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Cultivation of the four durum wheat varieties grown in the Moroccan Oriental region

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Abstract: The study focused on the cereal chain of four durum wheat varieties registered in the national catalog in 2009. The agronomic behavior of these three- to four-week-old varieties (Ourgh, Prospero, Riyad and Vitron) in the face of water and osmotic stress was studied with a view to a rational and reasoned strategy for their use. During the period of water stress application, it appears that the leaf part is more sensitive than the root part, depending on the water balance. The accumulation of proline and chlorophyll pigments is evident in all varieties, especially during severe stress, which shows a 10-fold increase in glutamine synthetase activity compared with the control, with an accumulation of nitrates in the roots and a transfer of these nitrates to the stressed leaves. It seems that a cascading regulatory pathway apart from foliar proline accumulation is a consequence of severe stress behavior.

Keywords: *durum wheat; nutrients; water stress; proline*

1. Introduction

The Moroccan agricultural sectors in regions with a hostile bioclimate to develop their skills and improve their know-how, in all areas their know-how in all areas, from upstream (producers) to downstream (consumers). downstream (the consumer). Hence the importance of the Green Morocco Plan an opportunity and a roadmap for agricultural development, which integrates the data and imperatives of the national and international environment in relation to the various specificities of Moroccan agriculture. (DPA, 2012).

The "value chain" approach has been adopted by the Green Morocco Plan as a fundamental principle in the development of high-performance, integrated agriculture. At a time of globalized trade and heightened competition on the agricultural products market, the organization of supply chains and private sector ownership of the sector's development is a prerequisite for improving our competitiveness. Today, seven years after the launch of the Green Morocco Plan, we have witnessed the creation and development of 19 interprofessional federations, including 14 representing the plant sectors and 5 representing the animal sectors (Green Morocco Plan, 2015).

2. Methodology

2.1 Experiments

Seeds of four varieties of wheat, durum wheat (*Triticum durum Desf*.) were kindly provided by the O.N.S.S.A. in Oujda. The varieties used are: OURGH, PROSPERO, RIYAD and VITRON. Grain sterilization and seedling culture: durum wheat grains are sterilized with highly diluted bleach for 5 minutes. They are then rinsed thoroughly with running water and placed in blotters with blotting paper. The soaked grains are left to germinate for 2 to 3 days. Germinated seeds are transplanted into pots (6 cm diameter, 6cm high) containing peat, at a rate of 10 per pot. The seedlings were watered every three days with tap water, in quantities corresponding to the reference evapotranspiration (ET0) (Kutch, 1978), based on the reference evapotranspiration for the Moroccan climate (MARA, 1978).

Honeycomb plates were designed for each variety; and for each treatment (control, moderate stress and severe stress). Plants having grown under standard growing conditions until the appearance of three leaves, 21 were subjected to water stress by suspension of irrigation. Seedlings are divided into three batches:

ƒ Control seedlings watered every 3 days

ƒ Moderately stressed seedlings undergo resumption of irrigation after one week and watering at the beginning and end of the week.

ƒ Severely stressed seedlings stop irrigation completely. Seedlings reaching the 3-leaf stage served as the basic material for our study.

Plant organs, notably roots, leaves and stems, are sampled throughout the experiment to measure proline, chlorophylls, glutamine synthetase and various ions.

2.2 Estimated leaf area (cm^2)

The morphology of durum wheat leaves is delicate due to their small size, so leaf area is estimated directly using AUTOCAD 2010 software. Leaf area is determined after each leaf sampling and for each treatment. The unit area is multiplied by the number of leaves to estimate the total leaf area (results not shown).

2.3 Proline assay

The method used is that of Monneveux and Nemmar (1986), modified by Rascio *et al*. (1987) and Trolls and Lindsley (1995). It is soluble in water, methanol and benzene and easily oxidized by ninhydrin or tricetohydrindene (Denden *et al*., 2005). 100 mg of fresh leaf material is weighed and placed in a hermetically sealed test tube. Add 2 ml of 40% methanol and heat in a water bath at 85°C for 1 hour. After cooling, to 1ml of the extraction solution are added: 1ml acetic acid, 25 mg ninhydrin and 1ml of the mixture (120 ml distilled water + 300 ml pure acetic acid + 80 ml orthophosphoric acid, specific gravity 1.7). The mixture is the mixture is boiled for 30 minutes in a water bath, then cooled. 5ml toluene are added to the mixture, which is vortexed.

The contents of a spatula of Na_2SO_4 are added. After stirring, two phases are observed, the upper phase containing the proline will be recovered for the assay Absorbance is read at 528 nm (G. BOYER spectrophotometer, SP-2000 Spectrum vis spectroptoMETER). The proline content is determined using a standard range of Lproline.

2.4 Determination of chlorophyll pigments

500 mg of fresh leaf material, taken from the middle leaf blade, is ground in 80% acetone (Tran *et al.*, 1995). Grinding is repeated several times to extract all the chlorophylls. After filtration, the homogenate is stored in a dark, cool place to prevent oxidation. Absorbance is read at 646 nm and 663 nm (Bruinsma, 1961), and the chlorophylls assayed are Chl a and Chl b respectively. Total chlorophyll concentrations, expressed in mg/g MF, are given by the formula:

$$\text{Chl (a + b)} = (7.15\ DO663 + 18.71\ DO646)X\frac{\text{V}}{\text{M}}$$

OD : Optical density (nm)
V : Volume of total extract (in liters)
M : Mass of fresh material (g)
Chl : Chlorophyll

2.5 Determination of glutamine synthetase activity

50 mg fresh leaf material is ground at 4°C in 1 ml extraction buffer containing (25 mM Tris pH 7.8; 1 mM EDTA; 1mM MgCl2 and 0.1% (V/V) ù-mercaptoethanol. The extract is centrifuged at 8,000 rpm at 4°C for 20 minutes. Glutamine synthetase (GS) activity is determined on the crude extract. It is characterized by the reaction:

$$\text{L-glutamate} + NH_2OH + \text{ATP} \xrightarrow{\text{GS}} \text{Ỿ- glutamyl-hydroxamate} + \text{ADP} + \text{Pi}$$

Hydroxylamine (NH_2OH) is used as it has been shown that GS activities, measured colorimetrically, give the same results as in the presence of the natural substrate NH4+ (O'Neal and Joy, 1973). The enzymatic crude extract contains: 450 µl extract +100µl (20 mM $MgCl_2$, 80 mM glutamate, 60 mM NH_2 OH, 4 mM EDTA) +100µl ATP; the reaction takes place at 35°C for 30 minutes.

The reaction is stopped by adding a solution of $FeCl_3$ (0.37 M $FeCl_3$, 0.2M TCA, 0.67 M HCl) which, in the presence of L-glutamyl-hydroxamate, gives a brown-colored complex. After centrifugation at 2400 g for 5 min at 4°C, absorbance is read at 540 nm. The quantities of products formed are determined by a standard range performed with increasing concentrations of -Ỿ glutamyl-hydroxamate. Activity is expressed in µmoles/gMF.

2.6 Water content

500 mg of fresh root and leaf material are weighed to determine the weight of fresh matter (WFM). They are then dried in an oven at 80°C for 48 hours, then weighed to determine the dry matter weight (DMW) up to constant weight. Biomass is expressed in grams. The water content is calculated using the equation:

$$\boldsymbol{TE}\ (\%) = \frac{\boldsymbol{PMF - PMS}}{\boldsymbol{PMF}} \times \mathbf{100}$$

TE: Moisture content, PMF = Weight of fresh sample material, PMS = Weight of dry matter in the sample.

2.7 Germination rate

It consists of counting the number of germinated seeds in relation to the total number. Germination is defined as the emergence of the radicle from the seed coat with a length of at least 2 mm (Dirik, 2000). It is expressed as the ratio of the number of germinated seeds to the total number:

$$TG\ (\%) = \frac{NTGG}{NTGT} \times 100$$

N, NTGG: Total number of germinated seeds T (total),
NTGT: Total number of seeds tested (results not shown).

2.8 Ion extraction

Ions present in biological material or soil samples are extracted by hot acid treatment (70°C for 1 hour 30 minutes). Extraction is carried out using 0.1 N HCl for all assays (nitrates, nitrites, phosphates and sulfates).

Mineral ion assays

Nitrite (NO_2^-), nitrate (NO_3^-), sulfate ($SO4_2^-$) and phosphate (PO_4^{3-}) ions are extracted and measured spectrophotometrically. Ions are measured in irrigation water and pressed juice.

2.8.1. Nitrate dosage

The NO method3- is based on hydrazine sulfate reduction. Nitrates are reduced to nitrites by the addition of copper sulfate, hydrazine sulfate and sodium hydroxide. Nitrites are then determined colorimetrically by diazotization with sulfanilamide and coupling with Nnaphthyl-diethylene diammonium dichloride (NNED). 1ml of sample is added to 0.6 ml of copper sulfate ($CuSO_4$; 25mg/l) after stirring, 0.4 ml hydrazine sulfate (0.69g/l) is added followed by stirring at 37°C for 5 minutes. Finally, 0.6 ml NaOH (0.3N) is added with stirring at 37°C for 10 minutes. 1.5 ml of the mixture (sulfanilamide 40g/l and NNED 2g/l in 4.5N phosphoric acid) is added with stirring at 37°C for 10 minutes. Absorbance is read at 546 nm. NO concentrations3- are determined using a KNO standard range3- (0 to 0.2 mM).

8.1.2. Nitrite determination

The method for determining NO_2^- is by reduction with N-azide3- in acid medium or with sulfamic acid (Morris and Riley, 1963; Toei and Kiyose, 1977). The oldest method involves forming a red azo dye with sulfanilic acid and Ị-naphthylamine (Griess reagent). The formation of an azo dye from sulfanilamide and N-(naphthyl1)ethylene diamine. To 25 ml of solution containing 5 to 50µg of NO_2^-, add 1 ml of sulfanilamide and wait 5 minutes. 1 ml naphthyl-ethylenediamine is added, topped up with 50 ml distilled water. Absorbance is read at 550 nm.

8.1.3. Inorganic phosphate assays

Inorganic phosphate in the samples was determined by the Ames method (1966). The reaction medium consisted of 0.9 ml plant extraction medium and other extracts, 2.1 ml reagent (ascorbic acid 10%, ammonium heptamolybdate 0.42% in H_2SO_4 1N, (1/6) (V/V), prepared at the time of use and stored at 4°C. After a 20-minute incubation at 45°C (water bath), samples are cooled under a stream of water. The stateon range is 0 to 0.1 mM. Absorbance is read at 820 nm.

8.1.4. Sulfate dosage

To 25 ml of solution containing from 250 µg to 2 mg SO_4^{2-} is added 0.25 g barium chloride. Shake for 30 seconds and wait 30 minutes, then shake by inverting the bottle several times before measuring at 480 nm. The standard range is prepared with a solution of $K_2\ SO_4$. (Grunbaum and Pace, 1965; Wimberley, 1968).

8.1.5. Statistical analysis

The results are subjected to a descriptive statistical analysis and a one-way analysis of variance (ANOVA), using SPSS software, version 21. The values framed by their means and standard deviations are compared using the Newman and Keuls method (Dagnelie, 1999) based on the smallest significant value. Means are compared using Tukey's method. Results are considered significant when $P<0.05$. On the cereal figures, each mean is assigned a letter, and means followed by the same letter are not significantly different. The values obtained are the statistical averages of three repetitions.

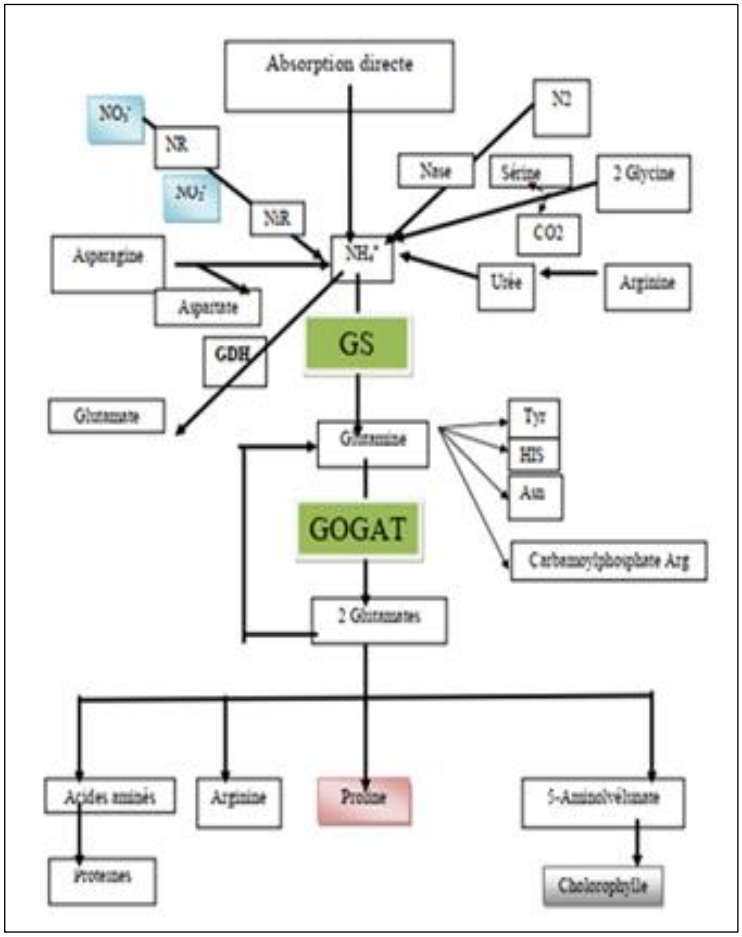


Figure a: The main metabolic pathways involved in ammonium assimilation and proline synthesis

3. Results and Discussion

3.1 Seedling growth

The leaf area of the seedlings showed a significant decrease in total leaf area as a function of the degree of water stress applied. The evolution of leaf area followed the same trend as that of leaf length and number, which are perfectly correlated. The more severe the water stress, the more these parameters progressively decrease. Figure 1a shows that the action of water stress is manifested by a reduction in stem, root and leaf length. Even rehydration does not fully restore this growth. The lengths of the whole seedling (leaf, stem and root) of the controls are greater than those of the moderately and then severely stressed plants. The same is true for the widths (Figure 1b) of these seedlings, the effect of which can be seen in all varieties. The results are verified by statistical testing using analysis of variance, which reveals that the water stress treatments have a very highly significant difference ($P\leq0.001$) on seedling growth.

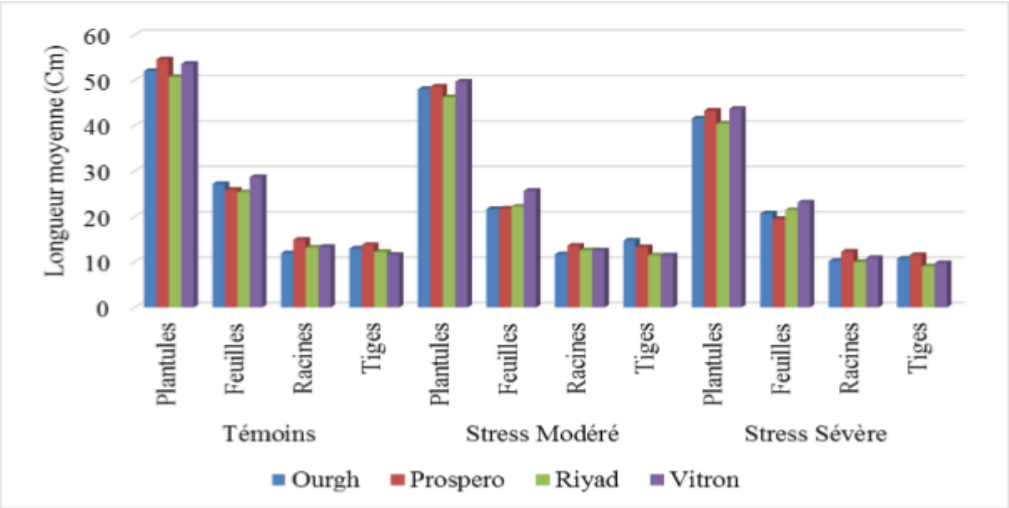


Figure 1a. Seedling length at different degrees of water stress.

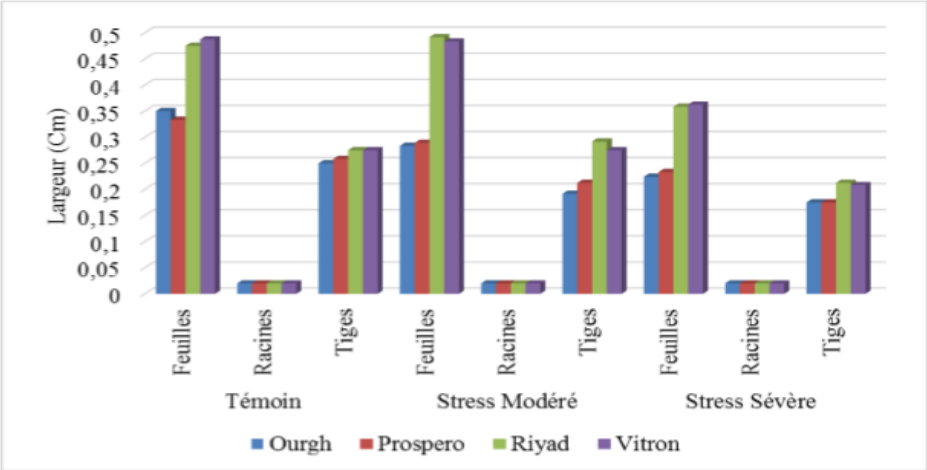


Figure 1b. Seedling width at different degrees of water stress

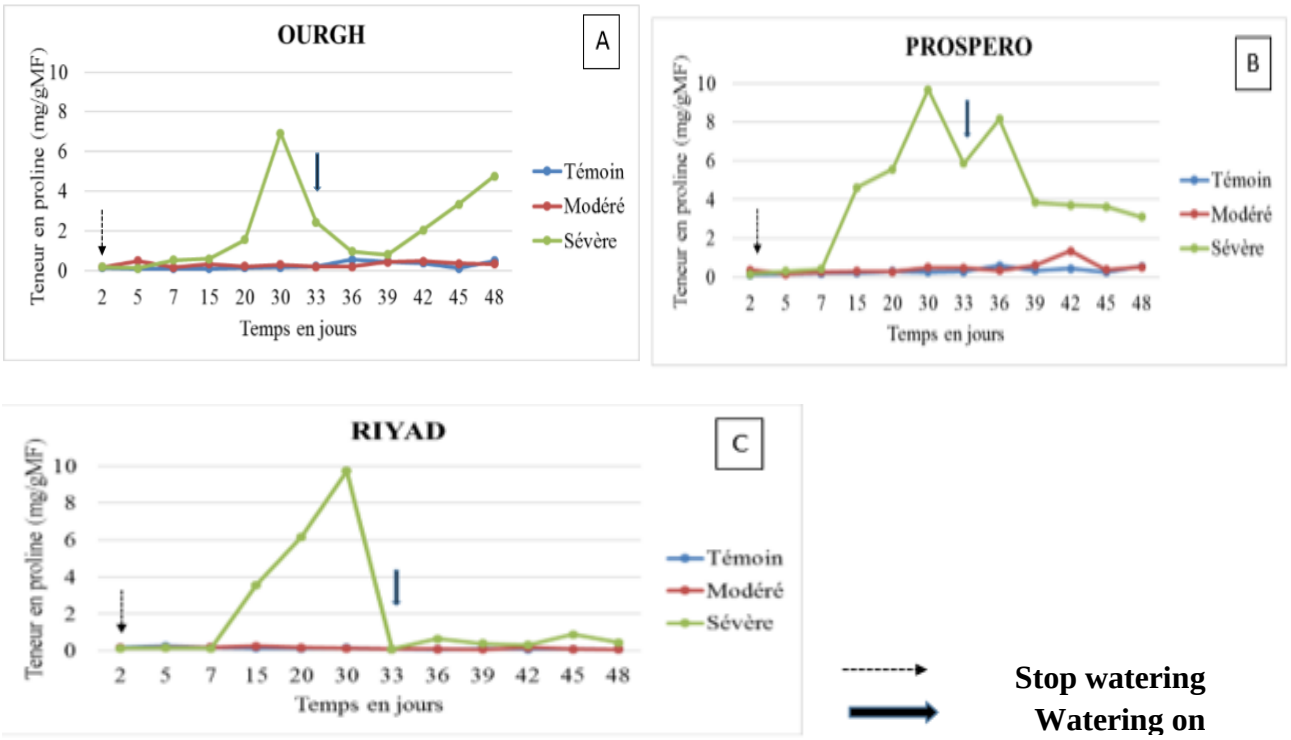
3.2 Leaf and root morphology

The morphological appearance of leaves (photos 1, 2 and 3) and roots shows that the more severe the stress, the more yellowing, leaf curling and reduction in leaf and root fresh weight are recorded. Severely stressed seedlings become increasingly dry. The control, on the other hand, retains its green leaves and almost constant fresh weight, followed by the moderately stressed treatment.

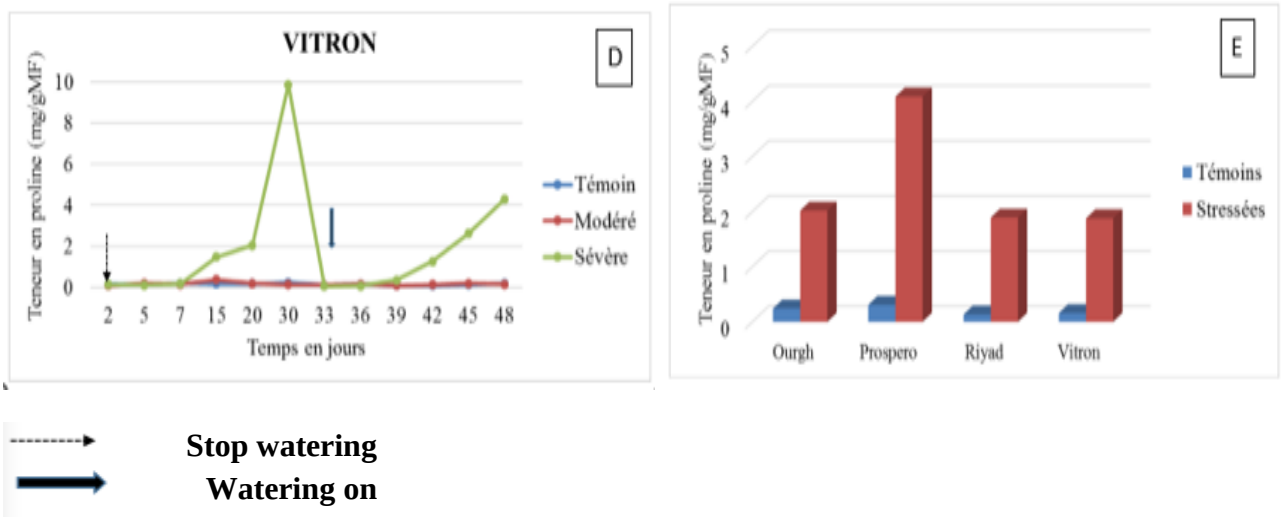
Photo 1: Control seedlings. **Photo 2**: Moderate seedlings **Photo 3**: Severe seedlings.

3.3 Proline accumulation

We tested the effect of water stress by stopping irrigation and then restarting on leaf proline content as a biochemical selection response for the durum wheat varieties listed in the materials and methods section. The figures below show the kinetics of proline accumulation during and after stress. The results show that for the 4 varieties studied, proline content increases significantly after water deprivation and decreases after irrigation resumes. (Figures 2 A, B, C, D, E)



Figures 2 (A, B, C): Proline content in plants of durum wheat varieties with and without water stress: A, B and C represent a comparison of varietal response to severe water stress and that of the control.



Figures 2 (D and E): Proline content in plants of durum wheat varieties with and without water stress: A, B, C and D. E represents a comparison of varietal response to severe water stress and that of the control

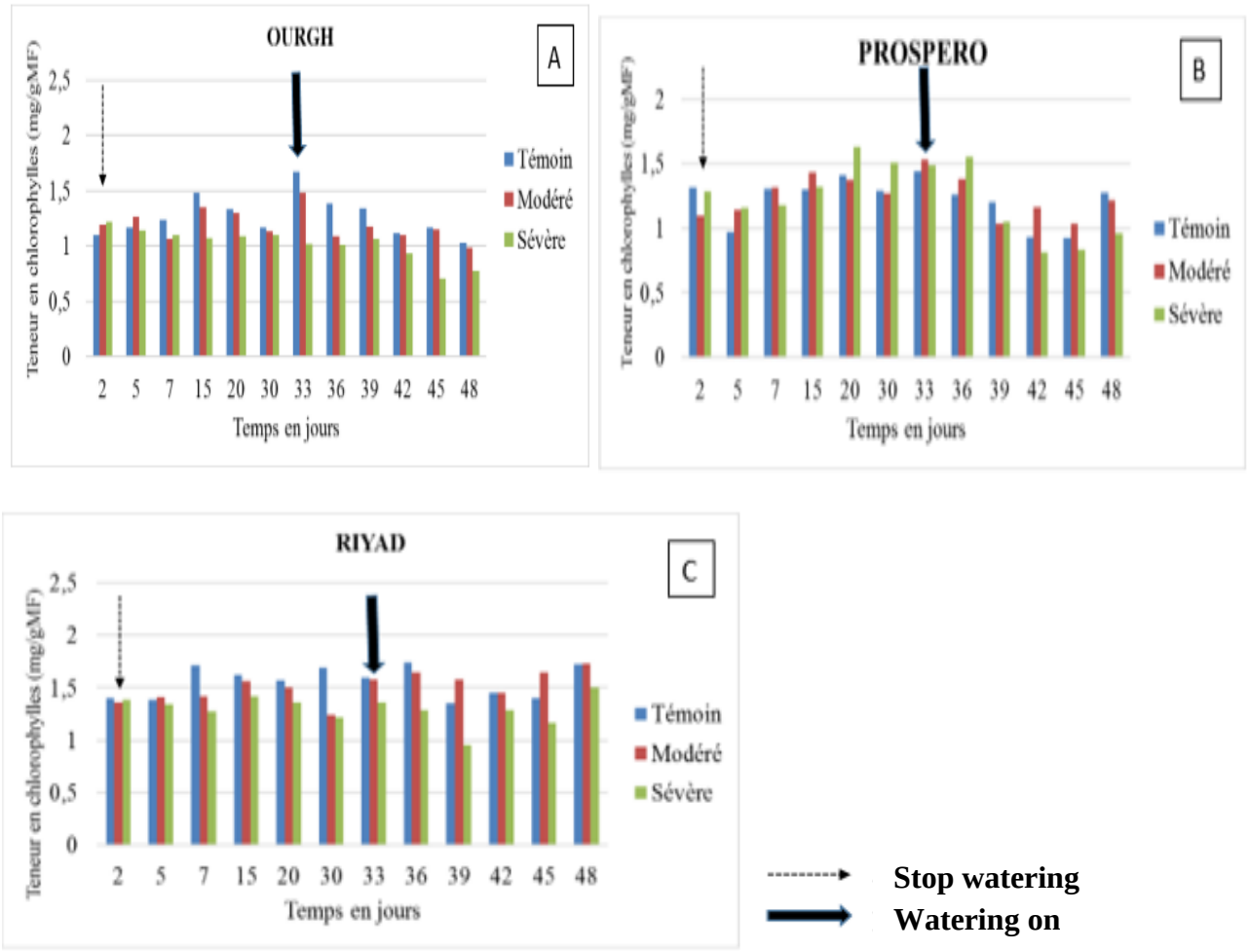
This increase appears after 15 to 20 days of stress, and becomes significant with time. After resumption of irrigation, a rapid decrease in proline content was observed after 33 days, approaching

that of the control at 36ème days. It should also be noted that the different varieties show variability in the rate and quantities of proline accumulated, this after 15 days of stress for Ourgh, Riyad and Vitron and 20 days for Prospero.

Different levels of proline are accumulated in all varieties. The prospero variety (Fig. 2B), Riyad (Fig. 2C) and vitron (Fig. 2D) show the highest levels. Plants undergoing moderate stress show proline levels almost comparable to those of the controls.

These results can be interpreted firstly as a biochemical response of proline synthesis following severe water stress, and that this synthesis is correlated with that of the main pathway (figure a) where photosynthetic activity is disrupted. The study of chlorophyll content may help to identify this regulation of synthesis under water stress.

3.4 Effect of water stress on chlorophyll pigment content



Figures 3 (A, B, C): Chlorophyll content in plants of durum wheat varieties with and without water stress: A, B, C represent a comparison of varietal response to severe water stress and that of the control.

Chlorophyll pigment levels decreased slightly in the 4 stressed varieties, compared with controls and moderates. This decrease is not proportional to the degree of stress, nor is it specific to any one variety studied.

Indeed, the ourgh variety shows a decrease at 30ème days of stress, then an increase after rehydration (33ème days) followed by a decrease. (**Figure 3 A).**

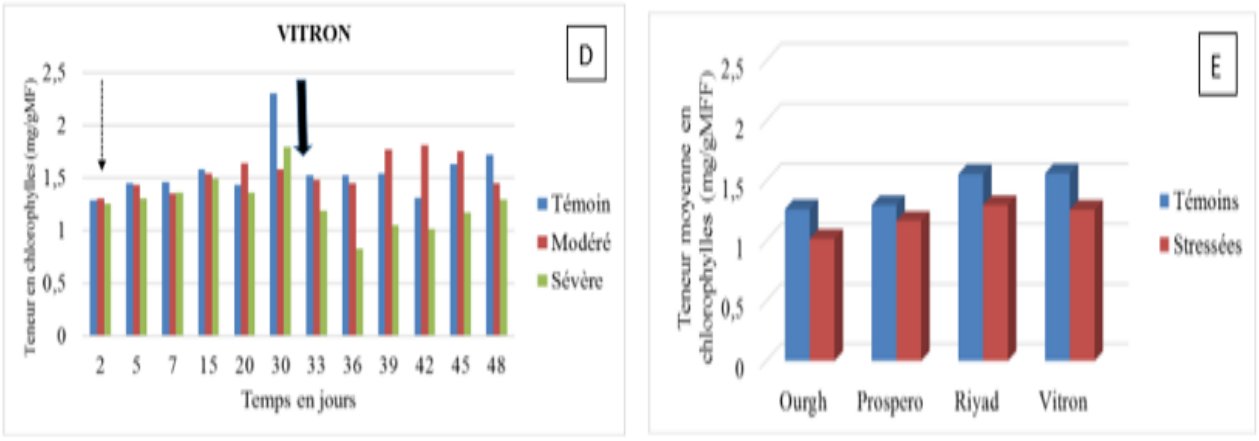


Figure 3: Chlorophyll content of waterstressed and unstressed durum wheat varieties: A, B, C, D and E represent a comparison of varietal response to severe water stress and that of the control.

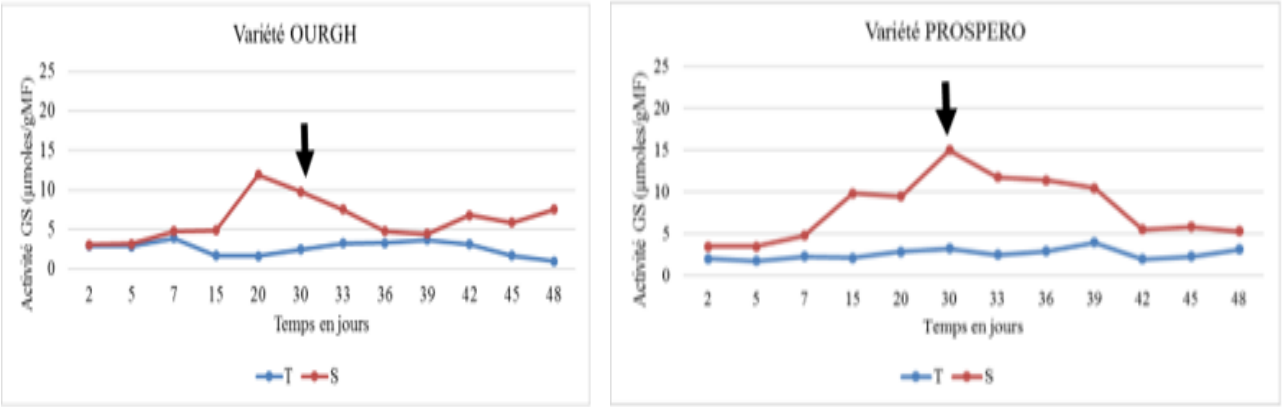
The prospero variety had roughly the same values in stressed and control plants. Reirrigation does, however, result in a drop in chlorophyll content **(Figure 3 B)**. The percentage of chlorophyll pigment accumulation in stressed plants compared with control plants shows that it is higher in the riyad variety, lower in the prospero and vitron varieties and lower in the ourgh variety (**Figure 3 E**).

These results, together with those for proline accumulation, show that stressed plants have higher proline concentrations and lower chlorophyll levels than control plants (**Figures 2 and 3**).

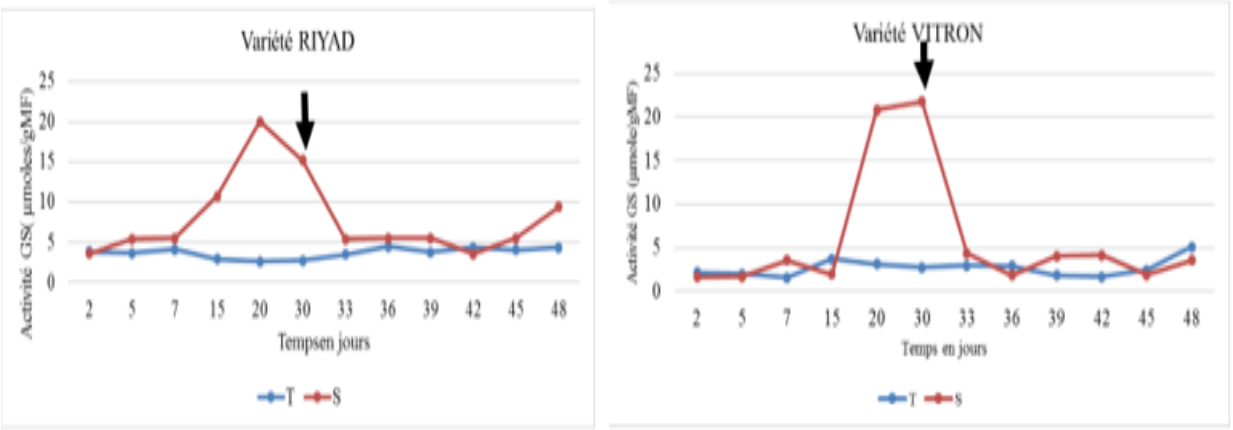
What's more, the varieties that accumulate the least proline are those that show the greatest increases in chlorophyll and vice versa. This suggests a relationship between proline and chlorophyll synthesis.

3.5 Effect of water stress on glutamine synthetase activity

As already mentioned, both proline and chlorophyll biosynthetic pathways use the glutamate pathway, mainly originating from the GS/GOGAT cycle, enzymes involved in the degradation of glutamate, the common precursor of proline and chlorophyll. This led us to study the activity of leaf glutamine synthetase. This leaf activity of GS increases following water deprivation compared to that in control leaves, while rehydration leads to a return to the values of control plants (**Figure 4 A, B, C and D**).



Figures 4 (A and B): Leaf glutamine synthetase activity in severely waterstressed varieties compared with the irrigated control.



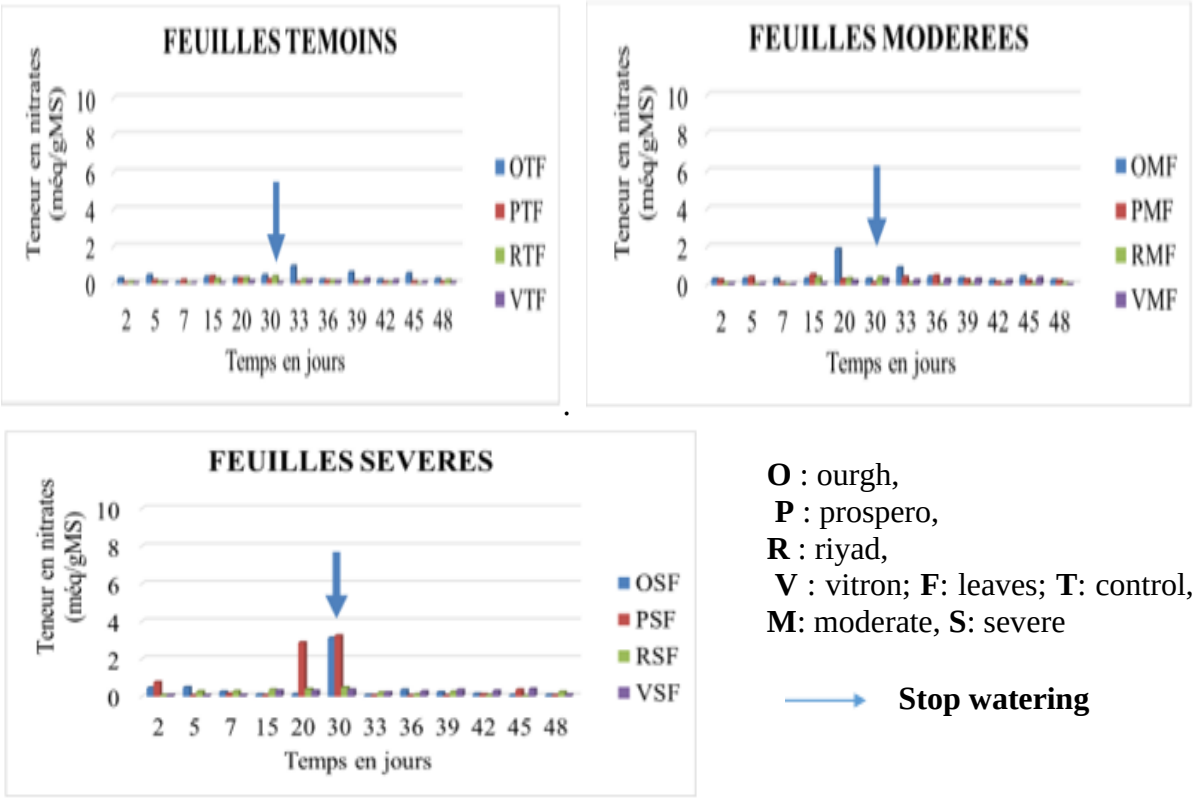
➔ **Stop watering**

T: Controls, **S**: Stressed, **GS**: Glutamine synthetase, **gMF**: gram fresh matter

Figure 4 (C and D): Leaf glutamine synthetase activity in severely waterstressed varieties compared with the irrigated control.

3.6 Effect of water stress on ion accumulation

This experiment was carried out following water stress by stopping irrigation followed by re-irrigation after 30 days. The accumulation of various ions as a function of time was monitored in the roots and leaves of stressed plants compared with control plants. Ion determination was carried out on dry material after hot acid extraction as described in materials and methods. We were interested in nitrate, nitrite, sulfate and phosphate ions, which are all anions and represent the bulk of the elements supplemented in the form of fertilizers in Eastern Morocco. A preliminary survey enabled us to quantify and qualify the nitrogen, phosphorus and sulphate fertilizers used by farmers



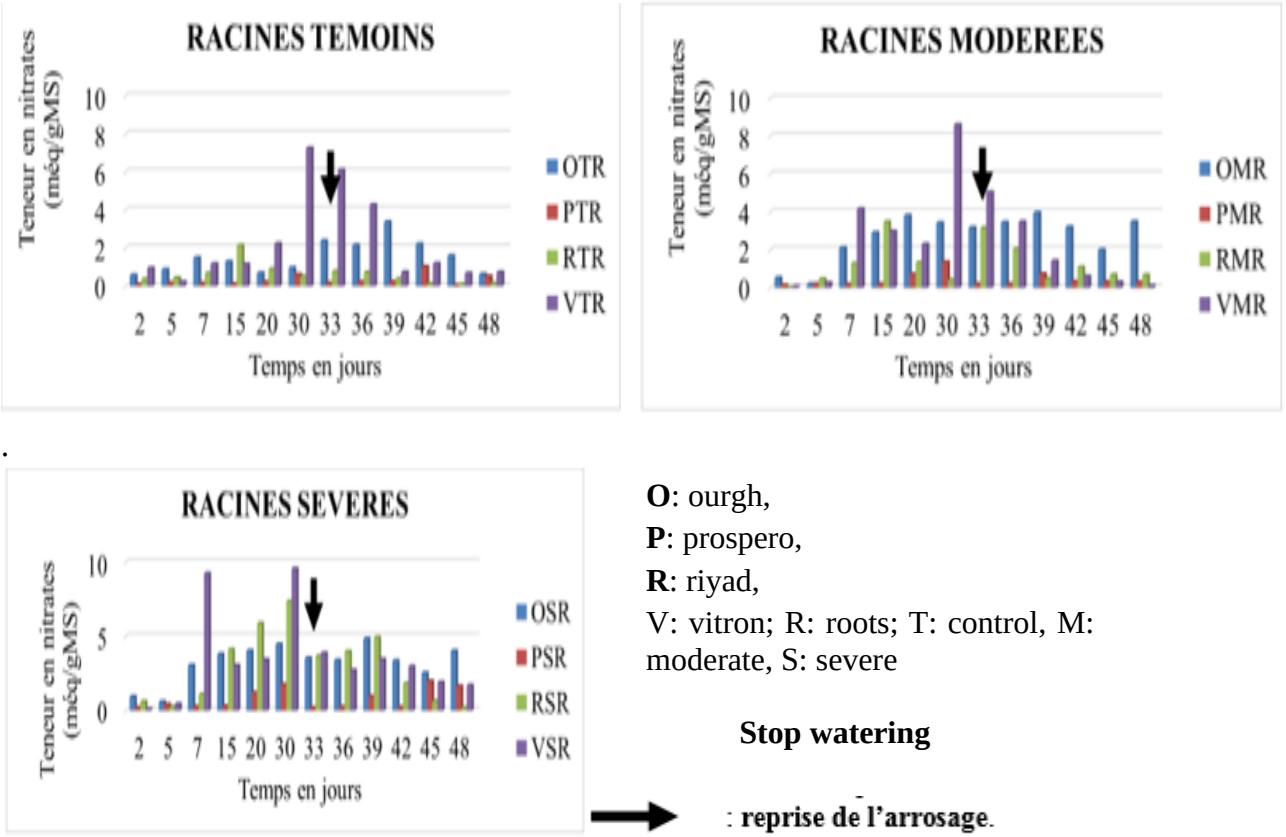
O : ourgh,
P : prospero,
R : riyad,
V : vitron; **F**: leaves; **T**: control,
M: moderate, **S**: severe

⟶ **Stop watering**

Figure 5 (A, B, C) : Leaf nitrate content of ourgh, prospero, riyad and vitron durum varieties following moderate and severe stress compared with a normally watered control.

NO_3^- ion accumulation occurs mainly in the root zone, compared with the leaf zone (**figures 5, 6**). Over time and under conditions of continuous watering, the root zone accumulates NO as early as day 7ème, with the levels in stressed plants rising slightly from the start of stress at day 7ème and then falling sharply compared with. control plants. Foliar levels are more or less the same before and after irrigation.

Nitrate ion accumulation shows that in the root zone of the vitron variety (**figure 5)**, a high peak is reached in 7-15 days, followed by a drop, after which levels remain constant even after re irrigation. In the foliar part, the rates of stressed plants are lowest until the end of re-irrigation. For the riyad and ourgh varieties, leaf content values are almost identical to those of the control.



.

O: ourgh,
P: prospero,
R: riyad,
V: vitron; R: roots; T: control, M: moderate, S: severe

Stop watering

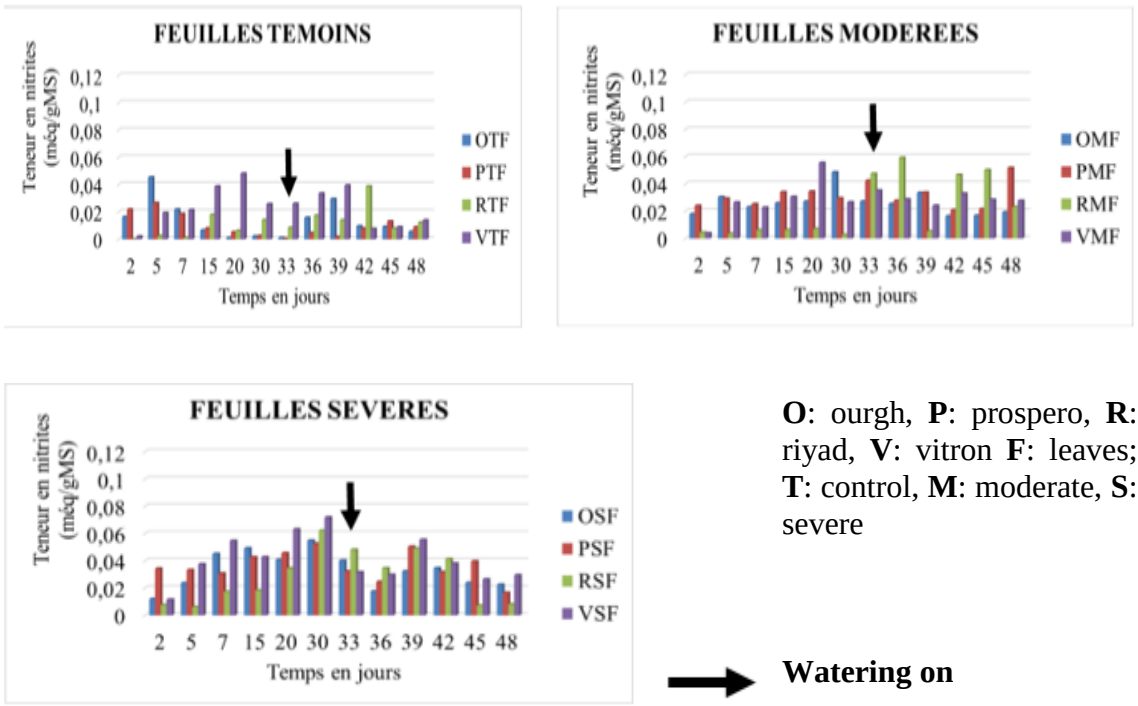
: **reprise de l'arrosage**.

Figures 6 (A, B, C): Root nitrate content of ourgh, prospero, riyad and vitron durum wheat varieties following moderate and severe stress compared with a normally watered control.

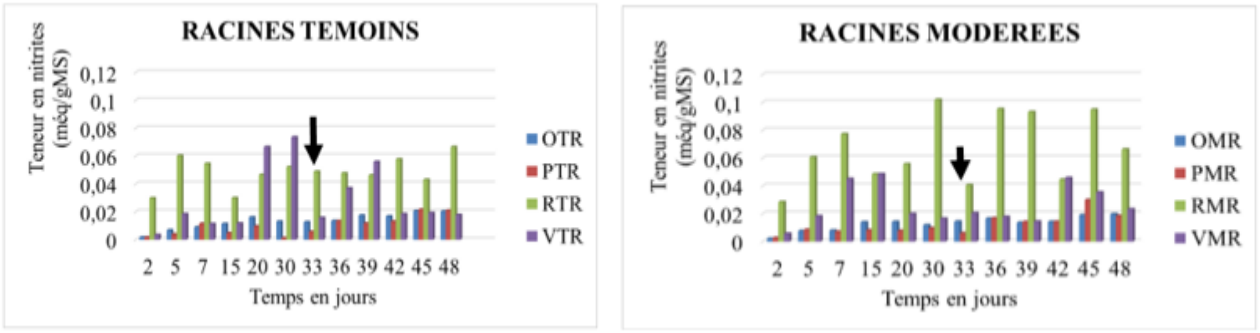
Nitrite accumulation (NO_2^-) occurs in both root and leaf parts of control plants (**Figure 6**).

It varies from one variety to another. This variability varies from one organ to another, with very low levels recorded in leaves (**compare figures 5 and 6**) compared with roots, in much the same way as for nitrates. This is consistent with the fact that the dependence of both elements is under the joint control of nitrate reductase and nitrite reductase activity, leading to the formation of ammonium.

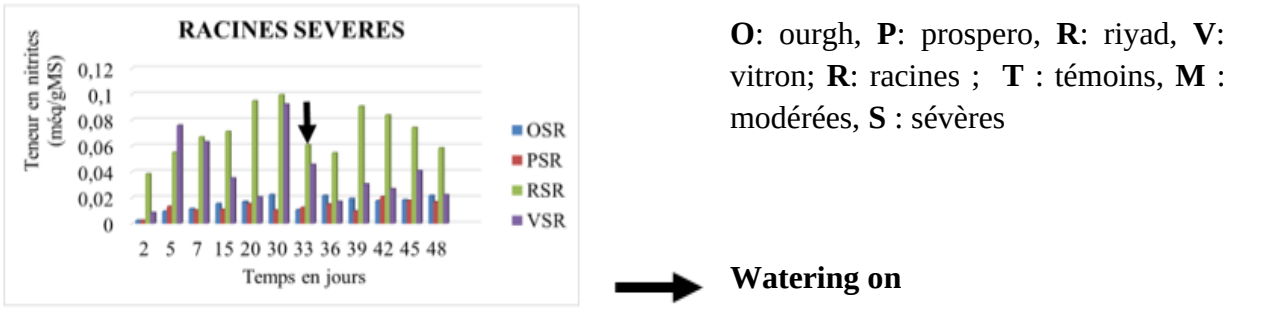
This situation prompts us to propose a physiological and biochemical response involving the bulk of nitrogen metabolism in both stressed and unstressed plants. The root zone shows irregularities in content in both stressed and control plants. In general, the evolution shows that control plants (root and foliar) have higher levels than stressed plants (**Figures 6**)



Figures 7 (A, B C): Leaf nitrite content of ourgh, prospero, riyad and vitron durum varieties following moderate and severe stress compared with a normally watered control.

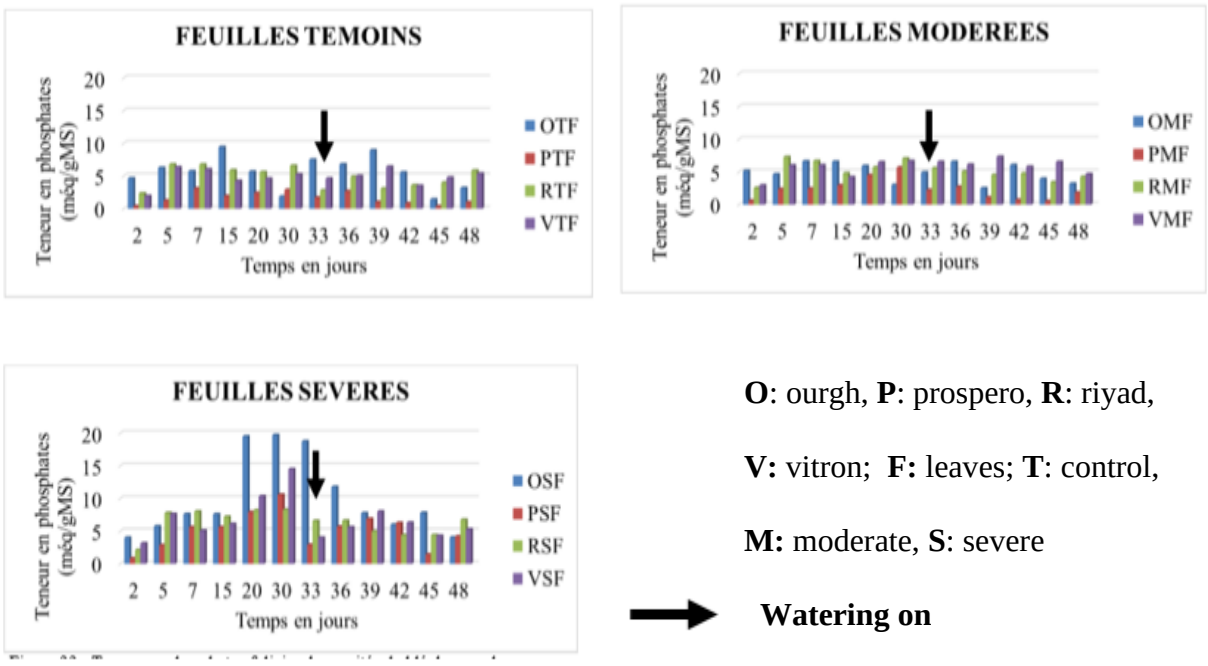


Figures 8 (A, B, C): Root nitrite content of ourgh, prospero, riyad and vitron durum varieties following moderate and severe stress compared with a normally watered control.

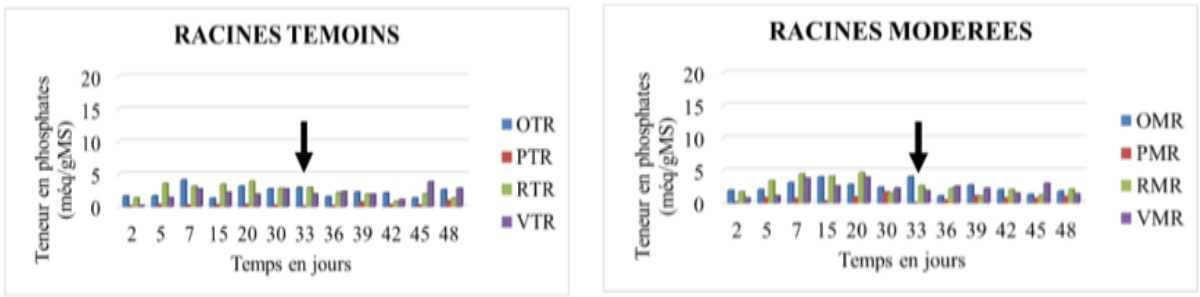


Figures 9 (A, B C): Root nitrite content of ourgh, prospero, riyad and vitron durum varieties following moderate and severe stress compared with a normally watered contro

Phosphate is accumulated more in stressed plants (foliar and root) than in control plants. Figures 9 to 10 show that the content is approximately the same for both root and leaf parts. This shows that this highly metabolizable ion is not organspecific.



Figures 10 (A, B, C): Leaf phosphate content of ourgh, prospero, riyad and vitron durum wheat varieties following moderate and severe stress compared with a normally watered control



Figures 11 (A and B): Root phosphate content of ourgh, prospero, riyad and vitron durum wheat varieties following moderate and severe stress compared with a normally watered control.

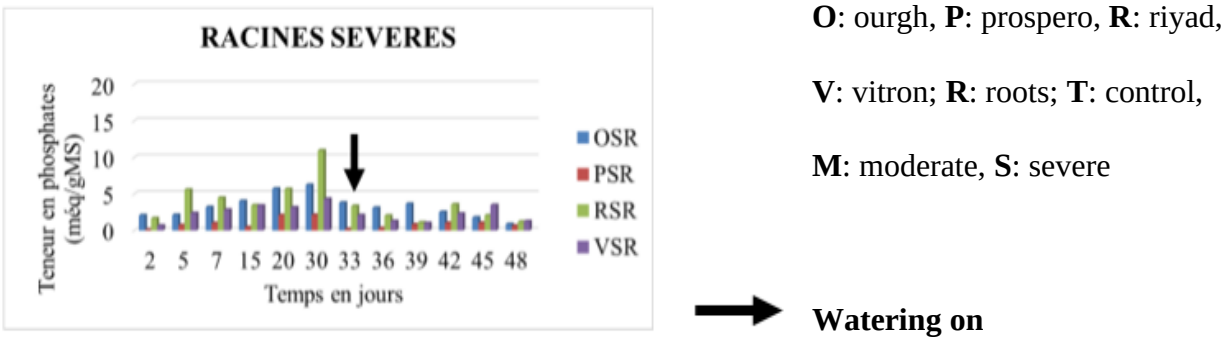


Figure 11 C: Root phosphate content of ourgh, prospero, riyad and vitron durum varieties following moderate and severe stress compared with a normally watered control.

The ourgh variety (figures 10 and 11) shows roughly equivalent quantities of root and leaf parts in controls and stressed plants, with only minor differences.

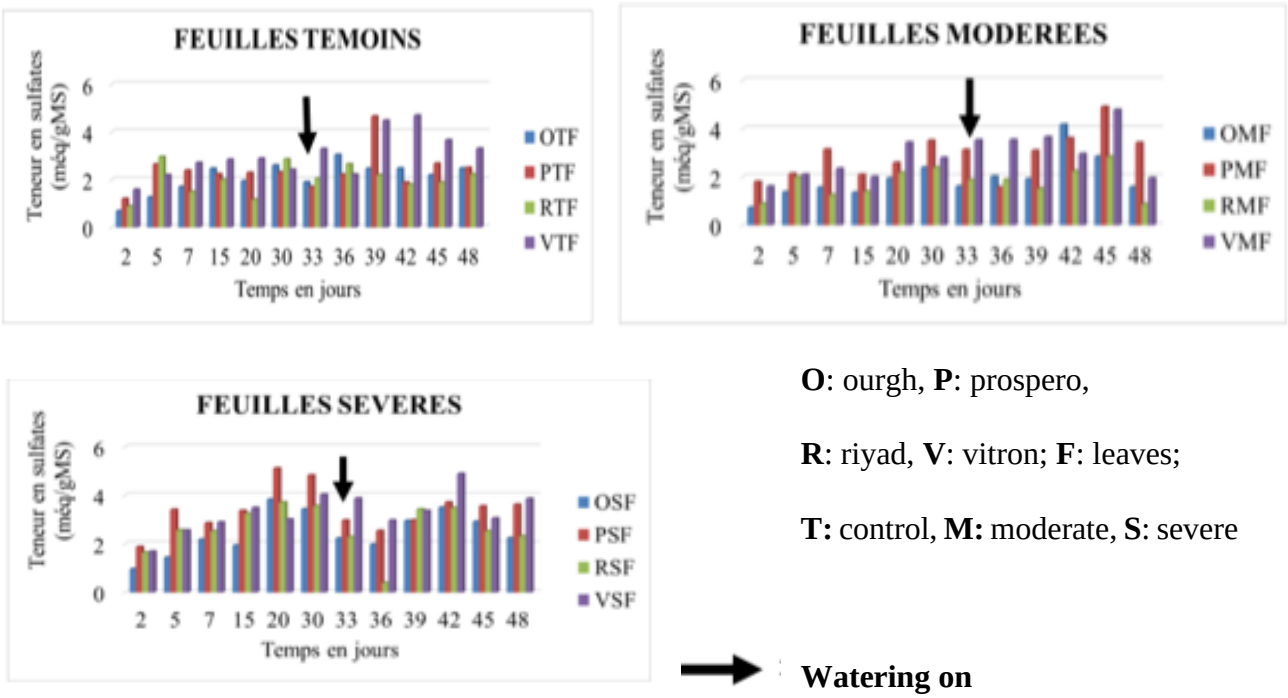


Figure 12: Leaf sulfate content of ourgh, prospero, riyad and vitron durum varieties following moderate and severe stress compared with a normally watered control.

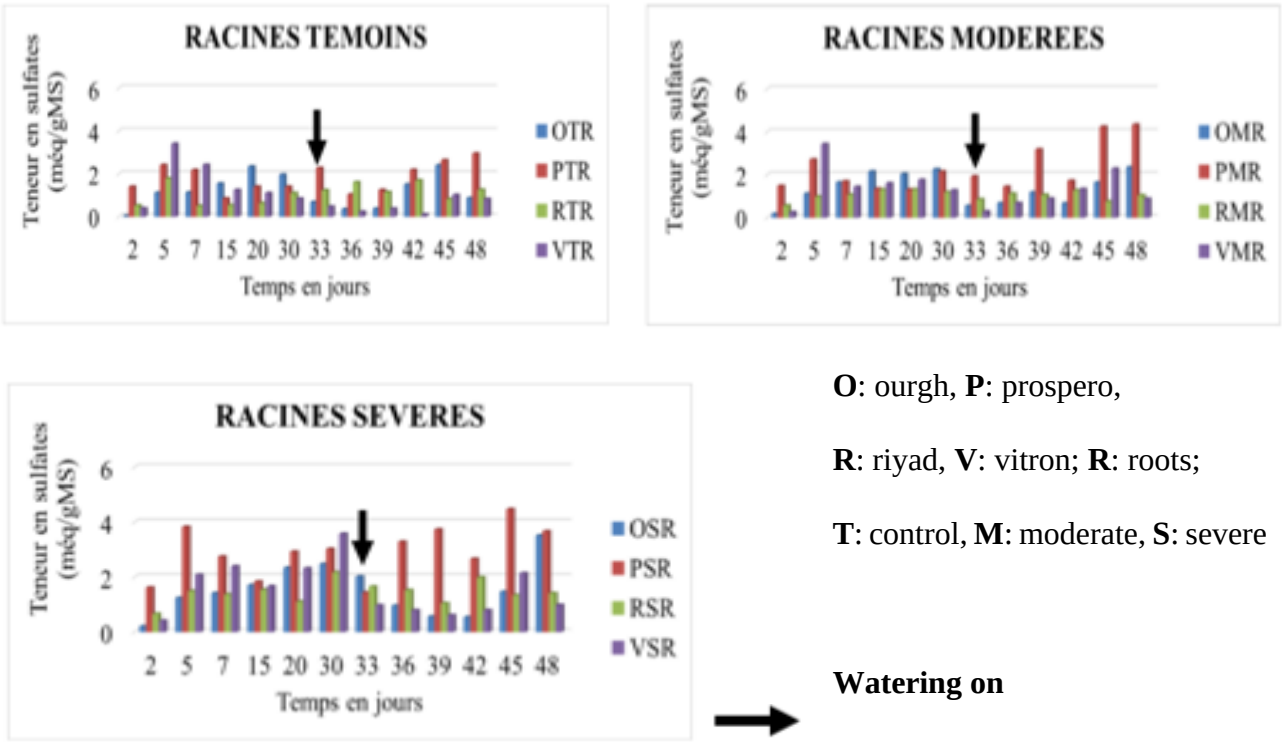


Figure 13: Root sulfate content of ourgh, prospero, riyad and vitron durum varieties following moderate and severe stress compared with a normally watered control.

3.7 Effect of water stress on plant water content

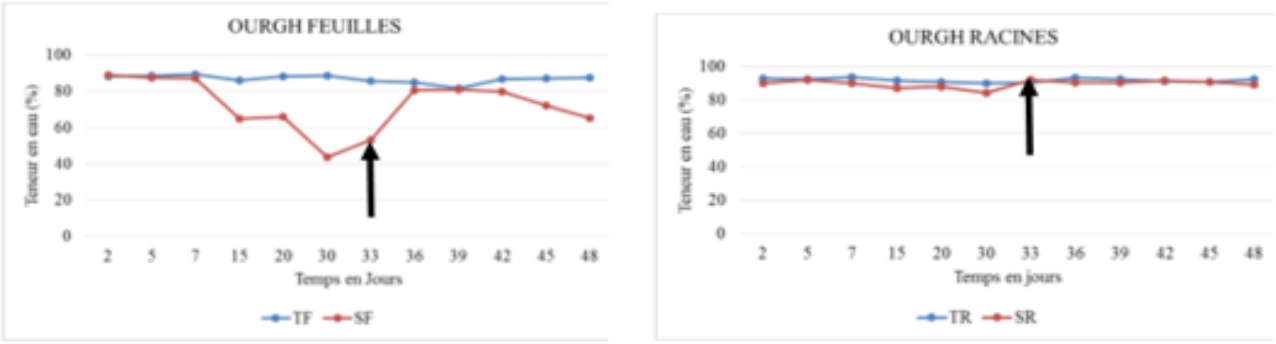
Water contents are determined after desiccation of root and leaf material (at 80°C for 48 hours), and are calculated by the ratio:

$$\boldsymbol{TE}\ (\%) = \frac{\boldsymbol{PMF - PMS}}{\boldsymbol{PMF}} \times \mathbf{100}$$

PMF: Weight of fresh matter
PMS: Weight of dry matter

The results, expressed as a percentage, are shown in figures 14-15-16 and 17. Examination of these figures clearly shows that water content decreases more significantly in stressed plants than in control plants, which remain constant in all varieties. All varieties show a very significant reduction in leaf water content in stressed plants.

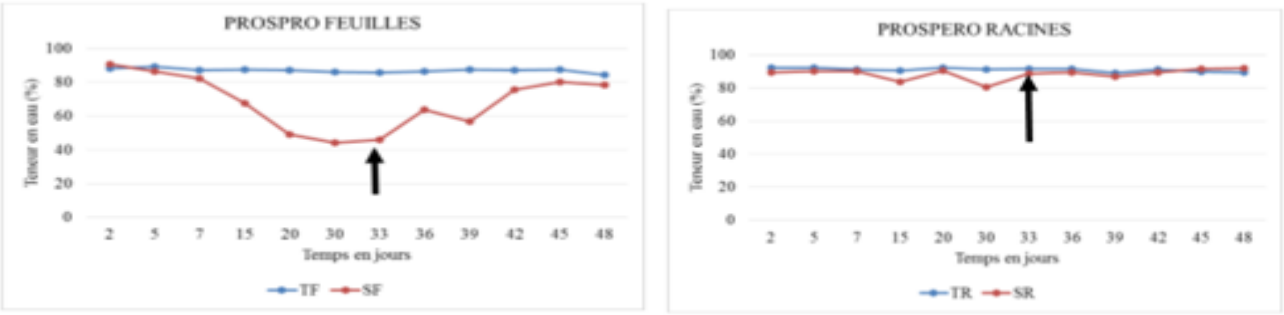
However, they are very low for stressed root parts. It should also be noted that, after irrigation was resumed, the water content increased and tended towards that of the control plants, showing that the plants were able to rehydrate once the stress had been lifted, and that the stress had not been so severe as to cause the phenomenon to become irreversible. Stressed plants show a greater reversal at the end of stress (30 days). In the prospero variety, leaf water content in stressed plants decreased until the 30ème days of stress. After rehydration, recovery is very spread out over time (**figure 14**) and is gradual compared with other varieties, which recover immediately.



: Reprise en eau

TF: Leaf controls, **SF**: Stressed leaves, **TR**: Root control, **SR**: Stressed roots

Figures 14: Leaf and root water content in % for the ourgh variety following severe water stress.



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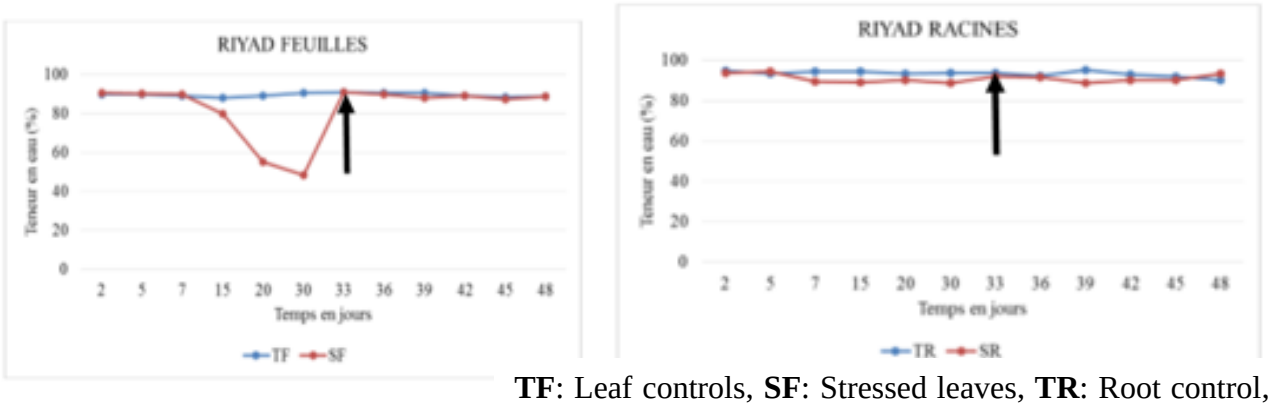
TF: Leaf controls, **SF**: Stressed leaves, **TR**: Root control, **SR**: Stressed roots

Figures 15: Leaf and root water content in % for the prospero variety following severe water stress



TF: Leaf controls, **SF**: Stressed leaves, **TR**: Root control, **SR**: Stressed roots

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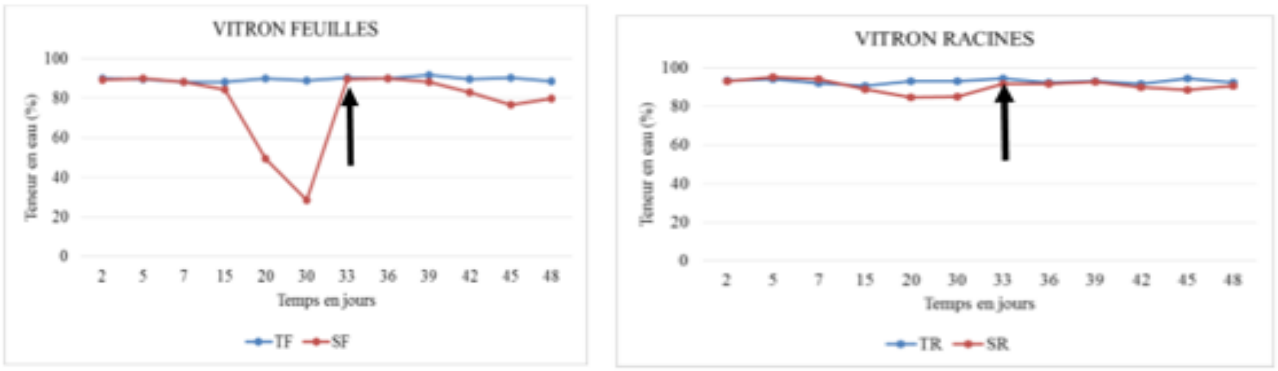
Figure 16: Leaf and root water content in % for the riyad variety following severe water stress.



TF: Leaf controls, **SF**: Stressed leaves, **TR**: Root control, **SR**: Stressed roots

: Reprise en eau

Figures 17: Leaf and root water content in % for the vitron variety following severe water stress.

Discussion

The results obtained show that all four durum wheat varieties show a loss of chlorophyll content after one month of water stress, and high proline content with a decrease in water content **(figs. 1 to 3)**. An increase in chlorophyll and water content was observed before rehydration. The phenotypic phenomenon noted was a reduction in leaf area at the time of stress (results not shown). This leaf area is an important consequence of transpiration. One of the first reactions of plants to water deficit is to reduce their leaf area to the point of curling.

This reduces water loss through cuticular transpiration, which is common in many cultivated plants (wheat, sorghum). This condition can be seen as an indicator of loss of turgidity and, at the same time, as a means of avoiding dehydration (ElHakimi, 1992; El-Jaafari, 1995; Zeghida et al, 2004).

The larger the surface area, the higher the rate of loss. Some varieties have the peculiarity of leaf curling during water deficit (Kirkham et al, 1980). Granier et al (2000) reported that the leaves of plants subjected to water deficit usually reach smaller apparent final sizes than those of controls. Depending on the adaptive strategy of each species or variety, the effect of water stress may be reflected in morphological changes affecting the above-ground or belowground part of the plant: reduction in leaf area and number of tillers, leaf curling and better development of the root system.

Vegetative development under conditions of limited water supply is severely disrupted (Ferryra et al, 2004; Slama, 2005; Laita et al., 2024). Prospero has a high proline content, while Ourgh has the lowest. All stressed durum wheat varieties (Vitron, Riyad, Prospero and Ourgh) show an increase in proline. Another form of drought resistance through the accumulation of proline. These results are in line with those obtained by Sadki (1996) and Tahri (1998). Proline can be used as a biochemical selection criterion for resistance to water deficit. Determination of proline in water-deficient leaves showed an increase in proline content in all four varieties studied. This increase was also observed in many species. After rehydration of durum wheat varieties, a decrease in proline levels was observed.

Our results suggest, subject to further experimentation, that resistance to water stress is regulated by a cascade phenomenon, particularly upstream in the chain. The accumulation of proline (which, according to all the literature without exception, is the essential component of the resistance response to water stress) is simply a consequence of this regulation.

The study of correlations between leaf proline content and tolerance to water stress has been the subject of numerous studies, particularly in cereals. The accumulation of proline results from the disruption of protein metabolism, which plays an important and remarkable role in water stress caused by membrane proteolysis and would be indicative of a certain resistance to drought. Our results concur with those obtained in the literature (N'da, 1984; Monneveux and Nemmar, 1986; Bellinger et al, 1991; Moulineau, 1993; Gorham ,1993; Tahri et al, 1998; Mekliche et al, 2003; Bezzala, 2005; Hamidou, 2006; Hireche, 2006).

The reduction in total chlorophyll content may be the result of reduced photosynthesis linked to tolerance to water stress. Wheat responds to water stress by increasing its stomatal resistance. Prolonged stomatal closure, aimed at limiting water loss through evapotranspiration, leads to the cessation of photosynthetic processes, resulting in low yields and increased proline production in the leaves.

On the other hand, the drop in chlorophyll content is the result of reduced stomatal opening to limit water loss through evapotranspiration and increased resistance to the entry of atmospheric CO_2 requiredfor photosynthesis. This shows the close relationship between proline and chlorophyll biosynthesis. The amount of chlorophyll in leaves can be influenced by many factors, such as leaf age, leaf position, and environmental factors such as light, temperature and water availability (Hikosaka et al, 2006).

The accumulation of proline is thought to be the result of a stimulation of synthesis from glutamate (Boggess et al, 1976 b), a decrease in protein synthesis (Stewart and Boggess, 1978) and an inactivation of oxidation reactions leading to the formation of glutamic acid (Stewart et al, 1977). The hypothetical role of proline supported by several authors is the osmotic regulation of the cytoplasm and the creation of a suitable environment for general metabolism in the event of stress. Tahri et al (1997 and 1998), show that the increase in leaf proline content under water stress is followed by a decrease in total chlorophyll pigment content.

The results reveal a certain inverse proportionality between accumulated proline and chlorophyll pigment levels. Glutamate molecules not converted to chlorophylls will follow the proline biosynthesis pathway. Under water stress, these two compounds (proline and chlorophyll) compete for their common precursor, glutamate. Proline biosynthesis is also regulated by enzymes involved in glutamate degradation, notably glutamine synthetase (GS). Glutamine synthetase (GS) associated with glutamate synthetase (GOGAT) is the most commonly used pathway, and GS has a strong affinity for ammonium, as it is central to nitrogen metabolism.

The accumulation of nitrate, nitrite, phosphate and sulfate ions was observed in stressed and control seedlings, in both leaf and root parts. They are highest at the beginning of the stress period. But also that the ability to accumulate different ions varies greatly from one variety to another. Nitrates play an important osmoticum role in most species, especially at root and leaf level. This suggests that nitrates and nitrites, as substrates of the nitrate reductase and nitrite reductase cycle, are upstream regulators of water stress in plants.

Plants subjected to unfavorable conditions seek adaptive strategies to maintain survival, and osmotic adjustment is a key mechanism in dehydration tolerance. Thanks to the phenomenon of adaptation, which appears to be a major mechanism of adaptation to ionic and osmotic stress and is expressed by a plant's capacity to accumulate ions actively at symplasmic level (Parida and Das, 2005; Navarro and Rubio, 2006; Munns et al, 2006; Teakle et al, 2007).

However, the plant's response to stress, the nature of the solutes accumulated and the variations in osmotic potential due to these solutes, depend on the species considered, the variety studied, the tissue examined, the stage of development and the intensity of the stress applied (Sadki, 1996; Laita et al., 2024b).

Conclusion

Our summary diagram (**figure 4**) shows that the Prospero variety is likely to be more resistant to water stress than the Ourgh, Riyad and Vitron varieties. Prospero may therefore be a potential candidate for bour cultivation, while the other varieties are likely to be grown in irrigated areas.

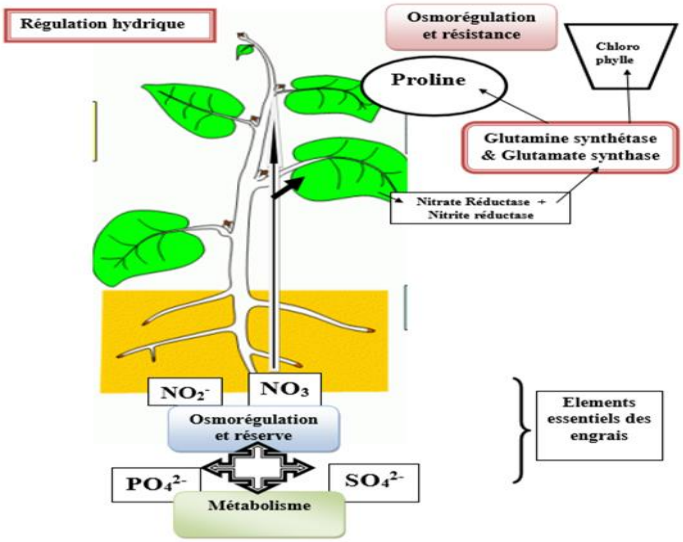


Figure 18: Summary of the different processes taking place following water stress.

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