



Asian Journal of Plant Sciences

ISSN 1682-3974

science
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Relationships Between Cold Tolerance, Grain Yield Performance and Stability of Durum Wheat (*Triticum durum* Desf.) Genotypes Grown at High Elevation Area of Eastern Algeria

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Abstract: To increase grain yield in the North African high plateaus, selection of durum wheat (*Triticum durum* Desf.) was accompanied by an early heading for effective utilization of the limited soil moisture and to escape terminal drought and heat stresses. Early genotypes suffer however seriously from low temperature damage during cold season. Developing cultivars resistant to low temperature stress appears critical to avoid crop failure and to stabilize grain yield. The objectives of the present study were to evaluate the genotypic variability for resistance to low temperatures using artificial tests and to investigate the relationship between the results of such tests and the agronomic performance and stability of a field grown set of durum wheat (*Triticum durum* Desf.) genotypes. The artificial laboratory tests employed enabled genotypes to be differentiated on the basis of their cold tolerance, results suggest that genotypes which possess the ability to cold acclimate during winter retain this feature until the booting stage. Means of agronomic traits varied significantly between seasons and genotypes, indicating the presence of significant genotypic variability and differential responses to the growth conditions experienced. No consistent relationships, between the tolerance to low temperatures and agronomic performances, were found due to the confounding effects of terminal heat and drought stresses, acting on the same yield components as the cold stress. Early, freezing tolerant and above average yielding cultivars were identified which serve as genetic source to improve tolerance to low temperatures in short cycle genetic background.

Key words: Durum wheat, agronomic performances, stability, cold tolerance, relationships

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) production encounters, in the North African high plateaus, cold damage from January through April and then is exposed to drought and heat stresses during May and June. Selection to increase grain yield was accompanied by an early heading for effective utilization of the limited soil moisture and to escape terminal drought and heat stresses. The selected cultivars were usually alternative and had a short growing cycle. Earliness proved to be beneficial in the favourable low land, winter mild areas, but early genotypes suffer seriously from low temperature damage during cold season, at the high elevation areas (Hadjichristodoulou, 1987; Bouzerzour and Bemmahamed, 1994; Annichiarico *et al.*, 2002a). Escaping spring frost damage was achieved, with some success, by developing cultivars with late emergence of ear primordia onto the ground surface and early heading to minimize the effect of terminal drought and heat stresses (Hoshino and Tahir, 1987; Villegas *et al.*, 2000).

Developing cultivars resistant to low temperature stress appears critical to avoid crop failure and to stabilize grain yield at the high elevation areas. Genetic variability for tolerance to low temperatures is then a key factor when selecting for areas where such climatic constraint is prevailing (Fletcher, 1983). Tolerance to low temperatures depends mainly on acclimation process that occurs when plants are exposed to low non freezing temperatures (Rizza *et al.*, 1994). The acclimation process itself involves changes at the cell level which are the expression of what is sensed by plant organs when subjected to low non freezing temperatures during hardening process (Fowler *et al.*, 1996). Tolerance to winter low temperatures is more present in winter than in facultative type cultivars and it is related to the genotype ability to harden (Kosner and Pankova, 2002). Artificial laboratory tests have been developed to evaluate the genotypic variability to tolerate stresses (Rizza *et al.*, 1994).

We employed artificial tests of crown survival rate and leaf cell membrane injury to evaluate the genotypic variability of durum wheat (*Triticum durum* Desf.) to

tolerate low temperatures and tested whether genotypes which possess the ability to harden, under field conditions, during winter, may retain some of this feature when they de-acclimate in the spring and tolerant spring frost events. The objectives of the present study were (1) to evaluate the genotypic variability for resistance to low temperatures using artificial tests and (2) to investigate the relationship between the results of such tests and the agronomic performance and stability of a field grown set of durum wheat (*Triticum durum* Desf.) genotypes, evaluated during four cropping seasons at high elevation area of eastern Algeria.

MATERIALS AND METHODS

Trials description: A field experiment was conducted during four successive cropping seasons, 1999/00 to 2002/03, at the agricultural experimental station of the National Institute of Agricultural Research (INRA) of Sétif (Lat 36°12'N, Long 5°24'E, alt 1081 m a.s.l.). The soil is a brown calcareous earth classified as a steppic brown soil. The experiment was sown during the second half of November at 250 seeds m⁻² rate, in a randomized complete block design with four replicates. Plot dimensions were 5 m long × 1.2 m wide (6 rows × 0.20 m apart). Twelve durum wheat (*Triticum durum* Desf.) genotypes were grown. Plots were fertilized with 100 kg ha⁻¹ of super phosphate 46% at sowing and 100 kg ha⁻¹ of urea 34% at the tillering stage. Weeds were controlled by application of Granstar [*Methyl Triberunon*] herbicide at 12 g ha⁻¹ rate mixed with 250 L of water.

Measurements: During the 3rd decade of January 2002, 100 field-hardened seedlings, 3rd leaf stage, were sampled from the 4th replication, taken to the laboratory for crown test processing according to the method outlined by Marshall and Kolb (1982). Seedlings were washed with tap water and potted up in plastic box filled with moist sand, at a rate of 25 seedlings per box. The boxes were entered into a programmable freezer, sets to reach the test temperature at a rate of -2°C h⁻¹. When the test temperature is reached, seedlings were allowed to stay for 60 min and then the temperature is raised at the rate of +2°C h⁻¹ to reach the ambient laboratory temperature of 22°C.

The seedlings were allowed to thaw; they were trimmed to leave only 3 cm above and below the crown and then potted up again for re-growth evaluation under laboratory conditions. Four temperature treatments -5, -10, -12 and -15°C were tested. Seedling survival, that is the number of plants which showed a significant re-growth above 3 mm out of the total number of seedling evaluated, was estimated 10 days after the seedling were transplanted.

Leaves, sampled at the booting stage, were tested at -1, -3, -6 and -9°C, for 60 min. The increase in the rate of cell electrolyte leakage was used as a measure of low temperature injury to leaf-cell membrane. Samples of 10 leaf segments 0.5 cm long per genotype and replication were placed in vials containing 15 mL of de-ionized water and maintained at room temperature for 3 h. A first reading of the vials was done with a digital conductivity meter, then they were autoclaved and a second reading of the conductivity of the bathing solution was done. Cell membrane injury was estimated using the following formula:

$$\text{Injury (\%)} = 100 (C1 - Cw) / (C2 - Cw)$$

where C1 is the conductivity of samples just after thawing, C2 is the conductivity of samples after autoclaving and Cw is the conductivity of de-ionized water.

The number of Days to Heading (DHE) was estimated as the number of calendar days from January first to the date when 50% of the spikes emerged. Grain Yield (GY), number of Kernels per Spike (KS) and Thousand-Kernel Weight (TKW) were determined from the combine harvested plots. Grain yield, kernels per spike, thousand kernel weight and number of days to heading across-season variances were estimated per genotype according to the formula given by Lin *et al.* (1986):

$$S^2_i = \Sigma (Y_{ij} - Y_i)^2 / (q-1)$$

where Σ = summation is done from season j = 1 to season j = q = 4

Y_{ij} = Yield of the ith genotype in the jth season

Y_i = Average yield of the ith genotype = $(\Sigma Y_{ij}) / q$

Data analyses: Data analysis was performed using the statistical software Irristat version 5. Percent crown survival and percent leaf cell membrane damage data were arcsine \sqrt{X} transformed and then were analyzed as well as grain yield, kernel per spike, number of days to heading and thousand kernel-weight according to a factorial experiment conducted in a completely randomized block design with three replications. Least Significant Difference (LSD), at 5% level, was used for means separation. Principal component analysis was performed on the basis of the correlation matrix among the measured variables.

RESULTS AND DISCUSSION

Genotypic response to freezing temperatures: The crown survival expressed by a given genotype is a reflection of the hardening process encountered during the autumn

Table 1: Monthly average minimum temperature (°C) and monthly rainfall (mm) recorded at the National Meteorological Office Station of Sétif during the 4 cropping seasons for the period extending from November to May

| Season | Temp | Months | | | | | | | Total Sep/June |
|---------|----------|--------|------|-------|------|------|------|------|-------------------|
| | | Nov | Dec | Jan | Feb | Mar | Apr | May | |
| 1999/00 | Temp. | 2.3 | 0.3 | -1.5 | -2.3 | 2.5 | 3.1 | 5.5 | - |
| | Rainfall | 23.9 | 80.9 | 5.9 | 5.7 | 21.5 | 28.9 | 61.9 | 384.6 |
| 2000/01 | Temp. | 4.6 | 2.2 | 1.5 | 1.0 | 2.66 | 3.5 | 7.5 | - |
| | Rainfall | 15.2 | 61.3 | 79.0 | 20.4 | 8.6 | 13.2 | 19.3 | 303.4 |
| 2001/02 | Temp. | 1.9 | -0.1 | -1.1 | -3.2 | 3.9 | 2.9 | 8.0 | - |
| | Rainfall | 35.3 | 8.5 | 22.7 | 24.0 | 29.3 | 8.8 | 24.2 | 215.9 |
| 2002/03 | Temp. | 5.3 | 5.0 | 0.3 | 1.3 | 2.6 | 4.1 | 6.3 | - |
| | Rainfall | 101.2 | 67.4 | 116.0 | 38.8 | 36.6 | 38.4 | 43.8 | 521.8 |

Table 2: Mean values (%) of crown survival of durum wheat genotypes field hardened and subjected to different temperature treatments during the 2001/02 season

| Genotypes | Temperature treatments | | | | Mean* |
|-----------------------------|------------------------|-------|-------|-------|-------|
| | -5 | -10 | -12 | -15 | |
| Mexicali75 | 97.67 | 80.00 | 65.33 | 22.33 | 66.33 |
| Marouani | 96.67 | 74.33 | 31.00 | 07.33 | 52.08 |
| Mohamed Ben Bachir | 98.00 | 90.00 | 59.00 | 17.00 | 66.00 |
| Heider/Martes/Huevos de Oro | 98.33 | 91.33 | 76.00 | 46.00 | 77.92 |
| Waha | 96.33 | 92.00 | 59.00 | 29.00 | 69.08 |
| Roqueno | 94.33 | 89.33 | 51.67 | 03.33 | 59.66 |
| Cyprus1 | 98.33 | 92.33 | 81.00 | 61.00 | 83.16 |
| Cyprus2 | 97.67 | 94.00 | 83.00 | 61.67 | 84.12 |
| Mazouna | 92.00 | 82.67 | 39.00 | 24.33 | 59.50 |
| Durum D'Oran | 95.00 | 90.00 | 74.00 | 55.67 | 78.66 |
| Beliouni3258 | 99.33 | 92.33 | 72.33 | 56.00 | 79.99 |
| Hedba3 | 93.00 | 47.67 | 20.67 | 08.33 | 42.41 |
| Average | 96.39 | 84.67 | 59.33 | 32.68 | 68.24 |

*Tested against Genotype × Temperature mean square variance, ANOVA done on arcsine \sqrt{X} transformed data; mean values and LSD 5% presented are back transformed to percentage survival, LSD 5% between genotypic mean values = 12.35%, LSD 5% between temperature treatments mean values = 7.13% and LSD 5% between any two combinations of genotype × temperature values = 8.45%

and winter period of the 2001/02 cropping season. During this 90-day period, from November to January, monthly mean temperature was within the threshold range needed to trigger the hardening process (Table 1). According to Szucs *et al.* (1999) the hardening temperature was below +4°C and the mean level of hardening achieved by durum wheat genotypes was within less than 30 day period.

Averaged over genotypes, temperature treatments had a significant effect on crown survival which decreased by a rate of 6.37% per °C decrease in the -5 to -15°C temperature range. The mean survival rates varied from 96.39% at -5°C to 32.68% at -15°C (Table 2). Averaged over temperature treatments, the crown survival rate indicated that Cyprus1, Cyprus2, Waha, Heider /Martes //Huevos de Oro, Durum D'Oran and Beliouni3258 were relatively low temperatures tolerant while Marouani and Hedba3 were susceptible and suffered severe damage. The remaining genotypes showed an intermediate tolerance to freezing temperatures (Table 2).

The significance of the genotype × temperature interaction indicated that genotypes responded differently to the tested temperatures. This interaction stems from the fact that the response varied between two

consecutive temperature treatments for the various genotypes (Table 2 and Fig. 1). -10 and -12°C temperatures enabled genotypes to be differentiated on the basis of their cold tolerance. At -15°C Cyprus1, Cyprus 2, Durum D'Oran, Beliouni3258 and Heider /Martes //Huevos de Oro expressed more than 50% crown survival, while Hedba3, Roqueno and Marouani showed almost zero survival (Table 2).

The tested temperature treatments were effective in inducing severe injury to the leaf cell membrane. The genotype × temperature treatment interaction was not significant; then the genotypic values averaged over temperature treatments give an overall indication about the leaf tolerance to low temperature. Genotypic mean values indicated that Cyprus 1 was the least injured while Marouani and Hedba3 showed more than 50% leaf cell membrane injury. Cyprus2, Durum D'Oran, Mohammed Ben Bachir and Beliouni3258 were equally resistant to low temperature as measured by the leakage test (Table 3).

At -6°C, Marouani, Waha, Mazouna and Hedba3, expressed over 60% membrane injury while Durum d'Oran, Mohammed Ben Bachir, Cyprus1, Cyprus2 had less than 40% membrane damage. The -6°C temperature treatment discriminated very well between the evaluated genotypes.

Table 3: Mean values (%) of membrane damage of field grown durum wheat leaves subjected, at the booting stage, to different temperature treatments during the 2001/02 season

| Genotypes | Temperature treatments (°C) | | | | Mean |
|-----------------------------|-----------------------------|-------|-------|-------|-------|
| | -1 | -3 | -6 | -9 | |
| Mexicali75 | 11.67 | 21.67 | 49.00 | 81.67 | 41.00 |
| Marouani | 23.33 | 41.00 | 61.67 | 91.67 | 54.42 |
| Mohamed Ben Bachir | 06.00 | 19.00 | 30.33 | 67.67 | 30.75 |
| Heider/Martes/Huevos de Oro | 12.33 | 35.33 | 49.33 | 73.67 | 42.67 |
| Waha | 18.67 | 36.00 | 68.33 | 82.67 | 51.42 |
| Roqueno | 23.67 | 33.67 | 42.33 | 92.67 | 48.08 |
| Cyprus1 | 10.00 | 20.67 | 28.67 | 52.33 | 27.92 |
| Cyprus2 | 09.00 | 21.33 | 36.67 | 58.00 | 31.25 |
| Mazouna | 19.33 | 46.33 | 60.33 | 78.33 | 51.08 |
| Durum D'Oran | 11.67 | 22.67 | 35.00 | 64.00 | 33.33 |
| Beliouni3258 | 10.00 | 27.67 | 49.00 | 67.67 | 38.58 |
| Hedba3 | 22.67 | 47.67 | 67.33 | 90.00 | 56.92 |
| Average | 14.86 | 31.08 | 48.17 | 75.03 | 42.28 |

ANOVA done on arcsine \sqrt{X} transformed data; mean values and LSD 5% presented are back transformed to percentage injury. LSD 5% between genotypic mean values = 3.14% LSD 5% between temperature treatments mean values = 2.29% and LSD 5% between any two combinations of genotype \times temperature values = 7.66%

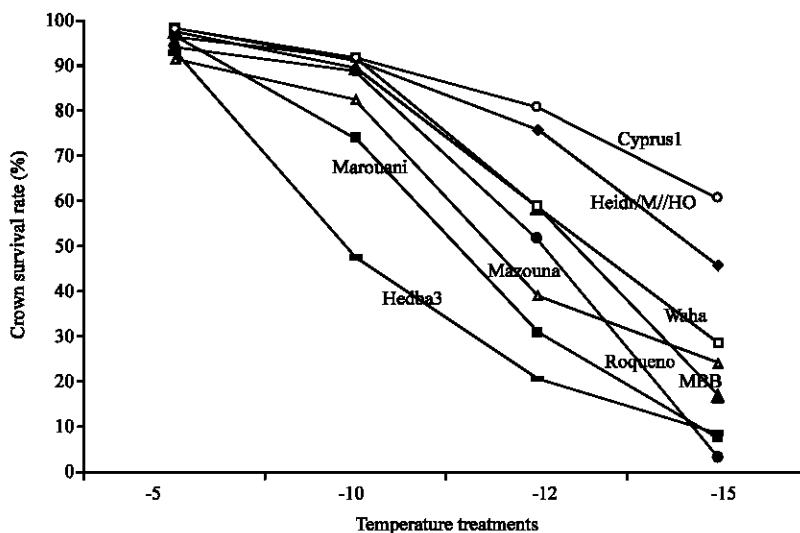


Fig. 1: Genotype \times temperature interaction of crown survival rate for some selected genotypes

The leakage test data were in good agreement with those of crown survival test in classifying the different genotypes as far as the tolerance to low temperature was concerned ($r = 0.576$, $p < 0.05$, $n = 48$).

Agronomic performances and stability: The agronomic performances of the set of evaluated genotypes during the 4 cropping seasons are presented in Table 4. The analysis of variance showed no significant genotype \times season interaction for the number of days to heading; but the interaction was significant for the remaining variables. The season main effect of the measured traits varied significantly. The number of days to heading varied from 117 to 128 days, thousand kernel weight from 33.9 to 46.2 g, number of kernels per spike from 15.4 to 36.3 and grain yield from 319.0 to 505.1 g m⁻² (Table 4).

The 4 cropping seasons presented quite different growing conditions with regard to rainfall distribution pattern and temperature regime (Table 1). These climatic conditions had an important bearing on genotype performances. Seasonal rainfall varied from 215.9 mm in 2001/02 to 521.8 mm in 2002/03. The period of January-February 2000 was very dry accompanied by a rainy April-May 2000 period. In contrast, December 2000-February 2001 period was rainy while the March-May 2001 period was relatively dry. The 2002/03 season showed a qualitatively good rainfall distribution pattern over all the cropping cycle (Table 1).

Averaged over years, the mean number of days to heading indicated that the set of evaluated genotypes could be grouped into a late group made of local land races, Mohammed Ben Bachir, Durum Oran, Beliouni3258, Hedba3 and Marouani with 125.0 days as average of

Table 4: Season and genotypic main effects of the measured variables and the across seasons variance

| | DHE (days) | σ^2_{DHE} - | TKW (g) | σ^2_{TKW} - | KS - | σ^2_{KS} - | GY (g m ⁻²) | σ^2_{GY} ** |
|------------------------|---------------|-----------------------|------------|-----------------------|---------|----------------------|----------------------------|-----------------------|
| Season main effect | | | | | | | | |
| 1999/00 | 117 | | 37.1 | | 15.4 | | 338.2 | |
| 2000/01 | 119 | | 36.8 | | 30.4 | | 320.1 | |
| 2001/02 | 128 | | 33.9 | | 32.9 | | 505.3 | |
| 2002/03 | 121 | | 46.2 | | 36.3 | | 497.2 | |
| Genotypic main effect | | | | | | | | |
| Mexicali 75 | 117 | 47 | 38.7 | 14.9 | 29.4 | 195 | 470.7 | 21.0 |
| Marouani | 125 | 30 | 40.2 | 46.0 | 28.0 | 144 | 403.2 | 13.2 |
| Mohammed Ben Bachir | 127 | 39 | 40.3 | 18.9 | 24.4 | 108 | 399.1 | 41.3 |
| Heider/Martes //Huevos | 119 | 13 | 38.0 | 49.6 | 31.2 | 16 | 446.3 | 2.4 |
| Waha | 116 | 24 | 35.2 | 47.3 | 27.2 | 50 | 414.2 | 37.9 |
| Roqueno | 118 | 32 | 40.6 | 20.6 | 27.9 | 209 | 469.5 | 13.6 |
| Cyprus1 | 117 | 19 | 36.3 | 53.5 | 30.0 | 87 | 418.2 | 22.5 |
| Cyprus2 | 119 | 27 | 37.6 | 76.0 | 31.4 | 78 | 475.6 | 6.2 |
| Mazouna | 123 | 34 | 38.3 | 47.3 | 27.4 | 48 | 369.2 | 15.6 |
| Durum D'Oran | 127 | 37 | 38.3 | 45.3 | 30.3 | 248 | 366.5 | 12.8 |
| Beliouni3258 | 124 | 43 | 39.8 | 20.8 | 27.7 | 95 | 400.3 | 7.6 |
| Hedba3 | 124 | 51 | 38.7 | 8.9 | 30.0 | 124 | 349.2 | 2.4 |
| Average | 121.7 | 33.0 | 38.5 | 37.4 | 28.7 | 116.8 | 415.3 | 16.4 |
| LSD 5%* | 3.7 | - | 2.5 | - | 5.8 | - | 32.2 | - |

DHE = Number of days to heading; TKW = Thousand Kernel Weight; KS = Number of kernels per spike; GY = Grain yield and their across seasons variances. *LSD 5% is based on genotype × temperature treatment interaction mean square for TKW, GY, KS and on residual for DHE. ** to be ×10³

number of days to heading and an early group made of the remaining genotypes with 117.7 days. Early heading group showed less across seasons variation for the number of days to heading with a mean variance of 19.2 while the late group exhibited an across seasons mean variance of 46.8 (Table 4). The stable cultivar for this characteristic was Heider /Martes //Huevos de Oro and the variable one was Hedba3. This variation stems from late drought and heat stresses which hasten specifically the late heading and maturing genotypes.

Marouani, Mohammed Ben Bachir and Roqueno showed above average thousand kernel weight while Waha and Cyprus1 had below average thousand kernel weight (Table 4). Mexicali75, Mohammed Ben Bachir, Roqueno, Beliouni3258 and Hedba3 had a low across seasons thousand kernel weight variance, while Cyprus1, Cyprus2, Mazouna, Heider /Martes //Huevos de Oro and Waha exhibited a high across seasons thousand kernel weight variance.

Little differences appeared among genotypes for the number of kernels/spike, on average over seasons, the cultivar Mohammed Ben Bachir had the minimal average with a mean number of kernels/spike of 24.4. Cyprus 2 was characterized by a high mean number of kernels/spike of 31.4. Differences between genotypes were noted for the magnitude of the across seasons variances. Durum D'Oran, Roqueno, Mexicali75, Marouani, Hedba3 and Mohammed Ben Bachir showed a high across seasons variance. The low across seasons variance for the number of kernels/spike was shown by Heider /Martes //Huevos de Oro (Table 4).

Best grain yielding cultivars were Cyprus2, Roqueno, Mexicali75 and Heider /Martes //Huevos de Oro with a mean grain yield, averaged over seasons, greater than

Table 5: Percentage of variation accounted for and latent vectors of the principal component analysis on the correlation matrix among the measured variables

| | Principal component | | |
|----------------------------|---------------------|---------|---------|
| | 1 | 2 | 3 |
| Variance accounted for (%) | 35.5 | 18.2 | 13.9 |
| Latent vectors | | | |
| DHE | 0.3556 | 0.1214 | 0.4910 |
| σ^2_{DHE} | 0.4500 | -0.0590 | -0.0407 |
| TKW | 0.3993 | -0.1116 | -0.1275 |
| σ^2_{TKW} | -0.4252 | -0.0388 | 0.2421 |
| KS | -0.2583 | -0.5734 | 0.1587 |
| σ^2_{KS} | 0.2847 | -0.1695 | -0.2618 |
| GY | -0.2455 | -0.1932 | -0.6612 |
| σ^2_{GY} | 0.0025 | 0.6474 | -0.3135 |
| Survival | -0.2887 | 0.3253 | 0.2186 |
| Injury | 0.1984 | -0.2189 | 0.0736 |

DHE = Number of days to heading, σ^2_{DHE} = Across seasons variance of the number of days to heading; TKW = Thousand Kernel Weight, σ^2_{TKW} = Across seasons variance of thousand kernel weight; KS = Number of kernels per spike, σ^2_{KS} = Across seasons variance of the number of kernels per spike; GY = Grain Yield, σ^2_{GY} = Across seasons variance of grain yield

440.0 g m⁻². Hedba3, Durum D'Oran and Mazouna were characterized by a low yield average (Table 4). Hedba3, a low yielding cultivar and Heider /Martes //Huevos de Oro, a high yielding cultivar, had a low across seasons variance. High variability was shown by Mohammed Ben Bachir and Waha grain yielding ability, with an across seasons variance almost 16 times higher than that of Heider /Martes //Huevos de Oro (Table 4).

Relationships between freezing tolerance, agronomic performances and stability:

The first 3 principal components accounted for 67.6% of the variation within the analyzed data set (Table 5). Principal component 1 (PC1) accounted for 35.5% of the variation and had large positive loadings for the across seasons variance of the

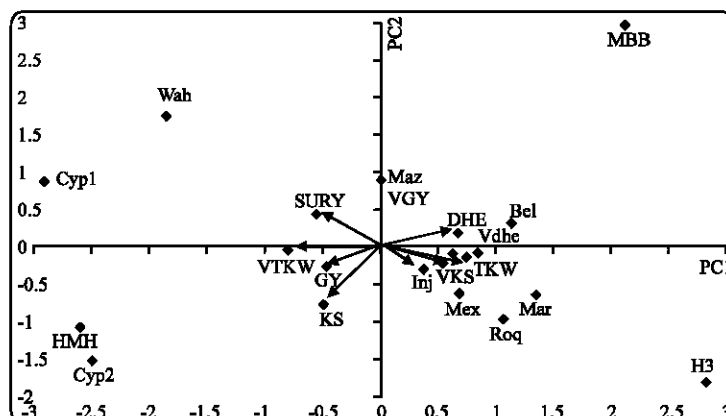


Fig. 2: Principal Component Analysis (PCA) projections on axes 1 and 2 accounting for 57.6%, of the total variance. The eigen values of the correlation matrix are symbolized as vectors representing the traits that most influence each axis and points representing the following genotypes H3 = Hedba3, MBB = Mohammed Ben Bachir, Oran = Durum D'Oran, Maz = Mazouna, Mar = Marouani, Cyp1 = Cyprus I, Cyp2 = Cyprus 2, Wah = Waha, HMH = Heider/Martes//Huevos de Oro., Mex = Mexicali75, Roq = Roqueno, Bel = Beliouni3258. Traits are GY = Grain yield, TKW = Thousand Kernel Weight, KS = Number of kernels per spike, DHE = Number of days to heading, Inj = Leaf cell membrane injury, Sur = Crown survival rate and VGY, VTKW, VDHE, VKS = Across season variance for the mentioned traits

number of days to heading, thousand kernel weight and the across seasons variance of the number of kernels per spike. It had a relatively large negative loading for the stability of thousand kernel weight.

The second principal component (PC2) accounted for 18.2% of the variation and represented crown survival rates and yield stability toward its positive direction and leaf cell membrane damage and number of kernels/spike toward its negative direction (Table 5 and Fig. 2). The third principal component (PC3) accounted for another 13.9% of the variation and had a negative loading for grain yield and a positive loading for the number of days to heading (Table 5 and Fig. 3).

From a biological point of view PC1 can be interpreted as the contrast between kernel weight, the stability of number of kernels per spike and that of the number of days to heading against the stability of thousand kernel weights. PC2 represented mainly the genotypic responses to freezing temperature, as measured by the crown survival rate and the leaf cell membrane injury, the number of kernels/spike and yield stability (Table 5 and Fig. 2). PC3 represented grain yield as opposed to the number of days to heading (Table 5 and Fig. 3).

PC1 and PC2 accounted for 53.7% of the total variation; this means that their bi-plot constitutes a good basis for genotype characterization. According to their relative position along the PC1 axis, cultivars Marouani, Hedba3 and Beliouni3258 had above average thousand

kernel weight, a low across seasons thousand kernel weight variance and a high across seasons variance of the number of days to heading and the number of kernels per spike. Along the same axis, cultivars Cyprus1, Cyprus2, Waha and Heider /Martes //Huevos de Oro showed the opposite characteristics. They had below average thousand kernel weight, a high across seasons thousand kernel weight variance and a low across seasons variance of the number of days to heading and the number of kernels per spike (Fig. 2 and Table 4).

When considering the relative position of the genotypes along the PC2 axis, Mohammed Ben Bachir had above average freezing tolerance, a high across seasons grain yield variance and a low number of kernels per spike. Due to its position on the PC1-PC2 plan Mohammed Ben Bachir was characterized by a high thousand kernel weight, a high across seasons variance of the number of days to heading and the number of kernels per spike, traits related to PC1.

Furthermore Waha, Cyprus1 and Beliouni3258, being positioned on the same side of the bi-plot as the cultivar Mohammed Ben Bachir, were characterized by an above average freezing tolerance and instable grain yield. On the opposite side of the PC1-PC2 bi-plot, Cyprus2, Heider /Martes //Huevos de Oro, Marouani and Hedba3 showed also below average freezing tolerance, above average number of kernels per spike and a relatively stable grain yield (Fig. 2 and Table 3).

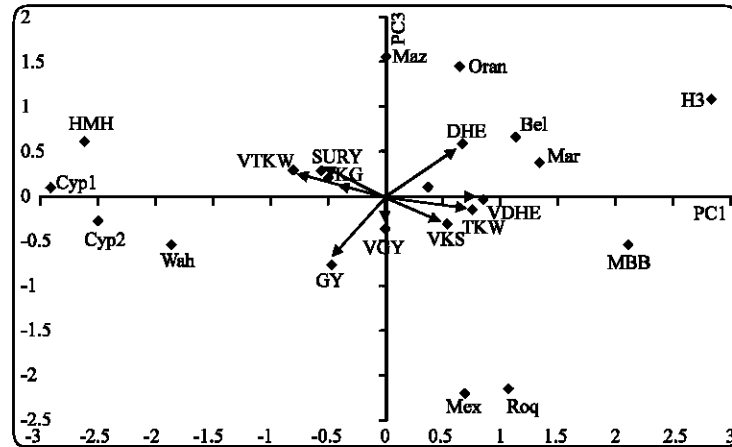


Fig. 3: Principal Component Analysis (PCA) projections on axes 1 and 3 accounting for 56.10%, of the total variance. The eigen values of the correlation matrix are symbolized as vectors representing the traits that most influence each axis and points representing genotypes as follow H3 = Hedba3, MBB = Mohammed ben Bachir, Oran = Durum Oran, Maz = Mazouna, Mar = Marouani, Cyp1 = Cyprus I, Cyp2 = Cyprus 2, Wah = Waha, HMH = Heider/Martes//Huevos de Oro, Mex = Mexicali75, Roq = Roqueno, Bel = Beliouni. Traits are GY = Grain Yield, TKW = Thousand Kernel Weight, KS = Number of kernels per spike, DHE = Number of days to heading, Inj = Leaf membrane injury, Sur = Crown survival rate and VGY, VTKW, VDHE, VKS = Across seasons variance for the mentioned traits

Mexicali75, Roqueno, Mazouna and Durum D'Oran were more related to PC3. Mexicali75 and Roqueno were characterized by a relatively high grain yield and earliness, while Durum D'Oran and Mazouna were characterized by a low average grain yield and lateness. All four genotypes were characterized by intermediate mean values for the variables related to PC1 (Fig. 3 and Table 3). Among the genotypes having high grain yield and early heading, Waha had an irregular grain yield associated with a low number of kernels per spike while Cyprus 2 and Heider /Martes //Huevos de Oro had stable grain yield and high number of kernels per spike. Cyprus 1 had intermediate values for these characteristics. Among the low yielding and late genotypes, Mohammed Ben Bachir exhibited an instable grain yield associated with a low number of kernels per spike while Beliouni3258, Marouani and Hedba3 had a more stable grain yield and a high number of kernels per spike (Fig. 3 and Table 3).

In durum wheat, as in other crops, grain yield depends on a number of environmental stress factors that can prevent the expression of the genetic potential. Severe yield reduction is often caused by low and high temperatures and drought stresses, acting separately or in combination. The high elevation area of Eastern Algeria has a semi arid, Mediterranean-type climate, in which 70% of the 250-500 mm annual precipitation falls during the winter months, this is followed by a rapid transition in weather conditions from cold and wet in winter and early

spring to hot and dry during the spring and summer (Bouzerzour and Dekhili, 1995). Genetic variability for tolerance to stresses plays, in this aspect, a major role in the development of genotypes adapted to this unfavourable environment.

The results of the present study showed that artificial laboratory tests were effective in sorting out the genotypes which were low temperatures tolerant those which were sensitive. The presence of a significant genotype \times temperature interaction indicated that genotypes responded differently to the tested temperatures. The -10 and -12°C temperature levels enabled genotypes to be differentiated on the basis of their cold tolerance appreciated through crown survival. The leakage test data, obtained from the leaf test realized at the booting growth stage, were in good agreement with those of crown survival test in classifying the different genotypes as far as the tolerance to low temperature was concerned. This result indicated that genotypes which possess the ability to harden during winter retain some of this feature until the booting stage.

The measured agronomic traits varied significantly between seasons and genotypes, indicating the presence of significant genotypic variability and differential responses to the growth conditions experienced during the cropping seasons test. It was possible to identify cultivars with high and stable yield and a good combination of yield components among late and early

groups of genotypes. Tolerance to low temperatures was present in late and early genotypes, but the results failed to show any relationships between the tolerance to low temperatures and agronomic performances.

An understanding of adaptation allows a better targeting of germplasm to specific environments to reduce the risks of crop failures to farmers. Stability and risk efficiency are generally explained by the differential genotype sensitivity to environmental variables such as minimum temperatures during the spike growth (Vargas *et al.*, 1998), spring frost hazards at the heading stage (Voltas *et al.*, 1999) and drought and high temperature during the grain filling period (Wardlaw, 2002). All the climatic factors explained a large portion of the grain yield genotype \times environment interaction in durum wheat (Annichiarico *et al.*, 2002b; Bahlouli *et al.*, 2005).

Sayre *et al.* (1997) mentioned that yield components which contributed the most to the decrease in yield under stresses were the number of spikes m^{-2} , and the number of kernels per spike; thousand kernel weight having less influence. Low temperatures reduced grain yield through their action on fertile tiller mortality and spike fertility. The effect of winter low temperatures is more pronounced on fertile tillers, while late frost events affect spike fertility (Fletcher, 1983). In the present study, variation in grain yield was not related to that of the number of kernels per spike, nor to the genotypic capacity to tolerate low temperatures, but to the number of days to heading. In fact grain yield ability, grain yield stability and tolerance to low temperatures were unrelated and were represented by separate PC components.

According to Annichiarico *et al.* (2002b) intermediate heading time, high level of the two yield component: Number of fertile tillers per unit square of soil surface and kernels per spike and a high level of tolerance to winter cold and late frost proved useful for adaptation to unfavourable environment. Evolutionary adaptation to these constraints had led Algerian landraces to develop a typical lateness combined with drought stress avoidance through a large rooting system, combined to straw tallness and the presence of long awn (Ali-dib *et al.*, 1992).

In order to verify whether variation in the genotypic ability to harden and its subsequent effect on tolerance to low temperatures is likely to be of significant adaptive value under natural environments, field based observation must be more reliable. In fact the low temperatures damage to genotypes, under the field conditions of the present study, could not be detected easily due the confounding effects of terminal heat and drought stresses, acting on

the same yield components as the cold stress. It may be therefore unreliable to expect a significant correlation between the laboratory tests results and the agronomic field performances.

Furthermore hardiness is a very complex trait and obviously a high score of leaf cell injury or crown survival *per se* does not induce high resistance to all stresses. However the use of artificial freezing tests allows one to monitor frost tolerance which represents a part of the plant hardening capacity. These considerations explain the lack of correlation between the results of the artificial tests and the field performances,

The results of the present study identified, however, early, freezing tolerant and above average yielding cultivars such as Waha and Cyprus 1 which can serve as genetic source to improve tolerance to low temperatures in short cycle genetic background. Facilities and protocols need to be developed to be able to apply artificial freezing tests at later growth stages which match the critical and sensitive crop stage at which late spring frost is most likely to hit the crop. Genetic variability plays a major role in determining adaptation to environmental stresses and hence in supporting the development of genotypes adapted to variable climatic conditions.

Enhancing resistance to various forms of stress by conventional breeding is being coupled to the precise molecular analysis of the genes involved in the stress response. Kosner and Pankova (2002) combined genes from spring and winter type cultivars to generate a wide range of variability in the vernalization and photoperiodic requirements and earliness *per se* leading to more opportunities to select for adaptation. The results of the present study demonstrated the presence of genetic variability of resistance to low temperature, it was possible to distinguish lines with low cold tolerance from the lines with medium or high cold tolerance, leading to a preliminary selection and monitoring of cold tolerant lines through the use of artificial tests.

The artificial laboratory tests employed were effective in classifying the evaluated genotypes according to their degree of tolerance to low temperatures. The -10 and -12°C temperature levels for crown test and -6°C for leaf membrane injury enabled genotypes to be differentiated on the basis of their cold tolerance. Data of booth tests were in good agreement suggesting that genotypes which possess the ability to cold acclimate during winter retain some of this feature until the booting stage. The measured agronomic traits varied significantly between seasons and genotypes, indicating the presence of significant genotypic variability and differential responses to the growth conditions experienced by the successive

cropping seasons. Tolerance to low temperatures was present in late and early genotypes, but the results failed to show any relationships between the tolerance to low temperatures and agronomic performances due the confounding effects of terminal heat and drought stresses, acting on the same yield components as the cold stress. The results of the present study identified, however, early, freezing tolerant and above average yielding cultivars such as Waha and Cyprus 1 which can serve as genetic source to improve tolerance to low temperatures in short cycle genetic background.

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