Lundy H. W., Lutrick M. C., Chapman W. H., 1973, *Comparison of three methods of recurrent selection in maize,* Crop Sci., 13, 485—489.
Jenkins M. T., Brunson A. M., 1932, *Methods of testing inbred lines of maize in cross bred combinations.* J. Am. Soc. Agron., 24, 523—530.
Lonnquist J. H., Lindsey M. F., 1964, *Top-cross versus $S_1$ line performance in corn (Zea mays* L.), Crop. Sci., 4, 580—584.
Lonnquist J. H., Castro M., 1967, *Relation of intra-population genetic effects to performance of $S_1$ lines of maize,* Crop Sci., 7, 361—364.
Miller J. F., Hammond J. J., Roath W. W., 1980, *Comparison of inbred vs. single- cross testers and estimation of genetic effects,* Crop Sci., 20, 703—706.
Putt E. D., 1966, *Heterosis, combining ability and predicted synthetics from a diallel cross in sunflower (Helianthus annuus* L.), Can. J. Plant. Sci., 46, 59—67.
Rawling J. D., Thompson D. L., 1962, *Performance level as criterion for the choice of maize testers,* Crop Sci., 2, 217—220.
Russell W. A., Eberhart S. A., Vega U. A., 1973, *Recurrent selection for specific combining ability for yield in two maize populations,* Crop Sci., 13, 257—261.
Skorič D., 1978, *Mode of inheritance of oil content in sunflower seed of $F_1$ generation and components of genetic variability,* Proc. 7th Int. Sunflower Conf., Krasnodar, U.S.S.R., 376—388.
Sprague G. F., Eberhart S. A., 1977, *Corn breeding.* In : Sprague G.F. (ed.), Corn and corn improvement. Agronomy, 18, 305—360, Am. Soc. of Agron., Madison, Wis.
Unrau J., 1947, *Heterosis in relation to sunflower breeding,* Sci. Agric., 27, 414—427.

## ÉVALUATION DES LIGNÉES AUTOFÉCONDÉES COMME TESTERS DANS UN PROGRAMME D'AMÉLIORATION DU TOURNESOL

*Résumé*

L'évaluation des lignées autofécondées dans les programmes d'amélioration du tournesol implique le choix des testeurs efficaces. L'utilisation de lignées comme testeurs est une pratique commune dans les programmes d'amélioration du tournesol bien qu'il n'existe pas beaucoup de travaux pour étudier son efficacité. L'objectif de ce travail a été la comparaison de la relative efficacité de deux lignées de tournesol avec une différente aptitude à la combinaison et avec différentes parentés avec les lignées à tester. Les hybrides essayés furent obtenus avec deux lignées mâles stériles cytoplasmiques, cms HA-89 et A-1, la deuxième provenant de la même population originale que les lignées restauratrices en évaluation. Les hybrides furent semés dans trois localités avec des conditions de climat semi-aride. L'interaction entre lignées restauratrices x testeur fut significative indicant que les testeurs n'ont pas classifié identiquement les lignées restauratrices pour rendements.

Le testeur le plus apparenté avec les lignées testées, A-1, semble plus efficace que cms HA-89 montrant plus de sensibilité à distinquer entre les lignées restauratrices. Les hybrides avec la lignée A-1 eurent un rendement supérieur dans toutes les localités probablement dû à sa precocité qu'améliore leur adaptation dans les conditions de l'essai.

On en conclue que les lignées autofécondées peuvent être utilisées avec efficacité comme testeurs pour l'amélioration d'autres lignées dans les programmes d'amélioration génétique du tournesol.

## EVALUACION DE LINEAS PURAS COMO PROBADORES EN UN PROGRAMA DE MEJORA DE GIRASOL

*Resúmen*

La evaluación de líneas puras en un programa de mejora genética del girasol lleva consigo la elección de probadores adecuados. La utilización de líneas puras como tales probadores es una práctica ampliamente extendida en casi todos los programas de mejora de girasol aunque no se hayan publicado muchos estudios sobre el tema. El objetivo de este trabajo ha sido comparar la eficiencia relativa como probadores de dos líneas puras de girasol con diferente aptitud combinatoria y diferentemente relacionados con las líneas a probar.

Los híbridos evaluados fueron obtenidos cruzando veintiuna líneas restauradoras con dos líneas androesteriles citoplásmicas : CMS HA-89 y A-1, esta última proveniente de la misma población originaria de las líneas restauradoras a evaluar.

Los íbridos fueron sembrados en tres localidades semiaridas en condiciones de secano.

La interacción líneas restauradoras x probador fue significativa, indicando que los probadores no clasificaron en el mismo orden a las líneas restauradoras para rendimiento.

El pobador mas relacionado, A-1, parece ser mas apropiado que CMS HA-89, mostrando mas sansibilidad, para discriminar entre líneas restauradoras. Los híbridos con A-1 rindieron mas en todos las localidades probablemente debido a su ciclo mas corto lo que mejora la adaptación en las condiciones del experimento.

Se concluye que las líneas puras pueden ser utilizadas razonablemente como probadores para la evaluación de otras líneas puras en los programas de mejora genética del girasol.