HEREDITY OF SUNFLOWER LEAF CHARACTERS USEABLE AS YIELD PREDICTORS

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Abstract

Seed yields are correlated with post-flowering activity of leaves. This paper reports about comparisons between total and residual leaf areas and nitrogen content in leaves, for a wide range of inbred lines and hybrids. For 10 inbred lines observations at two locations and two years were significantly correlated. Mean leaf areas of hybrids were twice those of inbred lines, whereas leaf nitrogen content was the same in both groups. In 2002, 51 hybrids and their parents were studied at one location and in 2003, 25 hybrids were studied at two locations. Total leaf area, residual leaf areas and leaf nitrogen content appeared heritable with significant correlation coefficients from r=0.41 to r=0.75. There appear to be more environmental effects on residual leaf area than on total leaf area. The possible use of these characters in breeding inbred lines which will lead to hybrids with increased yields is discussed.

Introduction

In Europe, sunflower is an important crop, with low input requirements and of environmental interest, but to remain competitive with other crops, and if possible accelerated, yield improvement is necessary. Genomic studies should in the long term permit marker assisted selection, but in the short term, phenotypic characters which are related to yield have been the subject of a research programme supported by the sunflower inter-profession in France. The results obtained so far (Triboi and Triboi-Blondel, 2002; Debaeke et al., 2004; Triboi et al., 2004) indicate that the photosynthetic activity of leaves during grain filling is of particular importance. This activity is made up of the leaf area remaining active after flowering and the efficiency of photosynthesis estimated by the nitrogen content of

leaves. To be used in breeding a character must be stable, heritable, and if possible measurable in segregating progenies or inbred lines. This paper reports the first results to determine whether leaf area and leaf nitrogen content are heritable characters which can be measured in breeding populations or inbred lines.

Materials and Methods

Sunflower Genotypes. A wide range of inbred lines bred by INRA, and hybrids made from them, were used in this study. In 2002, 51 hybrids in two series were obtained from crosses between two female tester lines and eight restorers and between two tester restorers and 16 females. In 2003, a partial factorial cross was used, chosen according to the 2002 results, with five females and eight restorers (detailed in Figure 1).

Method. In 2002, the hybrids were grown near Clermont-Ferrand, France, in a randomised block design, two replications and 100 plants per plot. The inbred lines were grown in the breeding nursery at Clermont-Ferrand, with two replications of 25 plants. In 2003, both the hybrids and the inbred lines were grown at two locations: near Clermont-Ferrand and near Toulouse, also in a randomised block design, two replications and 85 plants/plot at Clermont-Ferrand and 92 plants/plot at Toulouse. The blocks of inbred lines were adjacent to those of hybrids. In 2002, density was six plants/m sq. while in 2003 there were five plants/m sq. at Clermont-Ferrand and 6.7 plants/m sq. at Toulouse. All the usual observations for yield trials were made (plant height, flowering date, grain yield, seed water content at harvest). Total leaf area was obtained from measurements of length and width of all the leaves (the lower leaves were measured at flowering, the upper leaves two weeks later). Residual active leaf area three weeks after flowering was obtained by subtracting the areas of the leaves that were no longer green at this time from the total leaf area. At Clermont-Ferrand, in 2002, the leaf areas (LA, cm sq.) were measured on two mean plants (measured by stem diameter one month before flowering) in each plot. In 2003, six plants/plot were chosen and measured as in 2002. At Toulouse (2003), LA was measured on five plants per replicate (10 plants per genotype) using a similar method.

Leaf nitrogen contents were obtained by the Kjeldahl method at Clermont-Ferrand and the Dumas method at Toulouse on mixtures of five dried ground leaves, each one collected from a different plant in the plot concerned (8-12th leaves from capitulum for hybrids, 6-10th for inbred lines). These leaves were collected at the beginning of flowering.

Results

Table 1 presents a summary of total (TLA) and residual leaf areas (RLA) and leaf nitrogen contents (%N) of all the hybrids and inbreds studied in 2002 and 2003. For total leaf area, the mean values for the hybrids were equivalent to Leaf Area Indices (LAI) of 5.3, 4.6 and 4.1 for CF02, CF03 and TS03 respectively. It may be noted that there was considerable heterosis for total leaf area, the hybrids having 60-100% more TLA than female inbred lines and up to 150% more than restorer inbred lines (all of the last were branched, but only the leaves on the main stem were measured). For RLA three weeks after flowering, there was a similar difference, although the female lines appeared to mature relatively slowly, with a mean of about 75% of the RLA of their hybrids at this stage. In contrast, the restorer lines matured quickly, with their mean being less than half the mean RLA of hybrids in all

locations and some had very few green leaves 3 weeks after flowering (minima: 400-600 cm sq.). In spite of the increased leaf area in hybrids, their %N was in the same range as that of inbred lines.

Table 1. Summary of leaf areas and leaf nitrogen contents of sunflower hybrids and inbred lines studied in 2002 and 2003 (CF: Clermont-Ferrand, TS: Toulouse).

	Hybrids			Female lines			Restorer lines		
	CF02	CF03	TS03	CF02	CF03	TS03	CF02	CF03	TS03
Total Leaf Area (cm²)									
Minimum	6486	6842	4374	3235	3342	2058	2221	1302	2036
Maximum	14001	15758	10012	6706	7312	5536	6696	6290	6625
Mean	8881	9125	6096	4956	5440	4367	4099	3940	3153
s.e.	231	458	299	242	693	624	436	556	526
Residual Leaf Area (cm²)									
Minimum	2944	3941	2873	1489	2037	1041	463	594	780
Maximum	7270	11523	5240	5476	5689	3807	4425	3665	2703
Mean	4319	6532	4071	3346	3781	2917	2157	2430	1777
s.e.	130	325	167	241	678	487	404	398	251
% Nitrogen in leaves at flowering									
Minimum	4.01	3.63	2.52	3.87	3.49	2.85	3.97	3.62	299
Maximum	5.22	4.61	3.99	5.17	4.22	4.00	4.49	4.32	3.82
Mean	4.61	4.07	3.19	4.48	3.88	3.51	4.26	4.04	3.37
s.e.	0.03	0.05	0.08	0.09	0.12	0.19	0.06	0.08	0.15

Table 2 presents details of the results for the five female and five restorer lines studied in both years. These lines were chosen to cover the range of results obtained in 2002, especially for leaf areas. Correlations between locations and years were generally highly significant (Table 3) although some interactions between genotypes and locations were apparent; for example at Clermont-Ferrand, FNRM always had the smallest leaf area of the females whereas at Toulouse VDQ showed the least development. The 25 hybrids obtained from crosses of these lines were grown both near Clermont-Ferrand and near Toulouse in 2003. The correlation between TLA values at the two sites was highly significant whereas these hybrids appeared somewhat less stable for RLA, and %N was intermediate (Table 3).

Table 2. Leaf areas and leaf nitrogen contents of the 10 inbred sunflower lines studied in both 2002 and 2003 (CF: Clermont-Ferrand, TS: Toulouse).

Inbred lines	Total Leaf Area (cm²)			Residual Leaf Area (cm²)			% N in Leaves		
	CF02	**CF03**	**TS03**	**CF02**	**CF03**	**TS03**	**CF02**	**CF03**	**TS03**
XRQ	5937	6050	5379	4818	4625	3394	4.14	3.49	3.45
FNRM	3382	3340	4258	2588	2037	2978	4.85	4.02	4.00
VAQ	4132	6041	4605	3655	4471	3363	4.45	3.88	3.62
VHQ	6078	7312	5536	4108	5689	3807	4.77	4.22	3.64
VDQ	4481	4457	2058	3260	2442	1041	4.17	3.81	2.85
PSC8RM	3518	5119	2187	1627	2807	1490	4.03	3.62	3.48
PST5RM	3178	2773	3131	1721	1490	1237	4.25	4.03	3.44
PAR6	6082	6291	6626	3616	3664	2703	4.43	4.09	2.99
OPB4	6696	4687	3031	4425	2994	1612	4.34	4.03	3.11
PPR9	2221	2830	2381	436	1399	1701	4.49	4.31	3.82

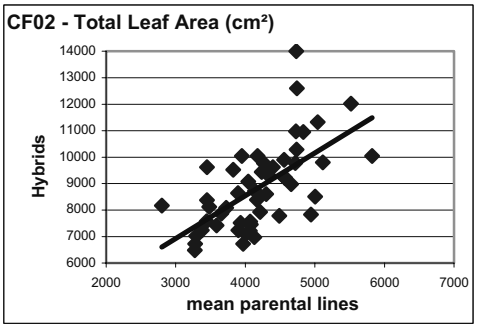
To determine whether observations on inbred lines can predict relative behaviours of hybrids, regression was calculated between the means of parental lines and the values of their hybrids in each location. The correlation coefficients are presented in Table 3. Figure 1 presents the

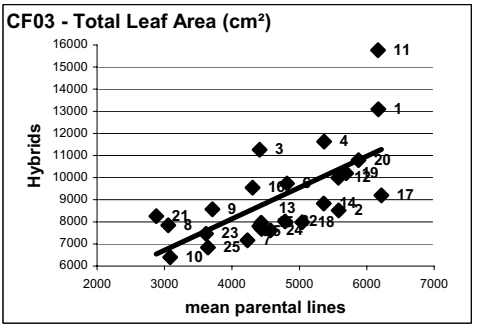
Table 3. Correlations between sites and years for inbred lines and hybrids and between parental lines and hybrids, for total leaf area (TLA), residual leaf area (RLA) and leaf nitrogen content (%N) (CF: Clermont-Ferrand, TS: Toulouse).

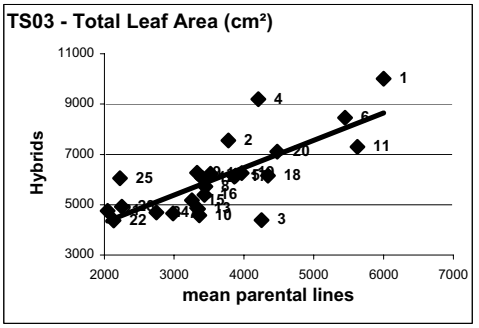
			CF02	**CF03**	**TS03**
TLA	Inbred lines	CF02	-	0.767 p<0.01	0.574 p=0.08
		CF03	-	-	0.663 p<0.05
	Hybrids	CF03	-	-	0.624 p<0.05
	Inbred lines - hybrids		**0.622 p<0.01**	**0.670 p<0.01**	**0.749 p<0.01**
RLA	Inbred lines	CF02	-	0.771 p<0.01	0.543 p=0.10
		CF03	-	-	0.778 p<0.01
	Hybrids	CF03	-	-	0.226 p=0.22
	Inbred lines - hybrids		**0.633 p<0.01**	**0.621 p<0.01**	0.405 p<0.05
%N	Inbred lines	CF02	-	0.695 p<0.05	0.663 p<0.05
		CF03	-	-	0.270 p=0.45
	Hybrids	CF03	-	-	0.385 p=0.06
	Inbred lines - hybrids		**0.669 p<0.01**	0.417 p<0.05	0.294 p=0.15

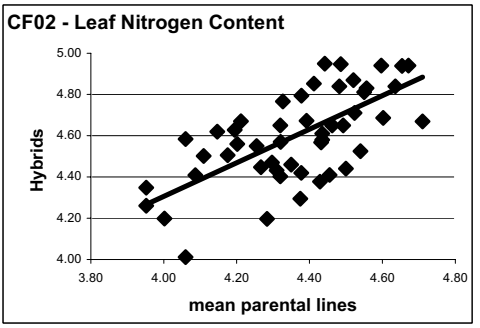
significant regressions. In 2002, for the 51 hybrids studied, differences between parental lines explained about 40% of hybrid variation for all three characters. As noted with the mean data presented in Table 1, heterosis was greatest for total leaf area with a slope of 1.61, whereas it was only 0.78 for %N. In 2003, for the 25 hybrids studied, although the inbred lines were grown adjacent to the hybrids in this year, the levels of correlation between the means of parental lines and their hybrids were similar or slightly lower than in 2002 (Table 3), especially for %N.

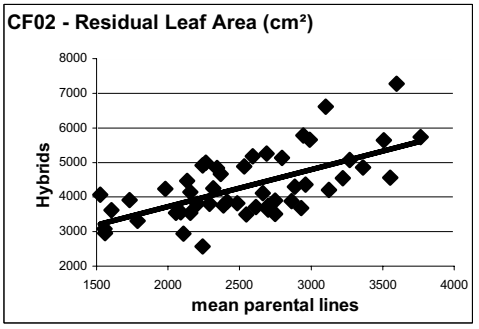
Although many of the genotypes grown in 2002 and 2003 were different, the levels of correlation between inbreds and hybrids were similar in all three locations/years for TLA. RLA also showed a similar level of correlation between the two years at Clermont-Ferrand, indicating that there was no gain in placing the inbred lines in the same trial as the hybrids. At Toulouse, although still significant, the correlation between the RLA of inbred lines and of hybrids was weaker than for TLA. For %N, the correlation between inbreds and hybrids was weaker in 2003, both at Clermont-Ferrand and Toulouse, than in 2002. This may have been related to the reduced variability among the 10 inbred lines studied in 2003, compared with the 28 studied in 2002. The difference between maximum and minimum for female lines was 1.30% in 2002, but only 0.73% in 2003, while %N of restorers varied 0.52% in 2002 and 0.70% in 2003 (Table 1).

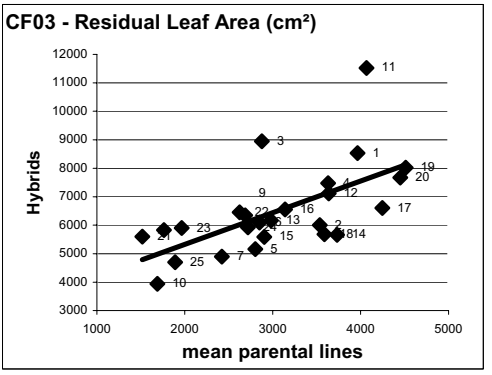












Codes of hybrids in 2003

Restorers	PAR6	PSC8RM	PST5RM	OPB4	PPR9	PST3	QPR6	PBT4
Females								
XRQ	1	2	3	4	5			
FNRM	6	7	8		10		9	
VAQ	11	12	13	14	15			
VHQ		17	18			16	19	20
VDQ		22	23	24(CF)	25	21		24(TS)

Discussion

Yield in sunflower appears to be largely dependent on post-flowering activity of leaves (Hall, 2000) and measurements of leaf area and nitrogen content were found to be good predictors (Debaeke et al., 2004). The present results show that modern genotypes show large differences for these characteristics. Mean leaf areas (both total and residual) show variation between locations and years, related to environmental conditions. For the common genotypes in 2003, differences between Clermont-Ferrand and Toulouse were probably mainly due different soil fertility (N in soil) and water retention capacities (in the context of the severe drought of 2003), with greater plant development in chernozem type soils at Clermont-Ferrand. The TLA, RLA and %N characters were lower at Toulouse in relation with more nitrogen and water stress in 2003.

However, relative values, especially of inbred lines appear to be quite stable. TLA appears to be under genetic control with good repetition between locations and significant correlation between inbred lines and their hybrids. The pattern of maturation of inbred lines and hybrids, as represented by RLA, also appears under genetic control but with greater influence of environmental conditions. However, there appears to be no advantage in placing the inbred lines adjacent to the hybrids as in 2003. In 2002, measurements on inbreds in a breeding nursery appeared to provide a good indication of the behaviour of their hybrids in similar environments. Further multi-location trials are necessary to determine how far measurements of leaf areas on inbred lines can serve to predict those of hybrids under a range of conditions. This could lead to including indicators of environmental conditions in the prediction of hybrid behaviour. Further studies will also be necessary to conclude which of the two measurements of leaf area is the most useful and at what date RLA measurement is most satisfactory. Total leaf area appears to be under the closest genetic control and when vegetative development is not very great, may largely determine residual leaf area, but this last characteristic is the one most directly linked to seed yield (Triboi et al., 2004), especially when growing conditions permit development of large leaf areas. It will be important to develop simplified methods and also, if possible, to model interactions with environmental conditions so that response to these can be predicted.

The nitrogen content in leaves at flowering is considered to represent possible photosynthetic activity in the leaf area remaining active during grain filling (in agreement with Connor et al., 1993). The present results show some variability between inbreds which is quite stable and heritable. However, according to the genotypes studied and the year, there may be negative correlations with TLA and RLA (data not shown). It may be that the smallest loss of %N during maturation is more important than %N of leaves at flowering. This would require two measurements and although it may be possible to replace the Kjeldahl method by a more rapid technique, further studies are necessary to determine the intervention of this character in seed yield, before recommending the use of this character in breeding programmes.

Conclusions

In conclusion, total and residual leaf areas and possibly leaf nitrogen content appear to be heritable characters which may be used in programmes breeding sunflower for yield.

Experiments are in progress to determine what gain in yield is possible in hybrids produced from F3 progenies selected for their residual leaf area.

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