IMPROVEMENT OF DROUGHT RESISTANCE IN CULTIVATED SUNFLOWER BY THE USE OF HELIANTHUS AGROPHYLLUS T&G. RESULTS OF A DIVERGENT SELECTION FOR PHYSIOLOGICAL PARAMETERS

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Abstract
The starting cross between Helianthus argophyllus T&G, as male parent and a cultivated (Helianthus annuus L.)inbred line (C) as female was made. Four randomly chosen F1 plants were self-pollinated and the F2 population was growed in the field with very limited water availability and, at flowering time, the evaluated characters were: leaf water potential, relative water content in the leaf (RWC) and transpiration and carbon exchange rate (CER). The F3 families, object of this study, were derived by self-pollinated F2 plants, which showing greater value (positive selection) and lower value (negative selection) than the average of F2 population increased or reduced of its standard deviation for each of the above characters. Besides for the mentioned characters, the F3 families were also analyzed for leaf area index (LAI), flowering - maturity period and biomass yield. This experiment was carried out in 1991 following a split-plot experimental design with three replications, with the wet and drought treatments in the main plots, and the 31 F3 families in the sub-plots. The drought treatment was obtained by the interruption of the irrigation at pre-flowering time. The genotypes showed a great variability, especially under stress conditions, for all the examined characters and some families showed a clear divergent physiological behaviour under drought. The efficiency of this selection is confirmed by the high values of heritability for the physilogical characters used as selection criteria, calculated as regression coefficient, between parents (F2) and progenies (F3) and as realized heritability. The significant and positive correlations between physiological characters and yield/growth components in the F3 families under drought underlines the presence of a character able to avoid the water stress, as a consistent root development and density under drought, indirectly selected from the source population. This approach could be very useful in breeding programme where wild species and also cultivated sunflower have to be evaluated in order to screen the potential parental genotypes of drought resistance hybrids, so limiting the number of crosses to make and evaluate.

Introduction
Previous studies indicate that H. argophyllus differs from cultivated sunflower for many physiological characters under wet conditions and many authors consider this wild species

useful to increase drought resistance in cultivated sunflower (Blanchet and Gelfi, 1980; Serieys, 1980; Seiler, 1988;). A direct selection for yield under drought conditions is not likely to be the most effective way of improving yields (Blum, 1985; Blum, 1988) especially in the exploitation of wild sunflowers (Sobrado and Turner, 1983). As information on the physiological background of drought resistance has been developed in recent years (Passioura, 1986; Turner, 1986;), the use of physiological selection criteria in breeding for improved drought resistance is becoming a realistic possibility in many crops (Sloane et al., 1990; Wright et al., 1983; Wright et al., 1988; Ritchie et al., 1990).
The aim of this work is to get some informations on the use of the physiological approach in a divergent selection programme for genetic improvement of drought resistance in sunflower.

Materials and Methods

The starting material was the wild species H. argophyllus coming from USDA-ARS North Dakota and the inbred C line selected at the Agronomy Department of Pisa University.
The initial cross was made on 1989 using H. argophyllus as male parent. On the F2 population grown during 1990 in drought conditions without irrigations, each plant (74 in total) was selfed and 56 days after sowing the following characters were recorded:

- Carbon Exchange Rate ($\mu mol\ m^{-2}\ sec^{-1}$)
- Transpiration Rate ($mmol\ m^{-2}\ sec^{-1}$)
- Leaf Water Content (%)
- Pre-dawn water potential (MPa)

The experimental scheme was a completely randomized block design, with plants as entries and leaves as replications.
Leaf gas exchange was measured with commercial ADC system described by Long and Hallgren (1985). Measurements in the mid section of the three top fully expanded leaves were made in each plant between 0.5 h before and 2 h after solar noon. Leaf water potential at the pre-dawn was measured with a pressure chamber using the methodoloy described by Turner (1981) in three top fully expanded leaves in each plant. Relative water content, was measured on the same leaves on which leaf water potential was measured , using the methodology suggested by Sobrado and Turner (1983)
The divergent selection, on this population, was made considering the plants wich had, for each character, upper (positive selection) and lower (negative selection) level than M+S and M-S, were M= mean value of F2 population and S= its standard deviation.
31 plants resulted selected and originated the 31 F3 families representing the material studied in this paper. The experiment was carried out at the Experimental Farm of Pisa University under rainout shelters. The plants were hand sown in a split-plot experimental design, with water regimes as main treatment, and genotypes as subtreatments, with two replicates.
Irrigation was manipulated to create two different water regimes: 1) dryland where irrigation was terminated 46 days after sowing ; 2) drip irrigation twice weekly up maturity in

order to maintain the soil near field capacity. The same physiological measurements above mentioned were made 74 days after sowing in all plots, with the same methodologies and were: pre-dawn leaf water potential, leaf gas exchange (transpiration and photosynthesys), relative water content (RWC) and in addition leaf area index (LAI), calculated using the relationship of Rawson et al.,(1980). Flowering to maturity period and plant height were also recorded in all plots. At maturity four plants per plots were harvested by hands and subdivided in above-ground matter and roots. Care was taken when pulling plants to recover as much of the root systen as possible, digging up a soil volume at 80 cm deep and 30/30 cm area. All vegetative components were oven dried at 80 °C for 48 h before weighing. Root index was calculated as proportion of root dry matter in total dry biomass. All acquired data were submitted to an analysis of variance which tests the effect of main treatments, F3 families and interaction. Simple correlation were calculated for variable means accross F3 families in the dryland treatment. Moreover two different heritability estimates were computed for the physiological characters used during the divergent selection (F2-F3). In one estimate heritability was evaluated using simple linear regression (parent-offspring regression, H). F3 families means of each character measured in 1991 were each regressed seperately on individual F2 plants values.
For the second estimate of heritability (realized heritability, Hr) was computed using results of F3 family expression as consequnce of divergent selection among F2 plants. The formula suggested by Farnham et al., (1990) to compute separately Hr for both positive and negative selection resulted appropriate:

$$Hr = \frac{[(x \text{ selected F3 families}) - (x \text{ stratified F3 sample})]}{[(x \text{ selected F2 plants}) - (x \text{ stratified F2 sample})]}$$

Results

Large and significant variability for the examined characters under stress conditions among the F3 families either as absolute value and as reduction compared to check, was found (Table 1). Particularly, mean CER over all genotypes were reduced in water stress to 54.3 % of the check, whereas transpiration rate was reduced to 79.1 % only. It is evident that the low water use efficiency of sunflower is determined by a low stomatal resistance of the downer leaf page, related to a progressive stomatal enclosure, during water stress increase (Planchon, 1990).
Mean root to total dry matter ratio, over all genotypes increases in water stress to 22.6 % of the control. This increase in root weight may indicate a greater density of roots or a greater depth of them, as observed, in sunflower, by Hsiao and Acevedo (1974). The variation accross a range of 98.5-156.2 % among families, as compared with control, can be considered as one character determining drought resistance among families obtained.

Table 1 - F3 families (n=31) means, standard error, ranges and significances of differences for physiological and agronomic characters measured under drought

				F3 Families range		
variable	unit	Mean	S.E.	Min	Max	Sig.
Carbon exchange rate	µmol m^{-2} sec^{-1}	11.5	7.2	3.8	22.7	**
Transpiration rate	mmol m^{-2} sec^{-1}	7.2	1.44	3.8	8.9	**
Leaf water content	%	0.57	0.086	0.4	0.7	**
Pre-dawn water potential	MPa	-1.45	0.05	-0.75	-2.6	**
Carbon exchange rate	% (a)	54.3	30.8	6.1	99.4	**
Transpiration rate	% (a)	79.1	12.9	48.9	95.1	**
Leaf water content	% (a)	82	9.3	63	99	**
Pre-dawn water potential	% (a)	242.7	90.1	130.8	542.1	**
Total dry matter	g plant-1	182	154.8	16.2	445.5	**
Root/total dry weight	g g-1	0.085	0.03	0.043	0.16	**
Plant height	cm	196.1	81.9	55	362.5	**
Flowering to maturity	days	31.6	6.3	21.6	46.2	**
LAI	m^2 m^{-2}	1.32	1.02	0.55	2.66	**
Total dry matter	% (a)	56.4	38.5	36.4	89.5	**
root/total dry weight	% (a)	122.6	48.6	98.5	156.2	**
plant height	% (a)	74.2	33.2	56.5	94.2	**
flowering to maturity	% (a)	77.2	16.2	56.2	91.2	**
LAI	% (a)	52.6	33.6	32.6	87.5	**

(a) = stress as per cent of non-stress
*, ** significance for P = 0.05 and 0.01 respectively

Table 2 - Phenotipic correlations of the main characters examined in the F3 families subjected to drought stress

Characters	Total dry weight	Root weight/ total weight	Plant height	Flow.-Matur. period	LAI
Leaf water cont.	0.662	0.408	0.753	0.691	0.743
Water potential	0.724	0.385	0.793	0.666	0.741
Transpiration	0.453	0.424	0.579	0.472	0.837
Photosynthesis	0.719	0.410	0.750	0.597	0.821

Degrees of freedom 29. F values P=0.01 0.449
P=0.05 0.349

Table 3 - Heritability estimation determined as parents/offspring regression coefficient (H) and performed basing on divergent selection (Hr) for the physiological characters examined.

Characters	Hr		H
	Positive selection	Negative selection	
Leaf Water Content	0.734	0.727	0.664
Transpiration	0.487	0.486	0.488
Photosynthesis	0.611	0.803	0.679
Water Potential	0.582	0.632	0.586

Mean pre-dawn leaf water potential over all families, as an expression of drought stress in the dryland treatment, varies from -0.75 to -2.6 MPa with mean level of -1.4 MPa.
The greater CER and transpiration (measured per unit leaf area), leaf water content and leaf water potential at pre-dawn resulted associated to a larger leaf area, later flowering time, higher plants and greater total dry matter per plant (Table 2). These relations show a more abundant utilization of soil water in those families F3 with unchanged physiology parameters during stress conditions, probably, due to the higher increment of root to shoot ratio under drought stress (Table 2).
High levels of estimated heritability for all the physiological characters used during this divergent selection either as heritability obtained as response of selection (positive and negative Hr) and as parent-offspring regression (H) were obtained (Table 3).

Conclusions

The first objective of this study, the obtaining of genetic variability for physiological characters under drought, among F3 families obtained, was met.
The resistance responsable mechanism, coming from H. argophyllus, may be hypothesized as the maintenance of water uptake by the increment of root/total dry matter ratio under drought; this fact determines an avoidance phenomenon permitting a more regular plant development thanks to the bigger soil volume explored by roots.
The physiological characters used during the selection, considered as the best parameters of plant phisiology during stress conditions (Planchon, 1990), have shown high heritability level; this result permits their use as selection criteria in breeding program for improving drought resistance in sunflower.

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