Letal deshydratation threshold of leaf area : interest of a test for breeding sunflower genotypes for the "stay green" character under water stress.

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Abstract :

As far as drought tolerance is concerned, leaf area resistance to dessiccation and senescence appears to be a positive character. The authors detailled a test built on single detached leaves of sunflower able to identify tolerance to leaf dessiccation. The results allow to plot the relation between the RWC (relative water content) of the leaves and the percentage of leaf area still alive after the dessiccation. Regarding the variability observed in the test, not only differences are shown for the letal deshydratation threshold, but also regarding the dessiccation curve and the rapidity of water losses according to the leaf area alive or death. The authors suggested that this simple testcould be one of the criteria helpfull for selecting genotypes able to keep leaf area intact under water shortages.

1. Introduction :

As far as sunflower is concerned by drought adaptation, a lot of works indicated clearly now that the first response of the crop to water limitation will bear upon leaf area, instead of photosynthesis (Merrien et al., 1981a ; Planchon, 1990 ; Merrien, 1992 ; Connor and Hall, 1996). More precisely, if water shortages occur very early in the growing cycle, during the leaf initiation period, the number of leaves could be reduced (Yegappan et al., 1980). Later, the plant will reacted by producing smaller leaves. The leaf area index (LAI) establishment appears to be well correlated with the water availabilities. To reach the optimum values for LAI (i.e. maximum of the light interception if LAI = 2.5/3, Picq, 1986) 160 mm of water availabilities [soil contributions + rainfalls + (irrigation)] will be required. Under french conditions, leaf area establishment could be limited only the driest years or when the crop is grown on very light soils. Many authors had indicated that under such conditions, leaf area survival after anthesis was more important that leaf area establishment from emergence to anthesis (Rodrigues Pereira, 1978; Merrien et al., 1981b ; Amiel, 1988). This character had been taken into account by the breeders as "the stay

green" character. This could lead to improvement in 1000-seeds weight, but also in the oil content. In this paper, we will presented a test built under controlled conditions to evaluate among some hydrids grown in France, if variation occurs in the leaf area capability to stay still alive when water dessiccations occur. Such a test could also be used for selections among lines.

2. Materials and methods :

Nine hybrids were gown in the field (silt-clay soil, 1.5 m depth), under a shelter to prevent from rainfalls and to get a better control of the water available to the crops. The plants were sown in order to get a regular plant density of 7 plants per square meter. The distance between rows was 0.5 m. A non-limitated fertilisation (N/P/K) was applied at sowing. In order to created a differenciation in leaf area development, 2 waters treatments were managed : the first one provided a limitation regime all over the cycle (300 mm) and the second one was an optimum regime that provided 160 mm (inc. soil contribution) before anthesis, 70 mm during anthesis and 180 mm for the ripenning period.
At the end of anthesis, homogeneous leaves (size, position on the stem) including petiols were sampled (2 trials, 20 leaves each time). Initial leaf area (Sfi) was determinated by image analysis (Optimas, -Imasys) and each leaf was placed in a water-flagon during one night at 20°C to get the maximum turgor in the tissue. The day after early in the morning, leaves were weighted to get the maximum weight at turgor (Pit).

Leaves were immediatly left to dessiccation under controlled conditions, on a table, lower face up under a steady climat (27°C and 500 $\mu E.m^{-2}.s^{-1}$). In order to control the steady state ot the evaporative demand, water cups were put on the table and weight regularly to check the evaporation. At regular intervals, according to the dessiccation speed, one leaf was sample, weight again (Pfd) and put to rehydration (petiol cut to refresh and put in a water-flagon) until the day after in the morning. There, leaf was weight again (Pfh) and the leaf area still alive (Sff) was controlled by cutting previously the senescence part of the tissus. The percentage of leaf area still alive $SF_{[0-100]}$ = Sfi/Sff*100, the relative water content $RWC_{[0-100]}$ = (Pfh-Pfd)/(Pit-Pfd)*100 were calculated. In some case, it's appears difficult to check the Pfh values (injuring of the petiol during the dessiccation process); in this case, we calculated only the leaf water content as $TRE_{[0-1]}$ = (Pit-Pfd)/Pit

3. Results :

a. Relation between RWC and leaf area still alive (SF).

The different kind of curves obtained are drown as example on figure 1a. Two typologies were identified. On one hand, linear models show for plants the capability to loss at least 60 % of the water content (RWC = 40%) in the tissue before any dammage in the percentage of leaf area still alive. Among this family of behavior, A, B, C was classified as leaves able to loss more water (A) or less (C) without any dammage in leaf area. After this threshold, called letal thesholf, the leaf area turn to die very quickly down to 0 for SF values.

Some hybrids differed in their behavior by a non linear response (see group classified on figure 1b as D^+ or D^-). In this case, we observed a progressive lost of water that induced very rapidly losses in leaf area. If such behaviors can indicated a small tolerance of the plant to keep the integrity of leaf area under water stress, on the other hand this could be interpretated as and adaptative strategy of the plant leaf area to water availability decreasing.

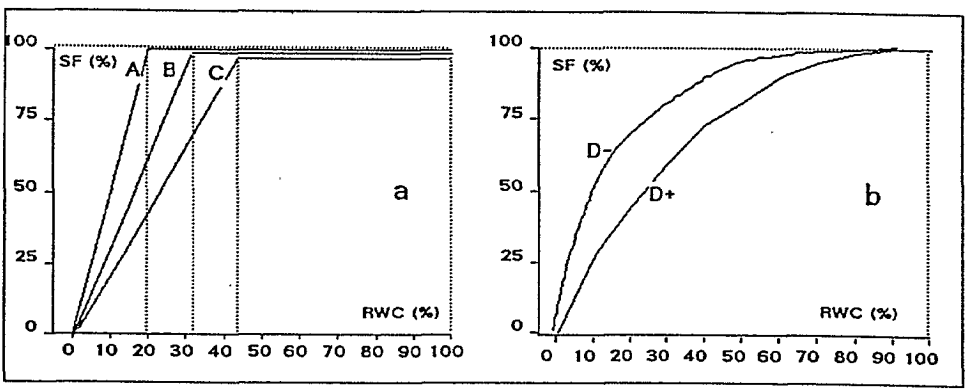

Figure 1: Typologies of different curves obtained from the relation between RWC and leaf area still alived (SF).

The table 1 indicated the classification for the 9 hybrids observed in this experiment according to the treatment irrigated or non irrigated. Euroflor, Flamme and Viki leaves caming from the irrigated plots were able to keep their leaf area alive (classification A and B) more longer than Frankasol, Select and Voltasol. According to the growing conditions of the plants before the test was applied (i.e., under water shortages or not), 5 cultivars indicated the same response to the test. The water treatment doesn't interacted with the capability of the plant

to maintain leaf area more or less alive. Three cultivars (Euroflor, Viki and X9210) interacted. The letal threshold value was lower for the leaves caming from plants grown under water limitation than the leaves from plant grown under irrigation. We don't succeded to classify the cultivar Santafé due to a very quick response of leaf area losses to the test.

Varieties	Non irrigated	Irrigated
Albéna	C	C
Euroflor	C	A
Flamme	B	B
Frankasol	D	D
Santafé	?	?
Select	D -	D +
Viki	B	A
Voltasol	D +	D -
X9210	D	C

Table 1: Classification established for the 9 hybrids of the experiment

b. Relation between the percentage of leaf area still alive after dessiccation and the TRE.

The data set was better when we calculated only TRE instead of RWC (due to difficulties to check carefully the Pfh). The models obtained for all the experiments fitted well with a sigmoïdal one. An example of such a result is shown on figure 2.

In order to compared those curves, a linearisation was done by a logarithmic model as : $Z = \log(100-SF+1) - \log(SF+1)$ and we plotted Z as a fonction of TRE. We get then a linear relation ($Z = aTRE + b$) with a negative slope. We made a comparition between the "a" and the "b" values for the 9 cultivars. An increase in the "b" value indicated that the SF value is affected for the highest values of TRE. An increase in the "a" value indicated that the leaf area stil alive (SF) decrease more rapidly. A statistical analysis was performed by using one modality as a reference.

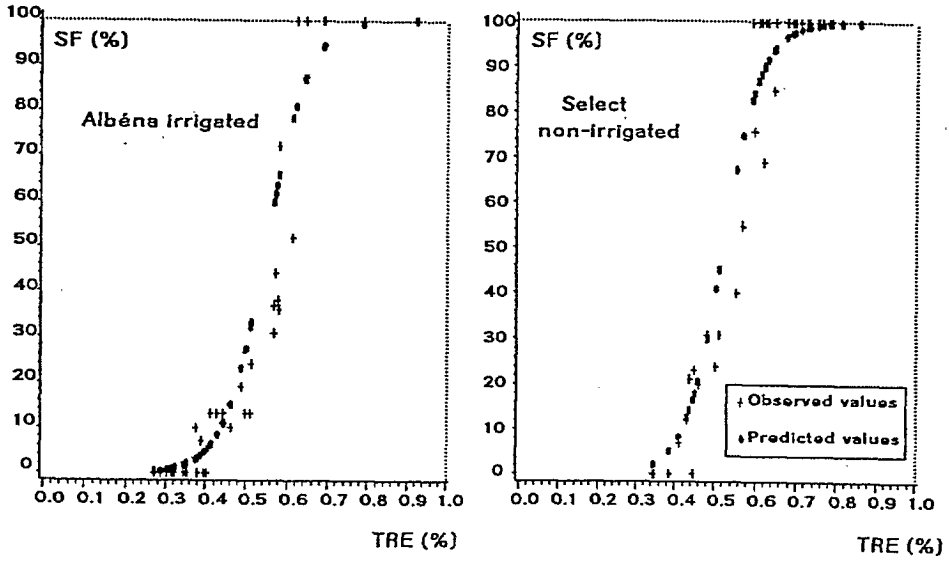

Figure 2 : Exemple of the sigmoïd model obtained between SF (% of leaf area alive after the test) and TRE (water content).

+ Effect of the water treatment applied : We do this comparision for each cultivar. In this case, we chose the non irrigated treatment as a reference. The results are indicated in table 2. Two cultivars (Viki and Select) were not treated here due to the lack of datas (only 20 points instead of 40 for the 7 others).

Varieties	Std		a1	b1	a2	b2	r2
	sec	Irrigué					
Albéna	0.76	0.70	-24.10 HS	13.08 HS	+4.19 PS	-2.15 NS	0.84
Euroflor	0.79	0.75	-20.86 HS	11.42 HS	+0.57 NS	-0.51 NS	0.80
Flamme	0.78	0.76	-21.62 HS	11.87 HS	-1.61 NS	+0.64 NS	0.82
Frankasol	0.79	0.71	-21.78 HS	11.34 HS	+0.30 NS	+0.40 NS	0.87
Santafé	0.73	0.74	-21.58 HS	11.93 HS	+0.40 NS	+0.59 NS	0.77
Voltasol	0.76	0.76	-21.20 HS	11.47 HS	-0.85 NS	+0.76 NS	0.80
X9210	0.74	0.73	-21.93 HS	12.29 HS	+0.11 NS	-0.15 NS	0.77

HS = Pr. F > 0.05 PS = 0.05<Pr. F<0.1 NS = Pr.F > 0.1

Table 2 : Standard deviation (Std), values for the slope (a1) and the .....(b1) from the regression lines for the irrigated modalities; a2 and b2 are the variation of the same parameters for the non-irrigated treatments; r2 = correlation coefficient for the regression obtained from the non irrigated combinations.

The values obtained for the standard deviation (Std) are quite small, allowing to say that the sigmoïd model used fits well with the observed values. Regarding the "a1" values, the fluctuation is very small. Only in the case of Albena a significative difference in this slope (0.05<Pr.

F<0.1) could be demonstrated, as shown on figure 3. For the others cultivars the growing conditions prior to anthesis had no effect on the result of the test.

+ Effect of the genotypes : The results are given in table 3. Compared to the reference Albena, only the cultivar Select shows a significative difference for the leaves of the plant caming from the plots under irrigation. Not ony Select had a higher letal threshold value, but the slope of the curve is smaller, as illustrated in figure 3.

Varieties	Non Irr. (r2=0.81)		Irrigated (r2=0.82)	
	a2	b2	a2	b2
Albéna	Z=-24.09xTRE+13.08		Z=-19.90xTRE+10.93	
Euroflor	+3.23 NS	-1.65 NS	-0.39 NS	-0.24 NS
Flamme	+2.48 NS	-1.21 NS	-3.33 NS	+1.59 NS
Frankasol	+2.31 NS	-1.74 NS	-1.58 NS	+0.82 NS
Santafé	+2.52 NS	-1.15 NS	-1.27 NS	+1.59 NS
Select	--	--	+3.30 NS	-3.43 S
Viki	--	--	-3.37 NS	+1.64 NS
Voltasol	+2.89 NS	-1.61 NS	-2.15 NS	+1.30 NS
X9210	+2.17 NS	-0.79 NS	-1.92 NS	+1.21 NS

S = 0.05 < Pr. F < 0.1 NS = Pr.F > 0.1

Table 3: Comparison to the reference Albena between the varieties irrigated or not (a2 = variation of the slope; b2= .....).

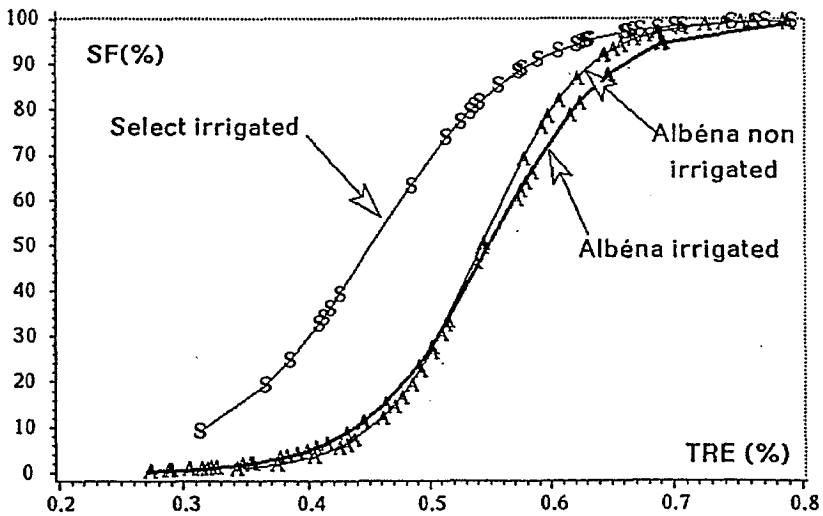

Figure 3 : Curves showing significative differences.

All the other varieties had a distinct behavior compared to Select and are not different from Albena. For the plants grown under dry conditions, due to the lack of datas for Select (and Viki), such a conclusion can't be done (Table 3).

4. Discussions-Conclusions :

From this experiment, we can concluded that it could be possible to classified behaviors of genotypes regarding their capability to maintain their leaf area alive under water shortages. Two parameters could be used to classified this behavior : on on hand the letal deshydratation threshold, that indicated how much water the leaves tissues are able to loss before wounded and leading to a reduction in leaf area. On the other hand, the speed at which the leaf area decline when water losses increase could also be obtained.
According to the choice we done in this experiment, Albena is the only cultivar showing a significative behavior between the plant issued from plots managed as irrigated crops and plants issued from plots managed under water limitations. From such a results could we concluded that the management of the crop (regarding water status for exemple) before anthesis had no effect on the leaf area duration after the anthesis ? This denies the results obtained by Blanchet et al., (1990).
On the genetic point of view, the only particular behavior identified here was Select's leaves grown under irrigation.
Nevertheless among the hybrids studied here, the genetic variability appears very narrow. In other trials with the same cultivars, conducted until harvest, any particular interactions between water treatments (inc. dry conditions) were obtained. This lead to conclude that there were no variability towards drought tolerance among those hybrids. It could be interesting to include in such a test wild genotypes (for ex. lines or hybrids obtained from *H. Argophyllus*) identified for their good behavior under water deficiency (Serieys, 1991 ; Vannozzi et al., 1992). More largely, this test could be helpfull for selecting genotypes able to keep leaf area intact under water shortages.

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