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## Relationship Between Flag Leaf Senescence and Grain Yield in Durum Wheat Grown under Drought Conditions

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**Abstract:** Reflectance was assessed along a line drawn from the basal part to the tip of the leaf, using the 'line profile' command of IPP software. Measurements were taken at twelve dates, expressed in sums of temperatures ( $\Delta t$ ) in degree-days after flowering. A highly significant correlation was noted between transmittance and Numerical image analysis (NIA) values averaged across cultivars ( $r = 0.998$ ,  $p < 0.001$ ). The linear adjustment of the function associating senescence, measured by (NIA) and transmittance to thermal time was also highly significant ( $r = 0.938$ ,  $p < 0.001$  and  $r = 0.931$ ,  $p < 0.001$  for NIA and transmittance measurements, respectively) justifying the calculation of the average velocity of senescence ( $V_a$ ). In the case of NIA measurements, the lowest  $S_a$  values were noted for Mexicali and Altar. Some cultivars as Mexicali and Waha had similar  $V_{max}$ , but highly differed for  $\Sigma 50$ . A highly significant correlation was noted between  $V_a$  and  $S_a$  calculated from transmittance data. A significant positive correlation was observed between  $\Sigma 50$  estimated by NIA and grain yield indicating that the stage when senescent area covered half of the flag leaf blade occurred later in cultivars that yielded more under drought. No correlation was noted between senescence parameters (assessed either by NIA and transmittance) and thousand kernel weight or grain growth rate. Negative (and in some cases significant) relationships were noted between 200 and 400 degree-days after flowering. In the case of the transmittance method, only the measurement made at 272°C days showed a significant positive relationship with yield.

**Key words:** Durum wheat, numerical image analysis, measurement, genotype, senescence, transmittance method

### INTRODUCTION

During grain filling, wheat photosynthesis is mainly related to photosynthetic activity and leaf green area duration. A substantial genetic variation for leaf senescence has been reported by Boyd and Walker (1972), Martin del Molino *et al.* (1986) and Saulescu *et al.* (2001) in bread wheat and by Hafsi *et al.* (2000) in durum wheat. Plants with delayed leaf senescence are preferred in many breeding programs as they are thought to have increased resistance to diseases, drought or lodging (Thomas and Smart, 1993). It was suggested that low rates of senescence could lead to a more regular translocation of assimilates to the grain and better grain filling. In bread wheat, slow rates of senescence were effectively found to be associated to higher yield by Spiertz *et al.* (1971), Rawson *et al.* (1983), Ellen (1987), Mi *et al.* (1999) and Pajevic *et al.* (1999). This is probably because senescence is coupled with remobilization (Yang *et al.*, 2001) that in some cases highly contributes to maintain grain yield (Gebbing and Schnyder, 1999). A number of factors influence the timing, extent and rate of senescence,

including environmental constraints and internal levels of hormones, in particular cytokinins (Ambler *et al.*, 1983).

Precise evaluation of senescence is difficult. The chlorophyll meter has several advantages over analysis of chlorophyll concentration in leaf tissue. Samples do not need to be sent to a laboratory for analysis, saving time and money. Irradiance, leaf water status and time of measurement may however interfere with transmittance measurements (Martines and Guaiamet, 2004). On the other hand, measurements are made on a limited area of the leaf blade and non-uniform chlorophyll distribution across the leaf surface is likely to affect their representativeness (Uddling *et al.*, 2007). Chlorophyll meters are relatively expensive for some national breeding programs in developing countries. Development of alternative methods is consequently needed for a more accurate and low-cost analysis of senescence. Numerical Image Analysis (NIA) methods have been explored and showed to be very promising. Dymond and Trotter (1997) and Clarke (1997) used digital cameras to assess crop greenness. Adamsen *et al.* (1999) developed this method to measure the senescence of wheat canopies.

Hafsi *et al.* (2000) modified the technique to evaluate senescence of flag leaf, which has been proved to be the main source of assimilates for grain filling (Wardlaw, 1990).

The objectives of the present study were to (1) Describe the senescence function with thermal time in durum wheat cultivars using the NIA method, (2) Analyze the relationship between transmittance and NIA based measurements of senescence and (3) Examine the association between senescence and grain yield in durum wheat under the strong stress conditions of the High-Plateaux of Eastern Algeria.

### MATERIALS AND METHODS

**Plant material and growth conditions:** The study was conducted at experimental fields of the Institute Technique Moyen Agricole (ITMA) of Sétif (5°21'W, 36°9'S, 1123 m above sea level), Eastern Algeria, during the cropping season 2008-2009. The soil at the experimental site is a rendzin, mollisol (Calcixeroll USDA) up to 0.6 m in depth, containing low organic matter.

Ten durum wheat cultivars (Table 1) were grown in randomized block design with three replicates. Plots were 10 m×4 rows with 18 cm row spacing and interplant space of 3 cm.

**Sowing density:** Sowing was done on Dec. 5 while harvesting was carried out on June 25. P (superphosphate 100 kg ha<sup>-1</sup>) and K (100 kg ha<sup>-1</sup>) were applied to all plots before sowing, while N (urea 150 kg ha<sup>-1</sup>) was applied at tillage to all plots. Weeds were removed manually as and when required.

Climatic conditions during the growth cycle are given on Fig. 1a. Rainfall was substantial during winter (147.8 mm during the months of Dec., Jan. and Feb.) and in Apr. (77.5 mm). Drought stress intensity (Fig. 1b) was calculated daily according to Doorenbos *et al.* (1979) as  $[1-(ET_a-ET_c)]$  with  $ET_a$  = actual evapotranspiration and  $ET_c$  = potential evapotranspiration. A slight stress was noted at booting stage, during the period March 15-Apr. 9 (625 to 856°C days after emergence) and strong and more prolonged stress from heading stage until maturity (1020 to 1984°C days after emergence).

**Measurements:** The number of days from sowing to heading (DH) was recorded. Grain yield (GY) and biomass (Biom) were determined at maturity from a 2.88 m<sup>2</sup> central area of each plot. The number of grain per spike (NGS) was evaluated on a random sample of 20 spike-bearing culms and the number of spikes per square meter was calculated. Thousand kernel weight (TKW) was determined from sub-samples taken from harvested grains of each plot.

Table 1: Brief description of the ten genotypes used in the study

Name	Information
Altar	CIMMYT cultivar, released in 1984
Bousselem	Vitron (Spanish cultivar)
Dukem12/rascon21	CIMMYT advanced line
Hogar	Heider/Marte/Huevo de Oro
Kucuk	CIMMYT cultivar, released in 1984
Mexicali	CIMMYT cultivar, released in 1975
Oued zenati	Local landrace
Polinicum	Local landrace
Sooty9/rascon57	CIMMYT advanced line
Waha	CIMMYT/ICARDA line (Sham 1) released in 1986

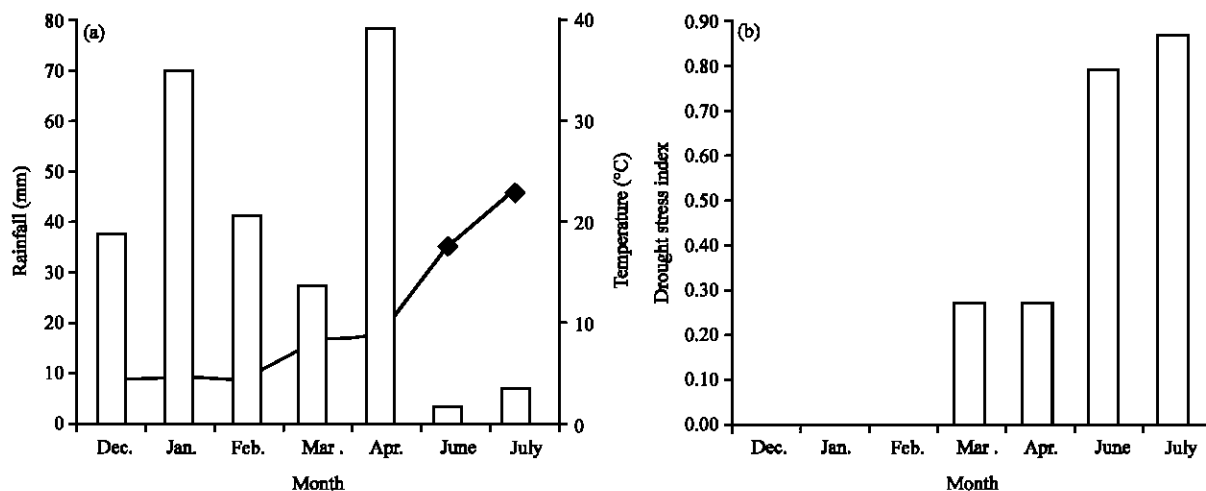


Fig. 1(a-b): Climatic conditions of the experiment (Institute Technique Moyen Agricole of Setif Cropping season 2008-2009. (a) Monthly rainfall and average temperature (b) Index of drought stress, estimated according to Doorenbos *et al.* (1979)

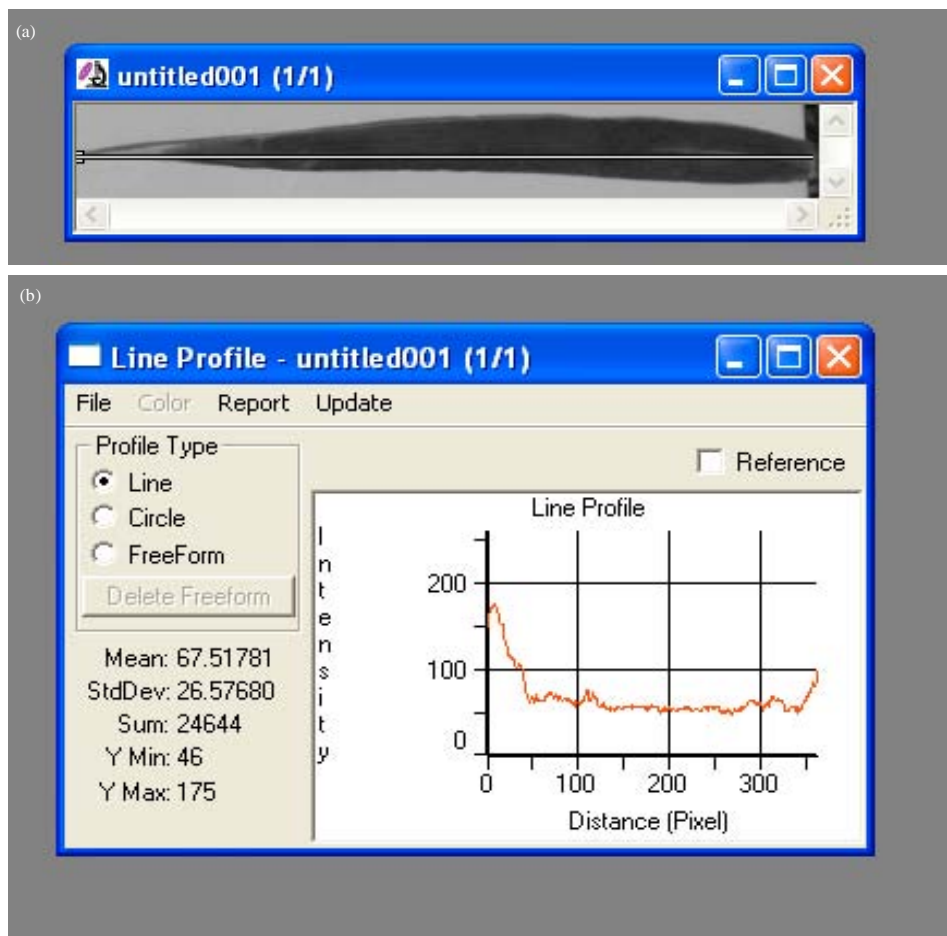


Fig. 2(a-b): Measurement of leaf senescence using the numerical image analysis (NIA) method. (a) Assessment of reflectance on stored images converted in gray (gray scale 8), along a line superposed to the leaf image and (b) Reflectance value along the line and calculation of an integrated value for the leaf

Leaf senescence was evaluated by transmittance and numerical image analysis (NIA). Three plants were chosen by plot at flowering and their main tiller was tagged. The flag leaf of these tillers was used for senescence measurements during the grain filling period. For each measurement, the leaf was photographed on a black surface between 11:00 and 12:00 solar time with a color digital camera (Sony SSC-C108P, Kyoto, Japan). Images were stored in a JPEG (Joint Photographic Expert Group) prior to downloading onto a PC computer and were analyzed using the IPP (Image Pro Plus, Version 4, Media Cybernetics, Silver Spring, MA, USA) software. In contrast to the protocol developed by Hafsi *et al.* (2000), stored images were first converted in gray (gray scale 8).

Reflectance was assessed along a line drawn from the basal part to the tip of the leaf, using the 'line profile' command of IPP software (Fig. 2).

An average reflectance value of the leaf was calculated for each measurement. Grayscale images are the result of measuring the intensity of light at each pixel (Noh *et al.*, 2005).

Measurements were taken at twelve dates, expressed in sums of temperatures ( $\Delta t$ ) in degree-days after flowering. Sums of temperatures ( $\Delta t$ ) were determined according to Higley *et al.* (1986) by adding the daily averages of maximum and minimum temperatures and subtracting the base temperature. Reflectance was assessed at 99.5, 148.4, 188.2, 205.5, 271.0, 290.2, 311.1, 330.8, 352.8, 399.6, 420.2 and 443.8°C days. Senescence (S)

was expressed for each sample as the ratio of the measured reflectance to the reflectance of a green leaf (in %).

Senescence was also estimated by following the decrease in chlorophyll concentration, using a SPAD-502 portable chlorophyll meter (Minolta, Tokyo, Japan) which measures leaf transmittance at red (650 nm) and near infrared (940 nm) wavelengths. Measurements were done on the same leaf used for NIA measurements, at five dates (148.4, 188.2, 271.0, 352.8 and 443.8°C days). Chlorophyll concentration was expressed in arbitrary absorbance or SPAD units (Dwyer and Tollenaar, 1989). Senescence at 188.2, 271.0, 352.8 and 443.8°C days was calculated as the relative decrease (in per cent) of SPAD values, relative to the initial value (at 188.2°C days).

Four parameters were calculated, according to Hafsi *et al.* (2000) to characterize the evolution of senescence, either measured by NIA or by transmittance using SPAD-502. Average senescence ( $S_a$ ) was calculated as the mean of all measurements. The date of mid-senescence ( $\Sigma_{50}$ ) was evaluated from the experimental curves  $S = f(\Sigma_t)$  as the sum of temperatures corresponding to an S value of 50%. The average velocity of senescence ( $V_a$ ) was calculated as the slope of the linear regression between senescence and thermal time. The maximum velocity of senescence ( $V_{max}$ ) was the highest value among the velocity values ( $V_s$ ) calculated for different periods as  $(\Sigma_{t_{i+1}} - \Sigma_{t_i}) / (St_{i+1} - St_i)$ , or in other terms, the highest value of the slope of the curve  $S = f(\Sigma t)$ .

Grain filling rate was estimated for each cultivar as the slope of the grain weight as a function of thermal time. For this purpose, five spikes were randomly collected at 123.1, 205.5, 311.1, 420.2, 466.7, 566.9, 621.5, 675.6 and 754.1°C days and over dried at 80°C for 24 h and then threshed by hand to estimate the average weight of 100 grains.

Data were analyzed using SAS, version 8.1. (SAS, 1987, Cary, NC, USA). GLM procedure was used for variance and correlation analysis.

## RESULTS

Highly significant genotype effect was found for all yield components (Table 2).

The two Algerian landraces (Oued Zenati and Polonicum) were characterized by higher biomass (9.11 and 7.86 t ha<sup>-1</sup>, respectively) and grain weight (2.55 and 2.47 t ha<sup>-1</sup>, respectively) and lower harvest index, fertile tillering and spike fertility than improved cultivars. The highest grain yield was noted for the cultivar Mexicali (3.19 t ha<sup>-1</sup>) and the lowest for Dukem12/Rascon21 (2.20 t ha<sup>-1</sup>). Altar, Hogar and Sooty9/Rascon57 showed the highest harvest index (0.40, 0.40, 0.38, respectively). The highest tillering was observed for Bousselem, Hogar and Kucuk and the highest spike fertility for Sooty9/Rascon57.

The tested cultivars highly varied for senescence, as measured by the NIA method, but the ranking of cultivars highly varied over time (Table 3). Senescence of the tested cultivars, measured by the NIA method at different stages of the grain filling period showed significant effect. The cultivars Bousselem and Oued Zenati showed the slower senescence (86.72 and 90.31, respectively) while the remaining genotypes have reached 100% of senescence.

Evolution of senescence during grain filling (measured either by NIA or transmittance) as a function of the sum of temperatures after anthesis followed a sigmoid curve (Fig. 3).

Equations of the sigmoid curves are  $y = -4e^{-6x^3} + 0.003x^2 - 0.850x + 59.01$  ( $r = 0.996$ ) and  $y = -6e^{-6x^3} + 0.005x^2 - 1.153x + 69.87$  ( $r = 0.993$ ) for NIA and transmittance measurements, respectively.

Table 2: Yield and yield components of the tested cultivars

Cultivars	Biomass	Grain yield	Harvest index	No. of spikes m <sup>-2</sup>	No. of grains per spike	Thousand kernel weight
Altar	7.29 <sup>bc</sup>	2.93 <sup>ab</sup>	0.400 <sup>a</sup>	271.900 <sup>ab</sup>	35.24 <sup>bc</sup>	37.70 <sup>bcd</sup>
Bousselem	8.74 <sup>ab</sup>	2.98 <sup>ab</sup>	0.340 <sup>ab</sup>	327.200 <sup>a</sup>	29.61 <sup>d</sup>	39.20 <sup>bc</sup>
Dukem12/Rascon21	6.24 <sup>c</sup>	2.20 <sup>c</sup>	0.350 <sup>ab</sup>	309.100 <sup>ab</sup>	35.15 <sup>bc</sup>	29.20 <sup>c</sup>
Hogar	7.46 <sup>abc</sup>	2.97 <sup>ab</sup>	0.400 <sup>a</sup>	319.700 <sup>a</sup>	35.65 <sup>bc</sup>	34.40 <sup>ab</sup>
Kucuk	7.56 <sup>abc</sup>	2.65 <sup>bc</sup>	0.350 <sup>ab</sup>	336.300 <sup>a</sup>	34.44 <sup>c</sup>	32.60 <sup>ab</sup>
Mexicali	8.64 <sup>ab</sup>	3.19 <sup>a</sup>	0.370 <sup>ab</sup>	312.800 <sup>ab</sup>	35.34 <sup>bc</sup>	35.10 <sup>ab</sup>
Oued zenati	9.11 <sup>a</sup>	2.55 <sup>bc</sup>	0.280 <sup>c</sup>	238.400 <sup>b</sup>	28.41 <sup>d</sup>	47.80 <sup>a</sup>
Polonicum	7.86 <sup>abc</sup>	2.47 <sup>bc</sup>	0.310 <sup>bc</sup>	244.100 <sup>b</sup>	33.96 <sup>c</sup>	41.90 <sup>b</sup>
Sooty9/Rascon57	6.91 <sup>c</sup>	2.66 <sup>bc</sup>	0.380 <sup>a</sup>	271.600 <sup>ab</sup>	39.48 <sup>a</sup>	30.20 <sup>c</sup>
Waha	7.76 <sup>abc</sup>	2.69 <sup>bc</sup>	0.350 <sup>ab</sup>	294.700 <sup>ab</sup>	38.68 <sup>ab</sup>	28.90 <sup>c</sup>
Mean	7.76	2.73	0.350	292.600	34.60	35.70
CV (%)	9.74	8.49	8.350	11.470	5.03	8.62
LSD (0.05)	1.09	0.33	0.042	48.710	2.52	4.46
Genotype effect	5.41***	6.37***	6.620***	4.210**	15.58***	15.53***

Means of a given column followed by the same letter are not significantly different at  $p > 0.05$  (LSD test). \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , LSD: Least significant difference, CV: Coefficient of variation

Table 3: Senescence of the tested cultivars, measured by the NIA method at different stages of the grain filling period

Cultivars	Thermal time of measurement (in degree days after flowering)										
	99.50	148.40	188.20	205.50	271.00	290.20	311.10	330.80	352.80	399.60	420.20
Altar	7.87 <sup>b</sup>	8.21 <sup>od</sup>	9.46 <sup>od</sup>	11.41 <sup>c</sup>	25.90 <sup>d</sup>	33.26 <sup>d</sup>	37.10 <sup>e</sup>	47.67 <sup>a</sup>	62.19 <sup>e</sup>	85.60 <sup>bc</sup>	100 <sup>a</sup>
Bousselem	10.44 <sup>a</sup>	11.47 <sup>a</sup>	13.23 <sup>ab</sup>	17.89 <sup>b</sup>	21.94 <sup>de</sup>	30.83 <sup>d</sup>	34.70 <sup>e</sup>	47.44 <sup>e</sup>	62.70 <sup>e</sup>	75.36 <sup>d</sup>	86.72 <sup>b</sup>
Dukem	4.39 <sup>d</sup>	5.89 <sup>e</sup>	6.82 <sup>e</sup>	11.66 <sup>c</sup>	31.10 <sup>f</sup>	48.71 <sup>b</sup>	51.94 <sup>b</sup>	61.37 <sup>c</sup>	81.25 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Hogar	6.41 <sup>c</sup>	7.55 <sup>ode</sup>	9.36 <sup>d</sup>	12.43 <sup>c</sup>	20.67 <sup>e</sup>	40.62 <sup>c</sup>	48.31 <sup>b</sup>	54.29 <sup>d</sup>	67.76 <sup>d</sup>	82.69 <sup>bc</sup>	100 <sup>a</sup>
Kucuk	7.89 <sup>b</sup>	8.92 <sup>bc</sup>	10.46 <sup>cd</sup>	16.72 <sup>b</sup>	37.54 <sup>b</sup>	52.84 <sup>b</sup>	62.97 <sup>a</sup>	72.82 <sup>a</sup>	88.23 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Mexicali	5.24 <sup>od</sup>	7.37 <sup>ode</sup>	9.79 <sup>od</sup>	10.46 <sup>c</sup>	13.26 <sup>f</sup>	14.51 <sup>e</sup>	38.62 <sup>e</sup>	43.83 <sup>e</sup>	61.04 <sup>e</sup>	83.43 <sup>bc</sup>	100 <sup>a</sup>
Oued Zenati	4.93 <sup>d</sup>	6.67 <sup>de</sup>	11.66 <sup>bc</sup>	21.28 <sup>a</sup>	26.09 <sup>d</sup>	49.41 <sup>b</sup>	59.26 <sup>e</sup>	67.61 <sup>b</sup>	73.45 <sup>c</sup>	81.07 <sup>c</sup>	90.31 <sup>b</sup>
Polinicum	5.71 <sup>od</sup>	7.85 <sup>ode</sup>	11.22 <sup>bcd</sup>	20.51 <sup>a</sup>	26.25 <sup>d</sup>	31.86 <sup>d</sup>	49.56 <sup>b</sup>	63.76 <sup>bc</sup>	71.38 <sup>d</sup>	87.59 <sup>b</sup>	100 <sup>a</sup>
Sooty	4.74 <sup>d</sup>	10.67 <sup>ab</sup>	13.22 <sup>ab</sup>	16.44 <sup>b</sup>	36.34 <sup>b</sup>	41.44 <sup>e</sup>	51.62 <sup>b</sup>	61.67 <sup>c</sup>	89.61 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Waha	6.31 <sup>c</sup>	9.16 <sup>bc</sup>	14.06 <sup>a</sup>	16.12 <sup>b</sup>	50.37 <sup>a</sup>	59.60 <sup>a</sup>	62.54 <sup>e</sup>	74.45 <sup>a</sup>	88.23 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Mean	6.39	8.37	10.92	15.49	28.94	40.30	49.66	59.49	74.58	89.57	97.70
CV (%)	12.98	14.67	12.09	9.18	9.82	7.24	5.48	4.94	3.80	3.55	2.45
LSD	1.42	2.10	2.26	1.33	4.87	5.00	4.66	5.04	4.86	5.45	4.11
Genotype effect	10.48 <sup>***</sup>	8.99 <sup>***</sup>	8.39 <sup>***</sup>	21.95 <sup>***</sup>	40.36 <sup>***</sup>	61.07 <sup>***</sup>	42.72 <sup>***</sup>	40.55 <sup>***</sup>	48.85 <sup>***</sup>	26.80 <sup>***</sup>	12.59 <sup>***</sup>

Means of a given column followed by the same letter are not significantly different at  $p > 0.05$  (LSD test). \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , LSD: Least significant difference, CV: Coefficient of variation. Data for the last date of measurement (443.8 degree days) is not shown, senescence being 100% in all cultivars

Table 4: Senescence parameters of the tested cultivars, calculated from NIA and transmittance measurements

Cultivars	NIA				Transmittance			
	$V_a$	$S_a$	$\Sigma_{50}$	$V_{max}$	$V_a$	$S_a$	$\Sigma_{50}$	$V_{max}$
Altar	0.333 <sup>b</sup>	38.96 <sup>e</sup>	333.5 <sup>a</sup>	0.75 <sup>c</sup>	0.330 <sup>ab</sup>	47.65 <sup>a</sup>	294.00 <sup>e</sup>	0.85 <sup>a</sup>
Bousselem	0.342 <sup>b</sup>	42.80 <sup>e</sup>	334.5 <sup>a</sup>	0.85 <sup>bc</sup>	0.310 <sup>b</sup>	36.98 <sup>e</sup>	329.60 <sup>e</sup>	0.73 <sup>ab</sup>
Dukem	0.387 <sup>b</sup>	40.31 <sup>de</sup>	298.6 <sup>de</sup>	0.92 <sup>bc</sup>	0.310 <sup>ab</sup>	43.47 <sup>e</sup>	297.60 <sup>e</sup>	0.46 <sup>b</sup>
Hogar	0.502 <sup>a</sup>	40.96 <sup>d</sup>	316.9 <sup>b</sup>	1.05 <sup>ab</sup>	0.310 <sup>b</sup>	41.24 <sup>e</sup>	323.10 <sup>e</sup>	0.82 <sup>a</sup>
Kucuk	0.415 <sup>ab</sup>	45.19 <sup>e</sup>	286.6 <sup>e</sup>	0.88 <sup>bc</sup>	0.320 <sup>ab</sup>	43.17 <sup>e</sup>	302.26 <sup>e</sup>	0.65 <sup>ab</sup>
Mexicali	0.382 <sup>b</sup>	34.32 <sup>f</sup>	338.9 <sup>a</sup>	1.07 <sup>ab</sup>	0.320 <sup>ab</sup>	41.81 <sup>e</sup>	309.26 <sup>e</sup>	0.32 <sup>a</sup>
Oued Zenati	0.380 <sup>b</sup>	49.30 <sup>e</sup>	290.9 <sup>f</sup>	1.21 <sup>a</sup>	0.320 <sup>ab</sup>	41.28 <sup>e</sup>	314.03 <sup>a</sup>	0.76 <sup>ab</sup>
Polinicum	0.390 <sup>b</sup>	43.24 <sup>e</sup>	312.8 <sup>bc</sup>	0.88 <sup>bc</sup>	0.330 <sup>ab</sup>	42.78 <sup>e</sup>	317.20 <sup>e</sup>	0.64 <sup>ab</sup>
Sooty	0.381 <sup>b</sup>	42.57 <sup>e</sup>	305.2 <sup>cd</sup>	1.27 <sup>a</sup>	0.320 <sup>ab</sup>	45.88 <sup>e</sup>	290.46 <sup>e</sup>	0.84 <sup>a</sup>
Waha	0.325 <sup>b</sup>	48.08 <sup>a</sup>	269.8 <sup>e</sup>	0.80 <sup>c</sup>	0.350 <sup>a</sup>	49.59 <sup>e</sup>	283.76 <sup>e</sup>	0.72 <sup>ab</sup>
Mean	0.384	42.67	308.8	0.97	0.242	43.38	306.12	0.74
CV (%)	16.900	2.09	1.46	14.48	6.510	17.00	12.46	28.08
LSD	0.111	1.53	7.75	0.24	0.036	12.65	65.45	0.35
Genotype effect	1.810 <sup>ns</sup>	65.23 <sup>***</sup>	76.75 <sup>***</sup>	4.67 <sup>**</sup>	1.017 <sup>ns</sup>	0.71 <sup>ns</sup>	0.46 <sup>ns</sup>	1.11 <sup>ns</sup>

Means of a given column followed by the same letter are not significantly different at  $p > 0.05$  (LSD test), LSD: Least significant difference, CV: Coefficient of variation. \*\* $p = 0.01$ , \*\*\* $p = 0.001$ , ns not significant

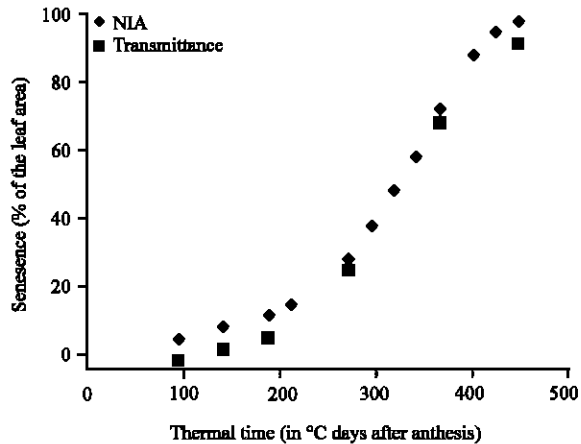


Fig. 3: Evolution of senescence measured by NIA and transmittance, as a function of thermal time

Equations of the sigmoid curves are  $y = -4e^{-6 \times 3 + 0.003 \times 2 - 0.850 \times + 59.01}$  ( $r = 0.996$ ) and  $y = -6e^{-6 \times 3 + 0.005 \times 2 - 1.153 \times + 69.87}$  ( $r = 0.993$ ) for NIA and transmittance measurements, respectively.

A highly significant correlation was noted between transmittance and NIA values averaged across cultivars ( $r = 0.998$ ,  $p < 0.001$ ). The linear adjustment of the function associating senescence, measured by NIA and transmittance, to thermal time was also highly significant ( $r = 0.938$ ,  $p < 0.001$  and  $r = 0.931$ ,  $p < 0.001$  for NIA and transmittance measurements, respectively) justifying the calculation of the average velocity of senescence ( $V_a$ ). The ranking of cultivars also varied according to the considered parameter (Table 4).

In the case of NIA measurements, the lowest  $S_a$  values were noted for Mexicali and Altar (34.32, 38.96, respectively). Altar also had the lowest  $V_{max}$  (0.75), together with Waha and the highest  $\Sigma_{50}$  (333.5), together with Bousselem and Mexicali (334.5 and 338.9, respectively). Some cultivars as Mexicali and Waha had similar  $V_{max}$ , but highly differed for  $\Sigma_{50}$ . Genotype effects were not significant for the senescence parameters measured by transmittance. Coefficients of variation were in general much higher for transmittance than for NIA measurements (Table 4).

Table 5: Correlations between senescence parameters calculated from NIA and transmittance measurements

	NIA				Transmittance			
	$V_a$	$S_a$	$\Sigma 50$	$V_{max}$	$V_a$	$S_a$	$\Sigma 50$	$V_{max}$
<b>NIA</b>								
$V_a$	.							
$S_a$	-0.138 <sup>ns</sup>	.						
$\Sigma 50$	0.000 <sup>ns</sup>	-0.792 <sup>**</sup>	.					
$V_{max}$	0.381 <sup>ns</sup>	0.045 <sup>ns</sup>	-0.032 <sup>ns</sup>	.				
<b>Transmittance</b>								
$V_a$	-0.539 <sup>ns</sup>	0.356 <sup>ns</sup>	-0.442 <sup>ns</sup>	-0.375 <sup>ns</sup>	.			
$S_a$	-0.352 <sup>ns</sup>	0.130 <sup>ns</sup>	-0.454 <sup>ns</sup>	-0.219 <sup>ns</sup>	0.774 <sup>**</sup>	.		
$\Sigma 50$	0.425 <sup>ns</sup>	-0.095 <sup>ns</sup>	0.499 <sup>ns</sup>	0.095 <sup>ns</sup>	-0.604 <sup>ns</sup>	-0.920 <sup>**</sup>	.	
$V_{max}$	0.018 <sup>ns</sup>	0.488 <sup>ns</sup>	-0.142 <sup>ns</sup>	0.032 <sup>ns</sup>	0.148 <sup>ns</sup>	0.210 <sup>ns</sup>	-0.032 <sup>ns</sup>	.

\*\*\*p<0.001, \*\*p<0.01, \*p<0.05, ns: Not significant

Table 6: Correlations between senescence parameters calculated from NIA and transmittance measurements and grain yield, thousand kernel weight and grain growth rate

Measurements	Grain yield	Thousand kernel weight		Grain growth rate
<b>NIA</b>				
$V_a$	0.032 <sup>ns</sup>	-0.032 <sup>ns</sup>	-0.326 <sup>ns</sup>	
$S_a$	-0.476 <sup>ns</sup>	0.230 <sup>ns</sup>	-0.221 <sup>ns</sup>	
$\Sigma 50$	0.638 <sup>*</sup>	0.270 <sup>ns</sup>	0.316 <sup>ns</sup>	
$V_{max}$	-0.019 <sup>ns</sup>	0.148 <sup>ns</sup>	0.095 <sup>ns</sup>	
<b>Transmittance</b>				
$V_a$	-0.032 <sup>ns</sup>	-0.11 <sup>ns</sup>	-0.245 <sup>ns</sup>	
$S_a$	-0.200 <sup>ns</sup>	-0.485 <sup>ns</sup>	-0.251 <sup>ns</sup>	
$\Sigma 50$	0.292 <sup>ns</sup>	0.616 <sup>ns</sup>	0.054 <sup>ns</sup>	
$V_{max}$	0.032 <sup>ns</sup>	0.173 <sup>ns</sup>	-0.025 <sup>ns</sup>	

\*p<0.05, ns: Not significant. \*\*\*p<0.001, \*\*p<0.01

The relationship among the four senescence parameters ( $V_a$ ,  $S_a$ ,  $\Sigma 50$  and  $V_{max}$ ) is presented in Table 4. For both NIA and transmittance measurements, there was a significant negative correlation between  $S_a$  and  $\Sigma 50$  ( $r = -0.792$ ,  $p < 0.01$ ) (Table 5).

Conversely, there was no significant association between the two methods for any of the measured parameters. A highly significant correlation was noted between  $V_a$  and  $S_a$  calculated from transmittance data ( $r = 0.774^{**}$ ,  $p < 0.01$ ) (Table 5).

A significant positive correlation was observed between  $\Sigma 50$  estimated by NIA and grain yield ( $r = 0.638^*$ ,  $p < 0.05$ ) indicating that the stage when senescent area covered half of the flag leaf blade occurred later in cultivars that yielded more under drought (Table 6).

No correlation was noted between senescence parameters (assessed either by NIA and transmittance) and thousand kernel weight or grain growth rate. The association between senescence values and grain yield highly varied with the considered stage (Fig. 4).

Measurements made at the beginning or at the end of the grain filling period showed a positive but not significant relationship with yield. Negative (and in some

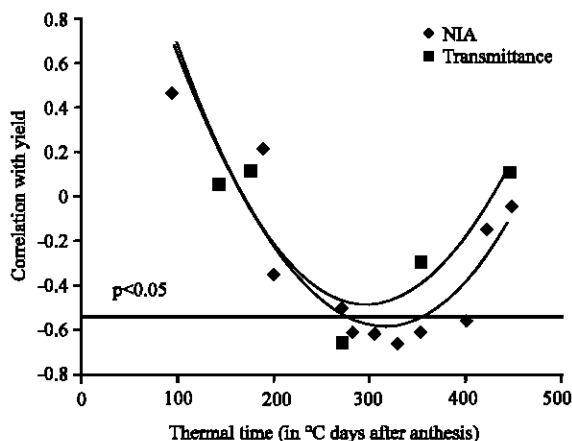


Fig. 4: Correlation between senescence measured by NIA and transmittance and grain yield, as a function of thermal time

cases significant) relationships were noted between 200 and 400 degree-days after flowering. In the case of the transmittance method, only the measurement made at 272°C days showed a significant positive relationship with yield.

## DISCUSSION

A broad genetic variation was found among the tested cultivars for senescence and senescence parameters measured by the NIA method. Differences between cultivars for senescence has already been reported in bread wheat by Boyd and Walker (1972), Martin del Molino *et al.* (1986) and Saulescu *et al.* (2001) and in durum wheat by Hafsi *et al.* (2000).

The sigmoidal time course of senescence, observed either by NIA or transmittance measurements, is in good accordance with previous works (Hafsi *et al.*, 2000, 2003). The level of senescence and its rate over time highly varied among the tested cultivars, suggesting that measurements at a given stage of the grain filling period

are not sufficient for accurately determining genetic variation for senescence. For example, some cultivars as Mexicali had low senescence values during the entire grain filling period and were obviously characterized by low  $S_a$  values. Others, like Bousselem or Altar, had relatively high senescence values at the beginning of the grain filling period, but had the lowest values at the end. This was reflected by lower  $V_{max}$  values in these cultivars, compared to the others. Finally, cultivars also differed for the onset of senescence, like Waha and Mexicali. Similar observations were previously made by Hafsi *et al.* (2000) who suggested that cultivars can reach similar senescence levels at the end of the grain filling period either after a long and slow or a short and sudden increase. Similarly, Thomas and Smart (1993) noted that cultivar with contrasted senescence can differ either for the (more or less) delay in the onset of senescence or for the (more slower or quicker) rate in progress of senescence.

Poor association was however found between the two methods for a given parameter. This can be explained by the fact that the transmittance method estimates the evolution of leaf color and chlorophyll concentration on a limited area of the leaf, while the NIA method integrates the change in color along the leaf blade (Hafsi *et al.*, 2000).

The tested cultivar showed a broad variation for yield components. Based on the yield components values, two groups of cultivars were clearly distinguished. The first included two Algerian landraces characterized by high biomass, low harvest index, tillering and spike fertility and high grain weight. The second involved the improved cultivars. Interestingly the highest grain yield was obtained by Mexicali, a cultivar released in 1975 but still largely cultivated in Algeria (Hafsi *et al.*, 2003). This cultivar also had the lowest senescence values, as measured by NIA, during most of the grain filling period as well as the highest  $\Sigma_{50}$  and the lowest  $S_a$  values. Significant correlations were found between senescence assessed by NIA and grain yield between 200 and 400°C days after flowering. A highly significant negative correlation was noted between  $\Sigma_{50}$  estimated by NIA and grain yield, indicating that senescence, as measured by NIA, was associated to grain yield in our experimental conditions. They are in good agreement with previous results from Spiertz *et al.* (1971), Rawson *et al.* (1983), Ellen (1987), Mi *et al.* (1999) and Pajevic *et al.* (1999). As mentioned above, this relationship however highly depends on climatic conditions, which can affect senescence differently according the cultivar. For example, Hafsi *et al.* (2000, 2003) observed a quick senescence for the cultivar Mexicali which showed

delayed senescence in the present study. This discrepancy is likely to be due to differences in climatic conditions and water availability between the different experiments.

Repeated and precise senescence measurements during the grain filling period using NIA revealed a high genetic variation for senescence parameters and showed that different cultivars can differ for average senescence level or for onset of senescence and maximal rate of senescence. This result indicated that assesment of senescence at different stages (or thermal time values) of the grain filling period is needed to accurately address the time curve of senescence and its genetic variation. It has been suggested that the NIA method that integrates the change in color along the leaf blade takes better into account the non-uniform distribution of chlorophyll and consequently provides a more exact assesment of senescence (Hafsi *et al.*, 2000, 2007). In the present study NIA measurements allowed to identify associations between grain yield and senescence that were not detected by transmittance assessments. NIA measurements are definitely slower than transmittance measurements (few minutes for each measurement instead of few seconds) but do not require specific equipment other than a digital camera and image analysis software that can eventually be free download. This method, less adapted to large-scale screening than the transmittance method, appears more convenient, in turn, for precision phenotyping and for precise comparison of a limited number of genotypes.

## CONCLUSION

It was suggested that low rates of senescence could lead to a more regular translocation of assimilates to the grain and better grain filling. Development of alternative methods is needed for a more accurate and low-cost analysis of senescence. For example, some cultivars as Mexicali had low senescence values during the entire grain filling period and were obviously characterized by low  $S_a$  values. Others, like Bousselem or Altar, had relatively high senescence values at the beginning of the grain filling period, but had the lowest values at the end. Cultivars also differed for the onset of senescence, like Waha and Mexicali. Similar observations were previously made which suggested that cultivars can reach similar senescence levels at the end of the grain filling period either after a long and slow or a short and sudden increase. Repeated and precise senescence measurements during the grain filling period using NIA revealed a high



genetic variation for senescence parameters and showed that different cultivars can differ for average senescence level or for onset of senescence and maximal rate of senescence. The result indicated that assessment of senescence at different stages (or thermal time values) of the grain filling period is needed to accurately address the time curve of senescence and its genetic variation. It has been suggested that the NIA method that integrates the change in color along the leaf blade takes better into account the non-uniform distribution of chlorophyll and provides a more exact assessment of senescence.

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