EVALUATION OF THE BIOCHEMICAL ASPECTS OF DROUGHT TOLERANT NEW WHEAT LINES DURING SEEDLING AND EARLY GROWTH STAGES.

BABEANU CRISTINA[1], MATEI GHEORGHE[2], PAUNESCU GABRIELA[3]
[1]University of Craiova, Faculty of Sciences, e-mail: cbabeanu@yahoo.com
[2]University of Craiova, Faculty of Agriculture
[3]Agricultural Research and Development Station Simnic

Key words: wheat lines, hydrolytic enzymes, chlorophylls, proline

ABSTRACT

21 wheat lines were investigated in the study, as well as 4 standard cultivars in order to evaluate biochemical indices that characterize the processes of growth and development of plants under drought conditions. The experiment was conducted at ARDS Simnic. Enzymatic activities of amylase, protease and phosphatase, reducing sugar, protein and proline content were determined at plant emergence and tillering stage. The obtained results indicate that all the investigated wheat lines are adapted to the ARDS Simnic conditions, thus recommending further testing for their selection in breding programs.

INTRODUCTION

Wheat production is frequently limited by insufficient water supply. Drought can manifest in many forms, dependent on its length and intensity or on the soil's water reserves. For this reason, drought resistance should constitute a primary objective of improvement programs. The main issue is therefore finding efficient selection methods. The complexity of the property of resistance to drought, as well as the likely negative correlations with production potential determine that the most recommended method to be to test the production capacity for a large amount of material, in several locations with high frequency of drought *(*Chorfi and Taibi, 2011; Kumar and Singh, 1998*)*.

Resistance to drought gained by wheat improvement programs applies only to climate conditions for which the selection was made. For this reason, the specifics of the droughts need to be well known. In the South of the country for example, droughts are characterized by high frequency, length and intensity, causing significant production loss (Mocanu et al., 2009; Mocanu et al., 2012; Paunescu and Boghici, 2008). The genetics of drought tolerance are complex, and the variability of rainfall make genetic progress towards drought resistance extremely difficult (Rajaram, 2001; Winter et al., 1988).

The responses of plants to water stress depend on the specie of plant, its age, phase of growth and development, the level and duration of drought as well as physical parameters (Dhanda et al., 2004). Plants develop different mechanisms (morphological, physiological and biochemical) which inhibit or remove the harmful effects of stresses (Chaves et al., 2002; Turner,1986)

In order to highlight the most performant and stable genotypes as well as identify genetic material resistant to drought, new wheat lines are tested in comparative culture. In this study 21 wheat lines were investigated, as well as 4 standard cultivars in order to evaluate biochemical indices that characterize the processes of growth and development of plants under drought conditions.

MATERIAL AND METHODS

Experiments were carried out on 25 varieties of wheat (1=DROPIA; 2=BOEMA; 3=FL 85; 4=EXOTIC; 5=S9917-6; 6=S9913-15; 7=S9916-12; 8=S0444; 9=S0449; 10=S072; 11=S0718; 12=S0719; 13=S0726; 14=S0743; 15=S0794; 16=S0795; 17=S0796; 18=S07102; 19=S07115; 20=S07166; 21=S07167; 22=S07170; 23=S07172;

24=S07175; 25=S07141*)* at Research and Agricultural Development Station from Simnic. These were chosen because they had a high productivity level in drought conditions. The „S.C.D.A." geographical region Simnic is known as a drought area, with reduced rainfall and extremely high summer temperatures (Mocanu et al., 2013; Paunescu and Boghici, 2008). The soil from the investigated area is characterised by an acid pH, by a low content of humus, nitrogen and potassium and by a significant supply of mobile phosphorus (Mocanu and Dodocioiu, 2010). The experiment was conducted under strictly controlled technology such that the differences between lines wasn't masked by the variation of nitrogen supply conditions, the previous crop, the sowing time, etc. factors which distort the comparisons made in farm conditions. The plants have been complexly fertilized 8-40-0, 200 kg/ha and treated with Divident 1L/t;

Biochemical analyses were realized on fresh leaves cut at different growing stages: stage I: plant emergence and stage II: tillering.

Fresh tissue was homogenated with 10mL/g of 0.1 M phosphate buffer (pH 7.0) containing 0.1 mM ascorbic acid and 0.1 mM EDTA. Homogenates were centrifuged for 20 min at 10,000 r.p.m. and the supernatants were used for biochemical analyses by suitable methods (Babeanu et al., 2003).

Amylolytic activity was determined by monitoring the increase in reducing sugar formation from starch with the dinitrosalicylic acid reagent at pH 6.8 in 100 mM potassium phosphate buffer. Quantitative results were obtained using a standard curve with glucose, and expressed as $U.g^{-1}$ FW. One unit (U) was defined as the amount of enzyme releasing 1 μmol of reducing sugar per minute and per mL of extract.

Protease activity was assayed in 50 mM Na-phosphate buffer, pH 7.5 using casein as a substrate. Quantitative results were obtained using a standard curve with tyrosine, and expressed as $U.g^{-1}$ FW. One unit (U) represents the amount of enzyme which catalyses the liberation of 1 μmole of tyrosine in a minute, at 25°C.

Phosphatases activities were assayed using 5 mM p-nitrophenyl phosphate as a substrate. Assay was performed in 50 mM Na-acetate buffer, pH 5.0 for **acid phosphatase** and in 50 mM glycine-NaOH buffer for **alkaline phosphatase**. Quantitative results were obtained using a standard curve with p-nitrophenol at 410 nm. Phosphatases were expressed as $U.g^{-1}$FW, where one unit (U) is the amount of enzyme which liberates 1 μmole of p-nitrophenol per minute at 25°C.

Proline content: was determined in 3% aqueous sulfosalicylic acid extract by spectrophotometry at 520 nm following the ninhidrin method of Bates et al. (1973) using L-proline as a standard. The results were expressed as μmol proline/ g fw.

Chlorophylls a and b and total carotenoids were extracted in 80% methanol, measured spectrophotometrically, calculated according to the formulae of Lichtenthaler and expressed as mg/g f.w (Lichtenthaler, 1987).

Reducing sugars and total soluble protein were extracted in distilled water (1:10 w/V) and assayed colorimetric (Babeanu et al, 2003).

All assays were performed in triplicate and the results presented here are the mean values.

RESULTS AND DISCUSSION

In the germination process, hydrolases transform reserve substances (starch and proteins) that are stored in the endosperm, into the simple substances, thus ensuring the necessary conditions for the process of embryo breathing, digestion and assimilation (Mak et al, 2009). In this process, a very important role is played by amylase and protease.

It can be observed that the enzymatic activity of amylase varies depending on the line, with values between 89.65 U/1g and 102.27 U/1g, registered in the case of line 25

(S07141) and 14 (S0743). In the case of standard cultivars the amylase activity displays an average of 98.17 U/1g (figure 1).

The protease activity of wheat lines varies between 24.12U/1g (12=S0719) and 46.76U/1g (19=S07115), all values being lower than that observed in standard 3=FL 85 (48.12U/g) and higher than that recorded in standard 4=Exotic; 23.68U/g (figure 1). The results have shown that amylase activity is three-fourfold higher then protease activity. This difference reflects the huge amount of starch in cereal grains compared to storage protein content.

Phosphatases catalyse the hydrolytic liberation of phosphate from phosphoric monoesters. Phosphate group is a structurally important part of certain molecules with energetic (ATP) and informational (nucleic acids) roles.

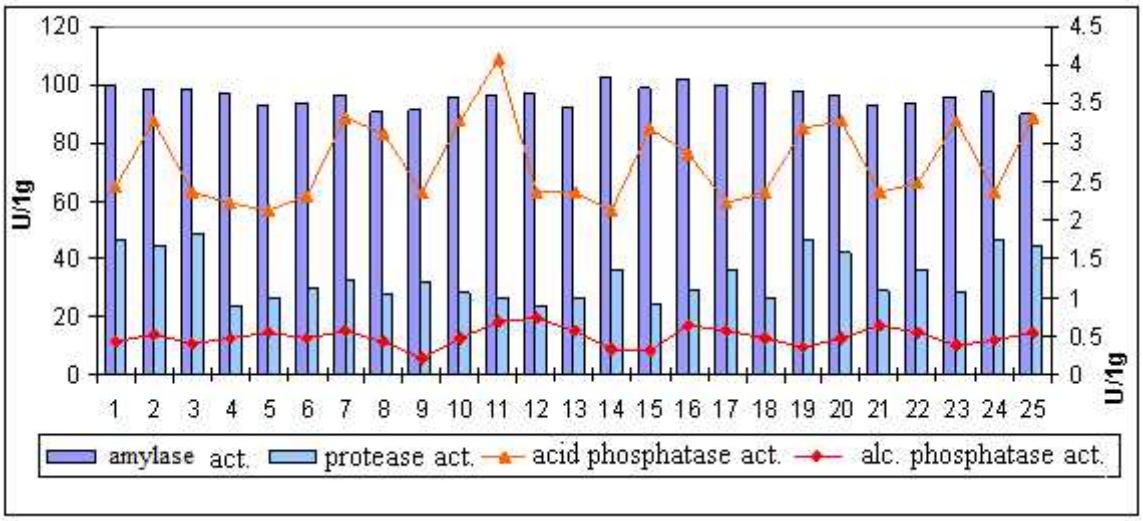

Figure 1. Activities of hydrolytic enzymes in seedlings of investigated wheat lines
(amylase and protease activity –left axe: phosphatases activities-right axe)

The enzymatic activity of acid and alkaline phosphatase differs per wheat line, without being correlated to other biochemical indices (figure 1). The phosphatase activity varies between 2,12U/1g (5=S9917-6) and 4.08U/1g (11=S0718) for the acid phosphatase and between 0.22U/1g (9=S0449) and 0.72U/1g (11=S0718) for alkaline phosphatase(figure 1). In the case of standard cultivars the phosphatase activity displays an average of 2.57U/1g (acid phosphatase) and 0.45U/1g (alkaline phosphatase) respectivly.

An extremely important process affected by water deficiency is photosynthesis, in particular the photolysis of water, with proper formation of ATP, NADPH + H^+ and O_2. Without water these processes are repressed, implicitly affecting carbohydrate synthesis (Cornic and Fresnau, 2002).To characterizes the photosynthesis process, the chlorophylls and carotenoids content of leaves was determined in the tillering stage. The obtained results are shown in figure 2. For all lines, the chlorophyll a content is lower than in standard cultivar 2 and 3, and higher than in standard 4. The same pattern is observed for chlorophyll b. The carotenoid content varies between 0.64 mg/1g and 1.15 mg/1g, which is lower than in standard cultivars 1, 2, 3 (average value 1.19mg/1g).

The variation in photosynthetic pigments content does not lead to significant differences in the carbohydrate and proteine content, displaying values close to standard cultivars. (figure 3).

Another parameter that has been studied is proline content. Under stress-free conditions, less than 5% of the total pool of free amino acids in plants is provided by proline. In many plants under various forms of stresses proline concentration increases up to 80% of the amino acid pool (Marcinska et al.,2013) The principal role of proline probably is to protect enzymes against dehydration and salt accumulation.

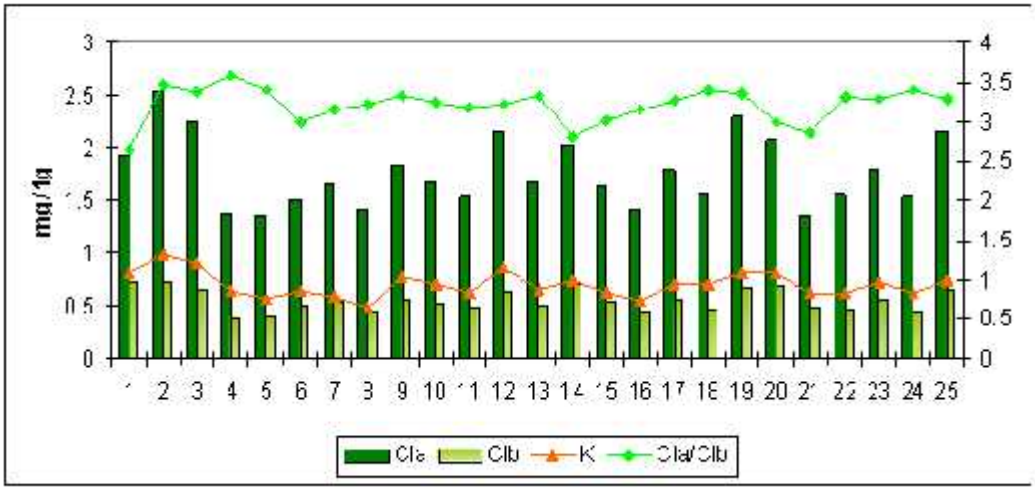

Figure 2. Photosynthetic pigments content in investigated wheat lines
(chlorophyll a and b content-left axe; carotenoid content and ratio Cl a/Cl b right axe)

For plants subject to various type of stress literature recorded the rise of the proline content. Proline has several functions during stress: osmotic adjustment, osmo-protection, free radical scavenger and antioxidant, protection of macromolecules from denaturation, regulation of cytosolic acidity and carbon and nitrogen reserve after stress relief. Proline can serve as a quick source of available nitrogen, carbon and reducing equivalents. Plants that accumulate proline, by either overexpression of proline synthesis enzymes, removal of enzyme feedback inhibition, or loss of catabolic enzymes, show also enhanced tolerance to other stress. However, doubts still persist whether the accumulation of this amino acid provides adaptative advantage or it is only a consequence of changes in the metabolism due to stresses. Increase in proline content in plants is either due to the inhibition of proline oxidation or to the more rapid biosynthesis of proline from its precursors.

The results obtained for proline content varies between 2.22mg/g (5=S9917-6) and 5.02 mg/g (24=S07175) all values being higher than that observed in standard 3=FL 85 (2.08mg/g) (figure 3). Higher values of proline content can be noticed in 9=S0449, 14=S0743 and 19=S07115 line with 4.78mg/g.

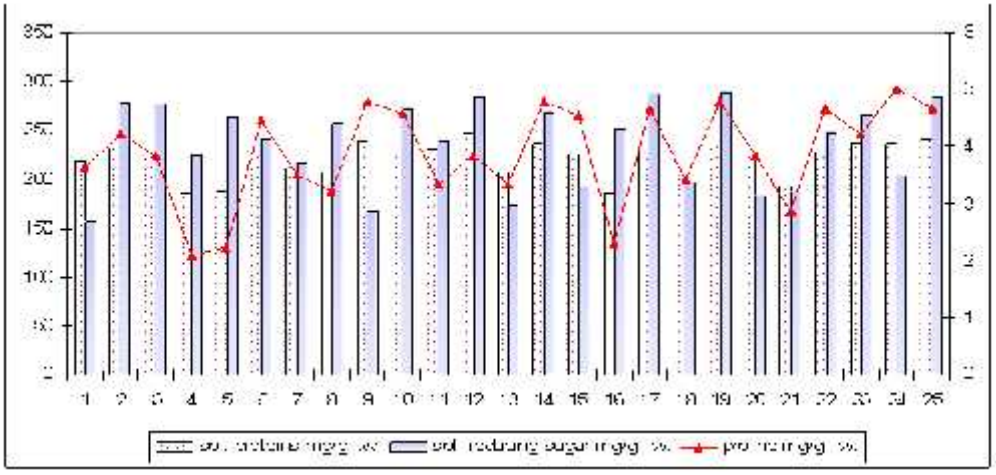

Figure 3. Soluble protein, reducing sugar (left axe) and proline content (right axe) in leaves of investigated lines

CONCLUSIONS

- The enzymatic activities in seedlings are differently modulated under conditions of field cultures. These showcase high values, ensuring the necessary conditions for starting the development and growth process.

- The variation in photosynthetic pigments content does not lead to significant differences in the carbohydrate and proteine content, displaying values close to standard cultivars.
- The results indicate that all the investigated wheat lines have a good metabolical status and are adapted to SCDA Simnic conditions, thus recommending further testing for their selection in breding programs.

BIBLIOGRAPHY

1. Babeanu C, Marinescu G, Glodeanu E., Ciobanu G., 2003 - *Biochimie vegetala practica, Ed INFO, Craiova*

2. Bates L.S., Waldren R.P., Teare I.D., 1973 - *Rapid determination of free proline for water stress studies, Plant Soil, 9, 205-207*

3. Chaves M.M., Pereira J.S., Maroco J., 2002 - *How plants cope with water stress in the field. Photosynthesis and growth, Ann Bot, 89, 907-916*

4. Chorfi A., Taibi K., 2011 - *Biochemical screening for osmotic adjustment of wheat genotypes under drought stress, Tropicult, 29, 2, 82-87*

5. Cornic G., Fresnau C., 2002 - *Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during a mild drought, Ann Bot, 89, 887-894*

6. Dhanda S.S., Sethi G.S., Behl R.K., 2004 - *Indices of drought tolerance in wheat genotypes at early stages of plant growth, Agron Crop Sci. 190, (1), 6-12*

7. Kumar A., Singh D.P., 1998- *Use of physiological indices as a screening technique for drought tolerance in oilseed Brassica species, Ann. Bot., 81, 413-420.*

8. Lichtenthaler H., 1987-*Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Methods of Enzymology,* 148, 350-382

9. Ludlow M. M., Muchow R.M., 1990 - *A critical evaluation of trait for improving crop yields in water limited environments, Adv. Agron., 42, 107-153.*

10. Mak Y., Willows R.D., Roberts T.H., Wrigley C.W., Sharp P.J, 2009- *Germination of Wheat: A Functional Proteomics Analysis of the Embryo, Cereal Chemistry***,** *86, 3, 281-289.*

11. Mocanu R.*,* **Dodocioiu A.M., Anton I***., 2009- The wheat yield quality with different fertilizers and pesticides applying when used to the wheat crop on cambic chernozem from ARDS Caracal, Lucrări Ştiinţifice, Universitatea de Stiinte Agricole Şi Medicină Veterinara" Ion Ionescu de la Brad" Iasi, Seria Agronomie* 52.1, 397-402

12. Mocanu R., Dodocioiu A.M., 2010 - *Phosphorus from the soil-An actual problem, Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series 40.1 495-498*

13. Mocanu R., Rosca D., Dodocioiu A.M., Susinski M., 2012 - *The effect of several fertilizer rates on winter wheat yield at ARDS Caracal, Research Journal of Agricultural Science, 44 (3),79-85*

14. Mocanu R., Dodocioiu A.M., Dobre M., 2013- *The effect of several fertilizer types on wheat yield and its quality, Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series* 42.1, 372-378

15. Păunescu G., Boghici O.N., 2008 - *Performance of several wheat cultivars under contrasting conditions of water stress, in central part of Oltenia, Romanian Agricultural Research, 25, 13-18*

16. Rajaram S., 2001- *Prospects and promise of wheat breeding in the 21st century. Euphytica, 119, 3-15.*

17. Turner N.C., 1986 - *Crop water deficits: a decade of progress, Adv. Agron, 39, 1-51.*

18. Winter S.R, Musick J.T., Porter K.B., 1988- *Evaluation of screening techniques for breeding drought resistant winter wheat, Crop Sci., 28, 512-516*