# Study of stomatal parameters for selection of drought resistant varieties in *Triticum durum* DESF

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## Summary

An analysis was made of the stomatal apparatus of the leaves of five Italian durum wheat varieties, grown under different natural rainfall regimes.

Rainfall had a significant influence on both development of the leaves and frequency and size of the stomata. Results were examined with respect to yield stability. Correlations between examined traits were calculated.

The contribution of each plant character to water stress tolerance was assessed, and suitability for selecting higher yielding genotypes in stress conditions was determined.

On examining the results it emerged that:

a) due to water stress, the transpiring/photosynthesizing area ratio increased;

b) cultivars which are able to mantain open stomata during water stress are more stable in terms of yield;

c) the dimension of stomatal aperture on the lower surface in plants grown under water stress is a useful trait for screening drought tolerant genotypes.

Abbreviations: LA – leaf area (mm<sup>2</sup>), N – number of stomata/mm<sup>2</sup>, SA – stomatal area (mm<sup>2</sup>), TA – transpiring area (mm<sup>2</sup>), TA % – percentage of transpiring area

## Introduction

Low rainfall reduces potential crop yield. Utilization of water stress tolerant cultivars would contribute to a minimization of yield losses in the Mediterranean region, which is characterized by low rainfall in many places (Blum, 1985).

Blum (1981) observed that genotypes which are able to maintain high water potential without stomatal closure are suitable for arid conditions. Stomatal closure, according to Planchon (1987), is not a water storage process, but a passive phenomenon related to the loss of turgor by guard cells and it shows the plant's incapacity to react against drought. Shimshi (1975) found that plants with open stomata gave higher yields without greater water consumption. Begg & Turner (1976) underlined the fact that leaf transpiration is the most powerful conductor of water and nutrient absorption from the soil, because it creates a lower water potential in the plant than the soil.

Another aspect to be considered is temperature. In fact, stomatal closure makes leaf temperature increase by about 5–6°C, due to the amplification of the difference of vapour pressure between leaf and atmosphere, followed by higher cuticlar tran-

spiration and consequent loss of part of the water retained by stomatal closure (Rawson et al., 1981). Moreover, Jones (1981) observed that the closure of stomata for a long time can cause the destruction of the chloroplast thylakoids.

The aim of the research was to identify cultivars with a better transpiration capacity, and use stomatal parameters for screening new water stress tolerant genotypes.

## Material and methods

In the cropping season 1988–89 five cultivars of durum wheat, included in the annually conducted Italian National trials (Boggini et al., 1989), were evaluated at two locations in East Sicily, viz. Mineo Lat. 37°15' Long. 2°15' (province of Catania) and Gela Lat. 37°00' Long. 1°45' (province of Caltanissetta). Sowing was carried out on 5-12-88 and 6-12-88 at Mineo and Gela respectively.

At flowering (Mineo 13-4-89 and Gela 3-4-89), flag leaves of 10 plants per cultivar were detached (Sapra et al., 1975). Leaf areas were measured with a Leaf Area Meter (Hayashi Denkoh), and the width of each leaf at the middle was recorded.

In the middle part of each flag leaf surface, prints of adaxial and abaxial surfaces were made by means of silicon resin. After drying at room temperature, the film of resin was peeled off and mounted on a slide by means of adhesive film (Miskin et al., 1970; Roselli & Venora, 1989). Of each sample, the number of rows of stomata per leaf and number of stomata on six microscopic fields between the midrib and middle bundle were recorded (Ledent, 1978).

Of 60 stomata per flag leaf, (30 stomata per leaf surface, 600 per cultivar), stomatal area, perimeter, length and width were measured by the image analyser IBAS 2000 Kontron-Zeiss connected to the microscope.

The transpiring area per leaf was computed as:

 $TA = N \times SA \times LA$ 

where N = number of stomata/mm<sup>2</sup>, SA = mean

stomatal area (mm<sup>2</sup>), LA = leaf area (mm<sup>2</sup>), TA = transpiring area (mm<sup>2</sup>).

Percentage of transpiring area was calculated as:

$$TA\% = (TA/LA) \times 100$$

where TA = transpiring area (mm<sup>2</sup>), LA = leaf area (mm<sup>2</sup>), TA% = percentage of transpiring area.

Data were analysed by analysis of variance, and differences among cultivars and locations were evidenced by cluster analysis (Scott & Knott, 1974).

Correlation coefficients among stomatal and leaf parameters were calculated, in order to identify useful traits for plant breeding.

## **Results and discussion**

Temperature and photoperiod were similar at both locations. Consequently, differences in plant growth were mainly due to the rainfall amount and distribution (Fig. 1). In Gela, average flag leaf area was 38.43% less than at the more favourable location of Mineo (Table 1).

Reduction in TA was less sharp than decrease in leaf area, which amounted to 27.97% and 31.64%, for the adaxial and abaxial surface respectively (Table 1). Consequently, TA% increased in unfavourable conditions by 13.93% at the adaxial and by 10.95% at the abaxial surface. Due to water stress, the transpiring/photosynthesizing area ratio increased (0.11 and 0.13 are the ratio values between leaf and total stomatal area, in favourable and unfavourable locations respectively). Such a mechanism can be considered a means of more efficient water use in dry conditions. Stomatal closure can affect yield because of the reduction of photosynthesis and assimilate production. This is confirmed by the stomatal characteristics and yield stability of each cultivar grown in locations differing in water availability (Tables 1 and 2).

Leaf area reduction differed in each cultivar. The highest value was found for Duilio, the lowest for Valnova (Table 1). Decrease in transpiring area (TA) also depended on the genotype. Increase in

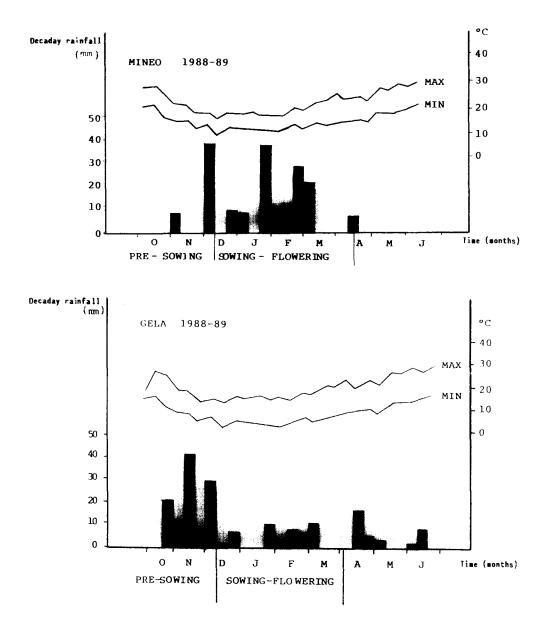


Fig. 1. Temperature and rainfall patterns recorded at Mineo (favourable) and Gela (unfavourable).

TA% was greatest in Duilio and Simeto, about zero in Trinakria and Vespro, and negative in Valnova.

In Table 2, yield indexes of the same 5 cultivars grown at seven different locations in Sicily (Boggini et al., 1989) have been presented. The locations were very different in rainfall and the mean yields were extremely diverse (from 0.97 to 4.09 t/ha). To evaluate the adaptability of cultivars to the different rainfall conditions, the standard deviation over all locations was computed.

In comparison to Gela, where rainfall was low, soil water deficit was less striking but fairly stressful for the plants at Caltagirone, Riesi and Caltanissetta (Fig. 2). On the other hand, rainfall patterns of Libertinia and Mineo were favourable (Fig. 2). The lowest S.D. values were found in the cultivars Si-

	Leaf Area			Transpirir	ng Area upper	surface	Transpiring	Area lower	surface
	Mineo	Gela	Difference %	Mineo	Gela	Difference %	Mineo	Gela	Difference %
Duilio	2599.9 a	1166.8 b	55.12	164.94 a	99.46 a	39.70	123.14 a	67.09 b	45.52
Simeto	2697.8 a	1854.7 a	31.25	171.12 a	150.33 a	12.15	138.16 a	103.34 a	25.20
Trinakria	2425.5 a	1350.3 Ь	44.33	171.16 a	108.29 a	36.73	116.03 a	72.67 b	37.37
Valnova	2559.9 a	1846.0 a	27.89	183.66 a	147.63 a	19.62	138.41 a	106.62 a	16.97
Vespro	3244.7 a	2111.9 a	34.91	201.92 a	145.38 a	28.00	143.19 a	100.74 a	29.63
Mean	2705.6 Aa	1665.9 Bb	- 38.43	178.56 Aa	a 130.22 Bb	- 27.07	131.79 Aa	90.09 Bb	- 31.64
	Trans	piring Area %	o upper surfa	ce	Transpiring A	rea % lower	surface	Mean diff	
	Minec	o Gela	Differe	nce %	Mineo G	ela D	ifference %	lower/2)	e (upper +
Duilio	6.31 E	3b 8.44 A	a 25.24		4.75b 5	.85 Aa	8.8	22.02	
Simeto	6.32 E	3b 8.14 A	a 22.36	i	5.11 a 5	.60 Aa	8.75	15.55	
Trinakria	7.09 A	Aa 7.89 A	a – 10.93	6	4.76 b 5	.37 Aa 🕺	1.36	0.21	
Valnova	7.16 A	Aa 7.93 A	a – 9.71		5.44 a 5	.82 Aa	6.53	- 1.59	
Vespro	6.18 <b>E</b>	Bb 6.85 B	b 9.78	3	4.34 b 4	.76 Bb —	8.82	0.48	
Mean	6.61 <b>E</b>	3b 7.86 A	a 13.93	\$	4.88 b 5	.48 a	.0.95	12.44	

Table 1. Values of the flag leaf area (mm<sup>2</sup>) and the transpiring area (mm<sup>2</sup>) of flag leaf of the 5 cultivars at Mineo (favourable) and Gela (unfavourable) locations

Means in a column and row (mean) not sharing a common letter are significantly different at the 0.01 probability level (capital letters) and at the 0.05 probability level (small letters) according to cluster analysis of Scott-Knott (1974).

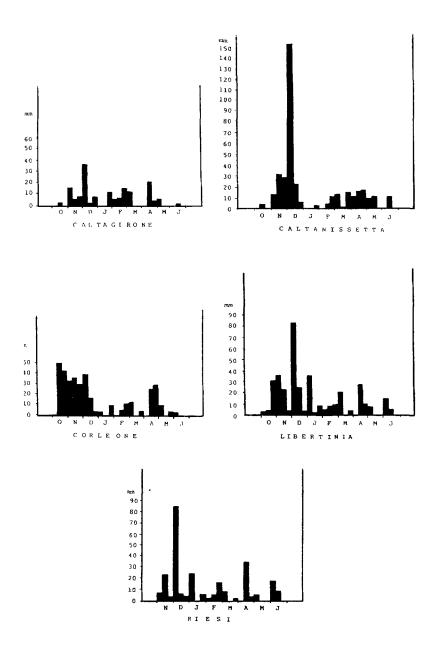
meto and Duilio, which also showed the highest yield indexes. In these two cultivars, the relative increase in percentage of transpiring area in dry conditions was the highest (Table 1).

On the basis of the above discussed data, the decrease in the transpiring area can be considered a survival mechanism in dry conditions (Planchon, 1987). To optimize yield, the plant must keep its

stomata open during stress, so that it receives better water and nutrient absorption from the soil. In this case, such genotypes can be considered drought resistant.

The correlation coefficients among all traits are showed in Tables 3 and 4. Transpiring area and percentage of transpiring area at both locations were positively correlated with flag leaf area and

Cultivar	Location								
	Caltagirone	Caltanissetta	Corleone	Gela	Libertinia	Mineo	Riesi	Mean	S.D.
Simeto	136	115	109	123	127	117	135	123	9.45
Duilio	122	93	94	108	93	117	108	105	11.08
Trinakria	95	107	109	130	107	90	95	105	12.38
Vespro	81	112	106	76	75	94	101	92	13.87
Valnova	66	73	81	62	99	82	61	75	12.60
Mean yield t/ha	1.71	2.16	1.49	0.96	4.09	3.04	1.86		



stomata area, and negatively with number and density of stomata rows. In agreement with others (Austin, 1982; Kutik, 1973; Maximov, 1929; Miskin, 1970; Salisbury, 1927; Sapra, 1975; Weaver, 1938), area, length and perimeter of stomata were found negatively correlated with stomata frequency. The increase in transpiring area in stress conditions cannot be obtained by selecting for high frequency and high density of stomata rows, because of the negative correlations between these param-

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								55.80	52.05		50.23										50.64
6									0.94		- 0.13										0.46
									88.51		1.71										20.81
10											-0.023										0.21
										8.73	0.06										4.56
==											0.935	-0.651		,					,		- 0.71
											87.43	42.38	25.65								50.45
12												-0.794	- 0.759	0.177							- 0.83
												63.12	57.66								68.71
13													0.608								0.61
													36.97								36.95
14														-0.621	1						0.07
														38.6	14.38						49.31
15															0.839	-	- 0.456				- 0.57
															/0.53						32.19
0																0.081					- 0.18
-																0.00					87.5 8
																	07.07 61.06				67 D
18																	07.10				K. 0
																		55.16	58.41	41.89	53.8
19																			0.991		0.88
																			98.37		77.39
20																				0.74	0.87
																				54.36	76.31
17																					0.97
																					93.83

Table 4. Regression coefficient (r) and explained variation, between twenty-two leaf and stomatal traits (mean value of each cultivar) in the unfavourable location of (Gela)

surface; 6 = Number of stomata/mm<sup>2</sup> abaxial surface; 7 = T otal number of stomata/mm<sup>2</sup> (upper + lower); 8 = Leaf width mm; 9 = Number of stomatal rows in adaxial surface; 10 = Number of stomatal rows in adaxial surface; 11 = Number of stomatal rows in abaxial surface; 11 = Number of stomatal surface; 12 = Number of stomatal surface; 13 = Length of the stoma ( $\mu$ m) in adaxial surface; 14 = Length of the stoma ( $\mu$ m) in adaxial surface; 14 = Length of the stoma ( $\mu$ m) in adaxial surface; 14 = Length of the stoma ( $\mu$ m) in adaxial surface; 15 = Width of the stoma ( $\mu$ m) in abaxial surface; 15 = V idth of the stoma ( $\mu$ m) in adaxial surface; 16 = Width of the stoma ( $\mu$ m) in adaxial surface; 17 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 18 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 18 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 18 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 18 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 10 = Transpiring area mm<sup>2</sup> of the adaxial surface; 20 = Transpiring area mm<sup>2</sup> of adaxial surface; 21 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 20 = Transpiring area a m<sup>2</sup> of adaxial surface; 21 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 20 = Transpiring area a m<sup>2</sup> of adaxial surface; 21 = Perimeter of of transpiring area of adaxial surface; 10 = T and  $\mu$  of the adaxial surface; 20 = T and  $\mu$  of the stoma ( $\mu$ m) in adaxial surface; 22 = Perimeter of the stoma ( $\mu$ m) in adaxial surface; 20 = T and  $\mu$  and  $\mu$  of the stoma  $\mu$  of transpiring area of adaxial surface; 22 = Perimeter of the adaxial surface.

eters and flag leaf area, length and perimeter, and transpiring area. These parameters can be useful for negative selection.

The leaf area, also positively correlated to the transpiring area, but very negatively influenced by water status, and other factors, cannot be a reliable selection trait. The stomatal area can be utilized as a selection parameter to increase the transpiring area. It is directly dependent on the stomatal perimeter.

The stomatal area measurements can be utilized to screen genotypes with a high transpiring area. In combination with an efficient photosynthetic apparatus, such genotypes can achieve high yields.

Such screening for high transpiring area, however, does not guarantee selection of drought tolerant germplasm: a characteristic of drought resistant plants is the capacity to maintain the stomata open during prolonged drought, and therefore, the stomatal opening is the most useful trait. However, it is not sufficiently closely correlated with stomatal area (r = 0.42 at Mineo and 0.01 at Gela) (Tables 3 and 4). This seems to be quite contradictory, because both stomatal opening and stomatal area are dimensional parameters of the same object (stoma), and should be (more closely) correlated.

Considering that the correlation coefficient was computed on the mean of all 5 cultivars, varying greatly in their responses to stress, it is possible to understand why there was no correlation. Table 5 presents measurements of stomatal aperture recorded for each cultivar in the unfavourable location of Gela. The highest value of stomatal opening can be found in Simeto and Duilio, followed by Valnova, Trinakria and Vespro, in decreasing order. Such a trend is similar to that shown for yield

Table 5. Stomatal aperture  $(\mu m)$  of 5 durum wheat cultivars recorded at the unfavourable location of Gela

	Adaxial	Abaxial	Mean
Simeto	24.85	24.13	24.49
Duilio	25.37	23.34	24.35
Valnova	24.60	23.17	23.88
Trinakria	23.46	22.15	22.80
Vespro	22.58	22.13	22.36

stability (Table 2) and for the transpiring area under stress (Table 1).

The data of stomatal aperture recorded on the abaxial surface reflect this trend more accurately (Table 5).

Therefore the dimension of stomatal aperture of the lower surface in plants grown under dry conditions is a useful trait for screening drought tolerant genotypes.

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