- Physiological, genomic and proteomic approaches of drought, saline and heat stress tolerance in durum wheat (*Triticum durum* Desf.)

- Biochemical and genetic approaches of cereal quality
Preamble

Climate change is exacerbating the challenges faced by the agriculture sector. In fact, it induced: increases in temperatures, rainfall variation and the frequency and intensity of extreme weather events which is adding more pressure on the global agricultural system. Unfortunately this system is already struggling to respond to rising demands for both food and renewable energy. The changing climate is also contributing to resource problems beyond food security, such as water scarcity, pollution and soil degradation. Cereals provide the majority of calories for human consumption, making this constraining scenario particularly challenging.

Investigating the molecular basis of drought tolerance mechanisms in cereals especially in wheat remain essential in order to cope with stress damages. Plant response relies on the expression of several genes which unleashes a variety of mechanisms, including the production of proteins and enzymes directly involved in stress metabolism, contributing to the specificity of the acclimation response to a given stress stimuli. In the last years, and because of the great interest for both basic and applied research, there has been an important progress in the understanding of the mechanisms and processes underlying abiotic stress adaptation and defense in different plant species. Recent technological advances and the aforementioned agricultural challenges have led to the emergence of high-throughput tools to explore and exploit plant genomes for crop improvement. These genomics-based approaches aim to decipher the entire genome, including genic and intergenic regions, to gain insights into plant molecular responses which will in turn provide specific strategies for crop improvement.

Another major progress in this area followed the determination of the genome sequence of Arabidopsis thaliana in 2000. Genome size has always been an important consideration for whole genome sequencing projects in plants, as such efforts have been limited historically to species with small genomes, which were cheaper and easier to sequence and assemble. Further angiosperm genome sequences, mainly those of crop species with small genome sizes, were also completed providing really valuable data. Yet, the problem in investigating the genomics of drought tolerance in species such as wheat is that: most pathways and candidates can be more effectively studied in model species with sequenced genomes and even amongst the cereals there are more extensive data available for rice and maize when compared with wheat.

However, recent technological advances and the imperative to ensure sustainable food production has driven research programs to improve this crop genetically despite the size and
complexity of the genome. The wheat genome is more than five times larger than the human genome and it is also more complex, comprising 21 chromosomes. The International Wheat Genome Sequencing Consortium (IWGSC) has now published a high-quality assembly of the genome. The two main articles have been published in Science, consisting of the description of the genome sequence, and an extensive exploration of the transcriptome. Work is now focused on delivering a high quality reference genome sequence that is anchored to the genetic maps, integrates different data resources, provides automated and manual annotation of genes and genomic features, and links genomic data directly to agronomically important traits.

In this context, our laboratory research is focused on wheat stress tolerance. We are using genomics, *in silico* analysis, proteomics, *in vitro* culture, biochemical, and physiological approaches to elucidate the mechanisms involved in abiotic stress tolerance in Algerian wheat varieties. The overall research goal is to discover genes and genetic variants that can be used to improve crop performance in sub-optimal growing conditions.

This seminar is going to be a good opportunity for our laboratory to confront our results related to drought tolerance and the technological quality of wheat. Hopefully this meeting will lead to future collaborations between us on subjects of common interest.
Presentation of the laboratory

Laboratory of Genetics, Biochemistry and Plant Biotechnology is a plant biotechnology research unit founded since July 2000. It brings together more than 27 researchers, 15 PhD students and 04 support staff, organized into 04 teams:

**Team 1:** Cytogenetics  
**Team 2:** Biotechnology and Plant Amelioration  
**Team 3:** Biochemistry Genetics and Proteomics  
**Team 4:** Molecular Physiology and Plant Biodiversity

Scientific research objectives of the GBBV laboratory focus primarily on the inventory and characterization of phylogenetic resources and their applications in the fields of agriculture and agro-food industry, varietal breeding for adverse environmental factors such as drought, salinity and soil degradation, gene expression analysis under stress conditions, the use of biotechnology tools to develop new varieties of crops, analysis of rhizosphere signals important in plant-microbial interactions in the case of associative nitrogen-fixing bacteria and actinorhizal symbiosis, genetic diversity of cereals and legumes, research and characterization of some wheat allergens by proteomics approaches.

**Research themes**

GBBV laboratory activities revolves 3 principal research axes:

1- Improvement of wheat growing in semi-arid regions based on knowledge gained in the fields of genetics, molecular biology, biotechnology and plant physiology. This is addressed by the study of adaptation and yield of wheat genotypes under water stress and high-temperature conditions.

2- The relationship among durum wheat plants and soil microorganisms (rhizosphere): effect of beneficial bacteria (PGPR) on growth and production, as well as abiotic stress tolerance.

3- Knowledge of the biochemical, genetic and molecular bases of wheat quality: genetic variability of cereals (wheat, rye, barley, triticale, related wheat species and Aegilops). Control of the identity and varietal purity of commercial batches and seed as well as control of GMOs. Characterization of genetic resources and plant material breeders, selection for technological quality (research and characterization of cereal allergens.)
Located at the University of Frères Mentouri Constantine 1, the GBBV laboratory collaborates with several research teams and universities worldwide including:

- Grenoble Alpes university – France.
- SAPIENZA University – Rome Italy.
- The French National Research Institute for Sustainable Development (IRD) - Laboratory of Tropical and Mediterranean Symbioses (LSTM) Montpellier. France
- The National Centre for Scientific Research (CNRS) - Institute of Plant Sciences (ISV) Montpellier – France.
- Center of Biotechnology of Sfax (CBS) - Tunisia.
- The BIA Unit (Biopolymères Interactions Assemblages) INRA Nantes. France
- Faculty of Pharmacy, Pharmaceutical UFR, Health-Environment-STIC, EA 4267 FDE / UFC, University of Burgundy, France.
- Team Ligands, complex architectures and catalysis (LAC2) LCC CNRS, Toulouse, France
- Catholic University Louvain-la-Neuve, Belgium
- The Algerian National Institute for Agricultural Research (INRAA) Algeria.
- Laboratory for the Improvement and Production of Seeds of Potatoes (LAPSPT) Tiaret – Algeria.

The GBBV laboratory has a range of scientific equipment needed to achieve research work: liquid nitrogen production station, greenhouse with microclimate control, microscopy room, plant growth chamber, physiology equipment (fluorometer, osmometer, phytotron, water vapour diffusion porometer, chlorophyll meter SPAD), molecular biology equipment (horizontal and vertical electrophoresis systems, thermocyclers, real time PCR instrument, NanoDrop spectrophotometer, Gel imaging system).

Master's and PhD programs are also backed by the laboratory. Students will learn how to carry out independent research that makes an innovative contribution to biological knowledge.
CONTENTS

Proteomic analysis of water stress in durum wheat (*Triticum durum* Desf).
KACEM N.S, MAURO S, MUHOVSKI Y, DELPORTE F, J. RENAUT, DJEKOUN A, WATILLON B

Somatic embryogenesis, somaclonal variation and salt stress in durum wheat (*Triticum durum* Desf.) Genotypic and molecular analysis.
BENABDELHAFID Zoheira, BOULDJADJ Ryma, YKHLEF Nadia, DJEKOUN Abdelhamid

Study of the polymorphism of Gamma and EMS mutants of durum wheat varieties by microsatellites SSR.
LOUALI Yamouna, BOULDJEDJ Ryma, BELBEKRI Nadir, YKHLEF Nadia and DJEKOUN Abdelhamid

Antioxidant enzyme gene networks involved in drought stress response and tolerance in durum wheat.
BOUCHEMAL Karima, DJEKOUN Abdelhamid

Effect of inoculation of cereals by PGPR and mycorrhizae under water deficit conditions.
NADJI Wassila, BELBEKRI Nadir, YKHLEF Nadia and DJEKOUN Abdelhamid

The interactions between wheat and PGPR: a review
KECHID Maya, MAOUGAL Rym.T and DJEKOUN Abdelhamid

PGPR, paranodules, growth stimulation and water deficit tolerance in durum wheat (*Triticum durum* Desf.)
BENMATI Mahbouba, LE ROUX Christine, BELBEKRI Nadir, YKHLEF Nadia and DJEKOUN Abdelhamid

The bioinformatic tools for the creation of wheat specific databases
HAMIDECHI Mohamed Abdelhafid and DJEKOUN Abdelhamid

Genetic Diversity of High and Low Molecular Weight Glutenin Subunits in Saharan Bread and Durum Wheats from Algerian Oases
BELLIL Ines, CHEKARA BOUZIANI Mohammed and KHELIFI Douadi

Genetic Variation and Geographical Diversity for Seed Storage Proteins of Seventeen Durum Wheat Populations Collected in Algeria
HAMDI Wahiba, BELLIL Ines and KHELIFI Douadi

Allelic Variation in *Glu-1* and *Glu-3* Loci of Bread Wheat (*Triticum aestivum* ssp. *aestivum* L. em. Thell.) Germplasm Cultivated in Algeria
BELLIL Ines, HAMDI Wahiba and KHELIFI Douadi
Diversity of five glutenin loci within durum wheat (*Triticum turgidum* L. ssp. Durum (Desf.) Husn.) germplasm grown in Algeria

**BELLIL Ines, HAMDI Wahiba and KHELIFI Douadi**

---

The genetic potential of a germplasm of interspecific crosses between durum wheats (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) and their relatives (*T. dicoccum* Schübl. And *T. polonicum* L.) in five glutenin loci

**BELLIL Ines, HAMDI Ouahiba, BENBELKACEM Abdelkader, KHELIFI Douadi**

---

Diversity of seven glutenin and secalin loci within triticale cultivars grown in Europe

**AMIOUR Nardjes and KHELIFI Douadi**

---

Allelic variation of HMW and LMW glutenin subunits, HMW secalin subunits and 75K gamma-secalins of hexaploid triticale

**AMIOUR Nardjes and KHELIFI Douadi**

---

Diversity of Seven Glutenin and Secalin Loci within Triticale Cultivars grown in France

**BELLIL Ines, KHELIFI Douadi**

---

Evaluation of two diploid Aegilops species variation in terms of HMW, LMW glutenin subunits and gliadins

**MEDOURI Asma and KHELIFI Douadi**

---

Polymorphism at High Molecular Weight Glutenin Subunits and Morphological Diversity of Aegilops geniculata Roth Collected in Algeria

**MEDOURI Asma, BELLIL Inès and KHELIFI Douadi**

---

Genetic Diversity of High and Low Molecular Weight Glutenin Subunits in Algerian *Aegilops geniculata*

**MEDOURI Asma, BELLIL Inès and KHELIFI Douadi**

---

The Genetic Diversity of Gliadins in Aegilops geniculata Roth from Algeria as revealed by RAPD and morphological markers

**MEDOURI Asma, CHEKARA BOUZIANI Mohammed and KHELIFI Douadi**

---

Assessment of the allergenic potential of wheat and identification of some allergens in the soluble fraction

**BELLIL Ines, KHELIFI Douadi**

---

Enzymatic degradation of gliadin by nigella sativa seeds protease: implications for new treatment of celiac disease

**BELLIR Nousseiba, BELLIL Ines, KHELIFI Douadi et ROUABAH Leila**
Identification of association between phenotypic and genotypic traits using Simple sequence repeat (SSR) markers in *Durum wheat*


HAMLAL Chourouk, BRINI Faical, AYADI Malika, MASMOUDI Khaled, DJEKOUN Abdelhamid and YKHLEF Nadia

TaSTRG gene expression under abiotic stress in interspecific lines durum wheat × Aegilops geniculata Roth.

KELLOU Kamel, DJEKOUN Abdelhamid and YKHLEF Nadia.

Morpho-physiologicals, biochemicals and transcriptomics markers of drought tolerance in durum wheat (*Triticum durum* Desf.)

MOUELLEF Adra, YKHLEF Nadia

Aquaporins gene expression and the control of water status in durum wheat

BENTAHAR Soumia, DJEKOUN Abdelhamid and YKHLEF Nadia.

Genetic diversity and molecular characterization of Algerian Durum Wheat (*Triticum durum* Desf) Using molecular marker

KHENAOUI Amina, DJEKOUN Abdelhamid and YKHLEF Nadia.

In vitro selection for saline and thermal stress tolerance in bread wheat (*Triticum aestivum* L.): Morpho-physiological, biochemical and molecular aspects

BENDERRADJI Laid, DJEKOUN Abdelhamid and YKHLEF Nadia.
Proteomic analysis of water stress in durum wheat (*Triticum durum* Desf)

Kacem N.S¹,², Mauro S¹, Muhovski Y¹, Delporte F¹, J. Renaut³, Djekoun A², Watillon B².

¹Department of Life Sciences, Walloon Agricultural Research Centre, Chaussée de Charleroi, 234, 5030 Gembloux, Belgium
²Laboratory of Genetic Biochemistry and Plant Biotechnology ‘Team II Biotechnology and Plant Amelioration’, Faculty of Nature and Life Sciences, University Frères Mentouri Constantine. Algeria
³Environmental Research and Innovation Department (ERIN), Integrative Biology Facility, Luxembourg Institute of Science and Technology, 41, Rue Du Brill, 4422 Belvaux, Luxembourg.

Email: kacem.nadia@umc.edu.dz

In the semi-arid high plains of Algeria, drought is a serious wheat production problem. The general effects of drought on plant growth are well known, but the effects of water deficit at biochemical and molecular levels are not well understood. Plant species possess distinctive indicators of stress tolerance at whole plant, tissue, or cellular level, reviewed by Ahmad et al. (2014). Understanding the drought tolerance mechanisms of wheat involves isolating and characterizing drought stress-related genes and proteins (Salekdeh et al. 2002). In our study, we started looking at the potential use of proteomic tools for identifying osmotic stress-related proteins in durum wheat calli. We describe a relatively simple and reproducible method that addresses the co-migration drawbacks (Kacem et al. 2016). The method provides new insights into the osmotic-responsive mechanisms at the cellular level of durum wheat.

The experiments were carried out on three durum wheat (*Triticum durum* Desf.) genotypes. Based on field trials, one genotype was classified as drought sensitive (Waha) and two as drought tolerant (Oued Zenati and Djenah Khetifa). The wheat germplasm was obtained from the Technical Institute of Field Crops (ITGC) Institut Technique des Grandes Cultures (Station El-khrroub Constantine, Algeria).

Callus from mature embryo explants was initiated on Murashige and Skoog (1962) medium supplemented with 2 mg l⁻¹ 2,4- dichlorophenoxyacetic acid (2,4-D) The calli were maintained by subculturing every 20 days on the same MS medium with different PEG 6000 concentrations: 0% (control), 10% (SI: -0.49 MPa) and 20% (SII: -1.2 MPa) (Kacem et al. 2017). *In vitro* screening for stress tolerance reveals the variety Djenah Khetifa as the most tolerant and Waha as sensitive. For proteomic and transcriptomic analysis, 20 % of PEG6000 was used, because this concentration had reduced more than half of the recorded values of all the characters studied (Kacem et al. 2017). However, cells that continue to grow under severe osmotic stress are considered as tolerant to water stress.
The total proteins were extracted from callus of the three-durum wheat genotypes. Bands were revealed with Coomassie Blue R-250 and analyzed using ImagQuant 5 and Photocapt 8 software. Bands 1D SDS-PAGE of the water-soluble protein fraction revealed outstanding quantitative and qualitative differences between control and stressed calli in Oued Zenati, more variation in Waha. No change in the protein relative abundance profile was observed in Djenah Khetifa, the most tolerant variety to PEG6000. These results indicate a positive correlation between the changes in the overall protein profile and water stress response at the cellular/tissue levels.

We chose to analyze the gel area between 25 and 100 kDa because this was the area where most changes in protein accumulation due to water stress were observed. In our study, we describe a new electrophoretic procedure that improves the separation, identification and relative quantification of interesting proteins. We combined two successive electrophoreses within a single experiment, resulting in a method we named «diagonal two-dimensional electrophoresis (D-2DE), which was subsequently combined with mass spectrometry (MALDI-TOF) (Kacem et al.2016). 1D SDS-PAGE Tris–glycine and Tris-tricine methods are widely used for separating high- and low-molecular mass proteins, respectively. The different separation characteristics of the two techniques relate directly to the strongly differing pK values of the glycine and tricine functional groups that define the electrophoretic mobilities of the trailing ions (glycine and tricine) in relation to the electrophoretic mobilities of proteins (Schägger 2006).

The results obtained showed very good reproducibility. Spots revealed with silver nitrate showed better resolution than with Coomassie Blue. We identified 16 conspicuous new spots in the cut area between 25 and 100 kDa in Oued Zenati stressed tissues, and this was compared with the control. Only the colored spots revealed with Coomassie Blue were identified by mass spectrometry. The system resolves most polypeptides and allows the rapid characterization of proteins with a wide range of molecular weights. This analysis method, that addresses the co-migration drawbacks, improved separation and gave highly reproducible gels with good spot resolution (Kacem et al. 2016).

Although staining with silver nitrate gives a better resolution than Coomassie Blue staining, the identified proteins were obtained only from gels stained with Coomassie Blue. These identified proteins included: glyceraldehyde-3-phosphate dehydrogenase (*Brassica juncea*) (spots 1 and 2); *Triticum urartu* globulin 1S allele (spots 1, 2 and 3), globulin 3-A (*Triticum aestivum*) (spots 3 and 4). Also, in spot 3 and 4 were identified predicted protein (*Hordeum vulgare*), we searched them against Genbank and it revealed a 95 % match with peroxidase (*Triticum urartu*), *Triticum aestivum* cDNA clone H01_1125_plate_3, mRNA sequence (spots 1 and 3) was identified as globulin-1S in *Setaria italica* or globulin 2 in *Zea mays*, with an 81 and 80 % homology, respectively, in spot 4 were identified as the hypothetical protein. Functionally, the identified proteins were related to protein...
synthesis, carbohydrate metabolism and drought tolerance. Some of the proteins that changed under dehydration induced by osmotic stress are considered to be allergens, such as globulin and GAPDH, suggesting that people sensitive to these proteins might be adversely affected by wheat products exposed to stress factors; the over expression of globulins under osmotic stress could improve grain nutritional quality (Kacem et al. 2016).

In order to confirm these results we tested three antibodies (REG 960, REG 100 and REG 410), initially directed against the rice globulin (Zi et al. 2012). We chose to work with REG 960 because it gave the best results, showing 68.4% positivity and 20.8 % identity with Glob S1 and Glob3A. With REG 410, however, the recognition rates were very low. Western blot analysis of the Oued Zenati callus revealed a significant increase in globulin under dehydration induced by osmotic stress. The results also indicated that there is a diverse group of globulins in wheat, some of which could be associated with osmotic stress tolerance.

Three proteins (GAPDH, globulin and peroxidase) identified by MALDI-TOF were examined at the mRNA expression level using qRT-PCR. Target gene expression was quantified using the comparative Ct (threshold cycle value) method of relative quantification ($\Delta\Delta$Ct), as described by Livak and Schmittgen (Livak and Schmittgen 2001).

Transcript accumulation on the three selected genes was quantified by qRT-PCR. The results showed that under osmotic stress, GAPDH, globulin and peroxidase were slightly up-regulated (no more than 1.74-fold) over the control, apart from Oued Zenati with GAPDH. Generally, the expressions of the GAPDH, globulin and peroxidase protein genes at the mRNA level were not in line with their expression at the protein level (Kacem et al. 2016).

Comparative transcriptomic approaches aimed at highlighting differentially expressed genes have been successfully used against both biotic and abiotic stresses (Ergen and Budak 2009, Kantar et al. 2011). The identification of differentially expressed genes solely, however, is generally not enough to unravel the underlying molecular mechanisms of water-deficit stress because several transcripts are known to undergo transcriptional, translational and posttranslational modifications. Thus, the comparative proteomics approach has emerged as a powerful and promising tool for investigating stress response in plants (Abdalla and Rafudeen 2012).

The analysis of organ-specific protein abundance provided rich information on the response mechanisms of plants to abiotic stress. Greater focus on these proteins at the cellular level (callus) could contribute to a better understanding of how plants respond and adapt to abiotic stresses and could help identify candidate genes for molecular breeding studies.
References


Somatic embryogenesis, somaclonale variation and salt stress in durum wheat
*(Triticum durum* Desf.)* Genotypic and molecular analysis.

Zoheira BENABDELHAFID, Ryma BOULDJADJ, Nadia YKHLEF, Abdelhamid DJEKOUN

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team II Biotechnology and Plant Amelioration’, Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria

Email: b.zoheira_28@yahoo.fr

The main objective of this work is to overcome the effect of salt stress on wheat through different techniques and biotechnology approaches, which are used to improve the behavior of different varieties of durum wheat and to select the best performing one. Somatic embryogenesis is currently integrated into many selection schemes since it can significantly reduce the length of improvement cycles.

Plant tissue culture plays an important role in plant amelioration and in the manipulation of plants for improved agronomic performance (Zale *et al.*, 2004). *In vitro* culture of plant cell tissues has shown a growing interest over recent years. This approach is considered as a powerful mean to study plant physiological, genetic process and to increase genetic variability which can be used in breeding programs (Karp *et al.*, 1987). Somatic embryogenesis is a process in which any somatic tissue is potent to regenerate into a whole plant through formation of embryo-like structures (Rao., 1996, Jiménez., 2005), and represents a unique developmental pathway that involves a number of characteristic events: cells dedifferentiation, cell division activation, reprogramming of their physiology, metabolism and gene expression patterns (Pathak *et al.*, 2008, Yang *et al.*, 2010). The phytohormones, auxins and cytokinins are important regulators and play a role in the developmental fate of pluripotent plant cells to regenerate plants which is well known, nevertheless, the mechanism is quite complicated (Ray *et al.*,1996, Pernisová *et al.*,2006.). Auxins are known to be associated with plants genetic instability, a phenomenon called somaclonal variation (Karp., 1989, Phillips *et al.*, 1994, Cullis., 1999). Although somaclonal variation can be used as a source for variation to obtain superior clones (Karp., 1993; Cassells *et al.*, 1999). This somaclonal variation can be caused by exogenous factors, as osmotic pressure or mutagenic treatment .The salt stress application during the regeneration process constitutes a convenient way to study the effects of salinity on the morphogenic step developments. Several studies showed that the selective pressure can be applied during the callus formation phase and/ or regeneration (Bouiamrin *et al.*, 2012, Soliman *et al.*, 2013,Balkishna *et al.*,2013). In wheat species, different explant sources have been used for embryogenic callus formation and plant regeneration including mature and immature embryos (Ozgen *et al.*,1996) immature inflorescences and coleoptiles (Benkirane,H *et al.*,2000), shoot apical meristems (Ahmed
et al., 2002) and others. Indeed, these tissues vary in their ability to regenerate into a whole drought stressed plants in durum wheat (Triticum durum Desf.) (Delporte et al., 2001). Embryo is the most frequently used explants for the initiation of wheat tissue culture either for callus production or direct DNA delivery techniques. Moreover, mature embryo are readily available all over the year and are used for transformation studies in the recent years (Patnaik et al., 2006).

The development of RAPD (Randomly Amplified Polymorphic DNA) has allowed simple, easy and less time-consuming genom analysis at the DNA level compared with RFLP (Restriction Fragment Length Polymorphism).

Development of a somatic embryos is obtained after culturing six varieties of durum wheat (Waha, Beliouni, Gumgoum Rkhem, Adna-2, Beni mestina and Adna-1) on a nutrient medium supplemented with 3.5mg/l of l 2.4-D.

The callogenesis was initiated from mature embryos, which are the most frequently used explants for the initiation of wheat tissue culture and DNA delivery techniques, moreover, mature embryos are readily available throughout the year, these results are confirmed by different works (Delporte et al., 2001, Salama et al., 2013, Almobasher et al., 2014). Selection of tolerant varieties in relation to salt stress has been realized by the addition of a selectiv agent: the NaCl (0,4,8 and 16g/l). Salts stress was applied during callus induction and regeneration phase the results showed that salinity causes a significant reduction in plantlets regenerative capacity. RAPD profiles analyses revealed the presence of somaclonal variations after regeneration of several seedlings.

Observation made during the incubation of cultures showed that the callus is induced after 5 to 8 days of seeding, NaCl have a great effect on regeneration capacity of callus medium. The regeneration rate of callus change with the different concentration of NaCl in the culture medium, callus initiated on MS medium added to 4g.l\(^{-1}\) NaCl produced a percentage of regeneration identical significantly to callus initiated on MS medium without NaCl (control), whereas, the percentage of callus initiated on MS medium added to 16g.l\(^{-1}\) was significantly different. Also, wheat has different behaviors in their ability to produce plants via callus cultivated in salt medium. The varieties Belioumi, Gumgum Rkhem and beni mestina have better responded better with high concentration of NaCl (16g.l\(^{-1}\)); Calli of Waha et Adnan-1 are more sensitive to salinity than others varieties. With 16g.l\(^{-1}\) NaCl the calli of Beni mestina Gumgum Rkhem and Beliouni gives a good callus regeneration rate.

The production means of somatic embryogenesis calli for all genotype in the medium added to 16g.l\(^{-1}\) (53.67%) are more important than the means obtained in medium added to 4 g.l\(^{-1}\) (42.94%) beside means obtained in control medium with a rate of (69.59%). the genotype effect and
concentration of NaCl is also remarked. The best regeneration percentage in control medium was observed on Beliouine (30.36%) and Adna-2 (24.74%) genotypes.

Molecular Genetic Marker analysis showed that the primer used and his amplification in the plant regenerated \textit{in vitro} under salt stress, is the proof that the six genotypes of durum wheat has developed a somatic variation under salt stress. We notice that there is a difference between the numbers of fragment amplified, it means that all durum wheat genotypes are not always identical in their DNA capacity to be amplified. The same results was fond in the work of (Hamaid et al., 2013).

\textit{In vitro} tissue culture could be an important means of improving crop tolerance and yield through genetic transformation as well as by induced somaclonal variation. Therefore, it is important to control well all the parameters during the \textit{in vitro} culture: The choice of the explant, the best concentration of regulator and the level of tissue culture to start the stress selection. The results of this study indicated that the effect of callus production, embryogenic callus induction and plantlets regeneration was influenced by addition NaCl in the culture medium, in a low concentration of NaCl (4g.l\textsuperscript{-1}), there is no a signifies effects on the tissue culture, but depressive effect accentuated by high concentration of salt (16g.l\textsuperscript{-1}). Differential genotypic response was also noted in callus ability to proliferate and regenerate plantlets under concentration of NaCl. Also, the results showed that all durum wheat genotypes were not always identical in their DNA ability to be amplified. RAPD analysis is useful molecular tools to indicate genetic polymorphism between the durum wheat genotypes under salt stress.

\textbf{REFERENCES}


Study of the polymorphism of Gamma and EMS mutants of durum wheat varieties by microsatellites SSR

Yamouna Louali, Ryma Bouldjedj, Nadir Belbekri, Nadia Ykhlef and Abdelhamid Djekoun

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team II Biotechnology and Plant Amelioration’, Department of Biology and Ecology, Faculty of Natural Sciences and Life, University of frères Mentouri Constantine, Algeria

Email: louali.yamouna@gmail.com

Abstract

A molecular analysis by twelve SSR markers was used to evaluate polymorphism, to study the nature and degree of genetic variation induced by gamma rays and EMS. The molecular study involved 48 samples including six varieties treated at different doses of gamma radiation (10, 25, 50, 100, 180, 220 Gy), and three varieties of the two generations M1, M2 treated with different concentrations of EMS (0, 0.24, 0.5, 0.7 %). The results revealed a polymorphism by the absence and the presence of new bands between the control and the treated varieties. Concerning the Gamma mutants among the 12 primers, 7 are polymorphic (84%), in the case of the EMS mutants, 6 are polymorphic (50%). The amplification product are tested on a 3% agarose gel in the presence of the 100bp size marker. The DNA samples were amplified by the majority of primers wmc134, wmc264, wmc24, wms6, wmc124, wms497, wmc177, wms131, X1, and X2. Except for BARC8 and wmc 322 where there was no amplification. The profiles that have been generated show the observed differences between mutants and their parents, where some show that the mutation affects the gene (absence of alleles). The mutations have affected several alleles of these markers, so the gene that is associated with these sequences will be unstable and as a result, its function changes. This study indicates that there is a possibility that SSRs detect gamma-EMS induced variations.

Key words: durum wheat, mutagenesis, EMS, gamma irradiation, molecular markers

Introduction

Cereals are an important part of human and animal food resources, among these cereals wheat is an important crop in the world in terms of the cultivated area contributing to the global food supply as well as the assurance of economic security. Its yield is strongly constrained, including drought, where nearly a third of the world's arable land suffers from this water deficit.

Induce of mutations in crop plants contribute by increasing genetic variability and enrich plants germplasm for direct selection and crossbreeding. In wheat, chemical and ionizing radiation
mutagenesis have been universally used to generate genetic variations for breeding researches and genetic studies (Cheng et al., 2015)

The exploitation of natural and induced genetic diversity is the basic condition for plant improvement in the development of varieties with desired traits. For this purpose the application of physical and chemical mutagenesis has been established.

In order to evaluate the polymorphism that gamma rays and EMS induced, twelve SSR microsatellite markers were used due to their instability characterized by induced mutations and mismatch repair defects in a repeated sequence, which makes this marker an excellent system for effective monitoring of spontaneous or induced mutation in plants(Kashi, 2006).

Molecular characterization of Gamma and EMS mutants by microsatellites

Subsequent studies have shown that SSRs are highly polymorphic in wheat (Manifesto et al., 2001, Zhang et al., 2002). But the mechanism of induction of mutation in plant microsatelites is still poorly understood. One of the characteristics of these repeated sequences is that they tend to have higher mutations that most of the time lead to length changes (Udupa and Baum 2001). Microsatellites have inherent spontaneous instability due to the susceptibility of one of two processes, ie, replication slippage or unequal recombination, and the high rates of these two processes are likely to be involved in the sensitivity of these loci to radiation induction (Bridges 2001), indeed some authors say that some non-targeted induced mutations cause instability in these repeated sequences and that the microsatellites are associated with coding regions of the genome, so the instability of these repeated sequences influences the function of the genes in some cases (Kovalchuk et al., 2000, Bridges 2001, Bouffler et al., 2006) . A comparative study of a single-generation wheat plant population in a highly contaminated area around nuclear central Chernobyl with a control population developed elsewhere indicated an increase in the frequency of germline mutations in microsatellite regions in the exposed population (Kovalchuk et al., 2000). Previous studies have shown that fast neutron and gamma mutations are mainly deletions larger than 5 Kpb (Shirley et al., 1992, Bruggemann et al., 1996, Cechini et al. 1998).

But recent reports have revealed a much more complex pattern of germline mutations that not only included the complete deletion of loci, but also showed a bias towards mutations with gains and losses from multiple repetition units. frequent insertions of DNA of unknown origin have also been observed (Kovalchuc et al., 2003)

The research work of Louali et al., 2016 showed that extracts of DNA samples obtained after CTAB extraction from one gram of plant leaves give a clear extract, after migration on agarose gel 1.5%, a band of average intensity without train were observed, this band indicates that there is a
satisfying quantity of DNA, pure and good quality extracts. The measurement of DNA and its quantification has been made by Nanodrop. Microsatellite markers were used to study the nature and degree of genetic variation induced by gamma rays and EMS and to detect polymorphism in six durum wheat varieties. Of the 12 primers used, two of them (BARC8 and WMC322) did not generate polymorphic profiles, unlike the other primers (X1; X2, wms131, wmc177, wms497, wms124, wms6, wmc24, wmc264, wmc134) produced polymorphic electrophoretic profiles, and showed variation between the mutant varieties (Louali et al., 2016)

Some differences were observed between the Gamma and EMS mutants and their parents, for the Gamma mutants among the 12 markers 7 are polymorphic (84%), and for the case of EMS among the 12 primers 6 are polymorphic (50%) (Louali et al., 2016). For amplified microsatellites, the intensity of the bands obtained varies from one microsatellite to another, and from one treatment to another. The degree of amplification of the microsatellites can be determined by the intensity of the bands obtained.

Amplification results by different primers according to (Louali et al., 2016)

Amplification of gamma-ray mutant samples with primer Wmc 134 shows the presence of 2 bands, one present in all the varieties with all the treatments of a molecular weight of 182.359pb, the other one which has a molecular weight lower than 100pb, another band is present that in the variety Mridj treated with the dose 220Gy, and at the GGR variety treated with dose 220Gy, the variety DK treated with dose 50Gy, the variety waha treated with 100Gy, with a molecular weight of 111,340bp, the presence or absence of bands is surely due to a mutation.

With the same primer, profile of EMS Mutants shows a band with a molecular weight 179.454bp present in all treatments except for the variety benimestina with the concentration 0.5%, 0.7% which reappeared in M2 with the concentration 0.5%, which sends us to say that the mutation was the cause of the loss of the band in the first generation, but with the concentration 0.7% this band is still absent. we also note other bands between 100 and 750 bp which are mostly in the M1 thing that explains that the mutation affected the first generation but after rearrangement genes have redone.

The DNA of the varieties treated with gamma rays was amplified with primer wms6 but not at the same distance, a band at 217,739 bp another 262,625pb, some DNAs were polymorphic, other monomorphic, and in variety waha treatment 100 Gy and 180Gy, the DNA was not amplified which may be due to a mutation. The intensity of the bands was different.

Amplification product of mutants gamma with primer wms 124 gave a band of the same distance less than 100pb, and other bands also were observed in some treatment between 100 and 500 bp, and absent in others, this is probably due to mutation or artifacts. concerning mutant EMS, DNA
of all varieties has been amplified by giving two bands with different intensity. For the first band a
weight of 255.136pb, it is absent in the DK variety treated with the concentration 0.24% in the second
generation, other bands of a very weak intensity appeared.

Products of amplification of gamma-ray mutant samples with primer wmc177 gave 2 bands, after
migration the size of the bands is of the same weight of 200.442 pb, this band is absent in the
GGR variety treated with the 100Gy dose, and another band is present with a molecular weight of
23.308bp, concerning Mutant EMS DNA was amplified and proliferated two bands of the same size
196.762 bp, the band is absent in the DK variety the first generation treated with 0.7% concentration
and the same variety in the second generation treated with concentrations 0.24%, 0.5%; and 0.7% did
not have amplification, the intensity is low. Another band below 100pb is observed.

The microsatellite amplification test shows that amplification conditions and parameters were
adequate, except for barc8 and wms 322, optimization of amplification conditions is essential.

The microsatellites used in this study for genetic variability, proved to be different from one
microsatellite to another, where some are monomorphic, other polymorphic for each variety. In order
to be sure that this polymorphism is due to a mutation and not a spontaneous mutation, the mutations
were tested on SC with certain primers, and each amplification was done several times.

Conclusion

This study indicates that there is a possibility that SSRs detect gamma-induced variations,
several patterns have been generated, where some show that the mutation affects the gene (absence
of alleles). The function of the drought tolerance genes is influenced by these repeated sequences.
The mutations have affected several alleles of these markers, so the gene that is associated with these
sequences will be unstable and as a result, its function changes. In perspective, the analysis of the
sequence of each polymorphism can also give an insight into the molecular nature of the mutation

References


mutants at the Arabidopsis thaliana HY4 locus. The Plant Journal10, 755–760.

Cecchini E, Mulligan BJ, Covey SN, Milner JJ.(1998). Characterization of gamma irradiation-
induced deletion mutations at a selectable locus in Arabidopsis. Mutation Research 401,199–206

mutant induced by gamma-radiation in wheat (Triticum aestivum L.). BMC Genetics, vol. 17: 112-
118.


Antioxidant enzyme gene networks involved in drought stress response and tolerance in durum wheat

Karima Bouchemal, Djekoun Abdelhamid

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team II Biotechnology and Plant Amelioration’, Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria
Email: karima.bouchemal@umc.edu.dz

Abstract

One of the inevitable consequences of drought stress is enhanced Reactive oxygen species (ROS) which are harmful to living organisms due to the potential oxidation of membranes, DNA, proteins, and carbohydrates. Reports have demonstrated that ROS can directly affect the signal transduction pathways and, consequently, the control of drought-responsive genes expression. An assortment of antioxidant enzyme genes with diverse functions is induced or repressed by this stress. SOD (superoxide dismutase), CAT (catalase) and POX (peroxidase), are the main components of antioxidative machinery for drought tolerance in higher plants. Many stress-inducible genes have been identified using transcriptomic approaches. Knowledge of antioxidant genes coding for SOD, POX and CAT could provide valuable information for understanding the drought tolerance related mechanisms in durum wheat. Identification of candidate genes responsible for higher levels of drought stress tolerance has become a major area of research, with a view to manipulating gene expression in durum wheat. The development of molecular markers for candidate genes in drought tolerance may allow marker-assisted breeding for the development of new drought-tolerant cultivars. In the long run, the interesting targets and molecular markers can be used to achieve more sustainable wheat production.

Introduction

Drought stress is well recognized as an environmental factor prevailing in the eastern high plateaus of Algeria with a Mediterranean-type climate where durum wheat is generally grown (Bouchemal et al., 2017). It is the major yield-limiting factor of wheat due to due to a lack of rainfall and it actively obstructs almost all feature of plants biochemistry and physiology. One of the inevitable consequences of drought stress is enhanced reactive oxygen species (ROS) (Cruz de Carvalho, 2008) which are harmful to living organisms due to the potential oxidation of membranes, DNA, proteins and carbohydrates. ROS damage membranes and macromolecules affect cellular metabolism and play a crucial role in causing cell injury under drought stress (Mattos and Moretti,
In fact, enhanced cellular ROS production lead to oxidative stress which is sensed by the plant as an alarm signal that triggers defense pathways and acclimatory responses, enabling the plant to adapt to the changing environment (Cruz de Carvalho, 2008).

An assortment of antioxidant enzyme genes with diverse functions is induced or repressed by this stress (Refli et al., 2014; Harb et al., 2015; Bouchemal, 2018). Most of their gene products may function in drought stress response and tolerance at the cellular level. The enzymes localized in the different subcellular compartments and comprising the antioxidant machinery include catalase (CAT), superoxide dismutase (SOD), peroxidase, and enzymes in the water-water cycle (Gill and Tuteja, 2010). Antioxidant enzymes have been shown to condition tolerance to drought stress (Hasheminasab et al., 2012; Ali et al., 2014; Bouchemal et al., 2017). SOD (EC 1.15.1.1) remove \( \text{O}_2^- \) by catalyzing its dismutation, one \( \text{O}_2^- \) being reduced to \( \text{H}_2\text{O}_2 \) and another oxidized to \( \text{O}_2 \). This removes the possibility of \( \text{OH}^- \) formation by the Haber-Weiss reaction. SOD has several isozymes, which can be classified by the location and catalytic metals; MnSOD in mitochondria, FeSOD in chloroplast, CuZnSOD in chloroplast and cytosol. A series of studies showed that the high activity of SOD identified in many plant species is a consequence of the protection against oxidative stress under drought conditions (Sharma and Dubey 2005; Hasheminasab et al., 2012). CAT (EC 1.11.1.6) is a tetrameric heme-containing enzyme responsible for catalyzing the dismutation of \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \) and \( \text{O}_2 \) and is indispensable for ROS detoxification during stressed conditions (Garg and Manchanda, 2009). POX (EC 1.11.1.7) decomposes \( \text{H}_2\text{O}_2 \) by the oxidation of co-substrates such as phenolic compounds and/or antioxidants (Blokhina et al., 2003). Changes in peroxidase activity have been frequently correlated to the response of tolerance of plants to abiotic stresses, such as drought (Zoz et al., 2013; Huseynova et al., 2015; Bouchemal et al., 2017).

The expression of antioxidant enzyme genes under drought stress was tested in a few studies. Recently, a number of these genes have been identified using a quantitative real-time PCR (qRT-PCR) and microarray analysis in durum wheat. This article provides an overview of the ROS signaling pathway under drought stress and discusses some recent results obtained through transcriptome analysis of drought-inducible antioxidant gene expression in wheat.

**ROS signaling under drought**

Drought stress will inevitably result in oxidative damage in plants due to over production of ROS, which include \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \), \( \text{OH}^- \) and \( \text{O}_2^- \) (Gill and Tuteja, 2010). As drought stress proceeds, stomatal closure induces the reduction of photosynthesis by limiting the entrance of carbon dioxide (\( \text{CO}_2 \)), and the use of energy for photosynthesis then becomes lower than the absorbed light energy. This will have direct and indirect effects on the reduction in net photosynthesis and on the overall
production of ROS by plants under drought stress (Mittler, 2002). In spite of their destroyed activity, ROS are represented second messengers in a diversity of cellular processes, including conferment of resistance to several environmental factor stresses (Mattos and Moretti, 2015).

There are several pathways involved in ROS-mediated signaling. Among them, H$_2$O$_2$ signaling, Mitogen-activated protein kinases (MAPK) pathway, sugars and abscisic acid (ABA) signaling are major genetic pathways (Cruz de Carvalho, 2008). H$_2$O$_2$ is likely to be a key component in the orchestration of plant drought stress responses, modulating Ca$^{2+}$ signaling, MAPK cascades and gene expression (Mazars et al., 2010). This H$_2$O$_2$-mediated Ca$^{2+}$ signaling is required for many biological responses, including stomatal closure, stress adaptation, and several types of programmed cell death (Pei et al., 2000; Rentel and Knight, 2004). Drought triggers the production of the phytohormone ABA, which in turn causes stomatal closure and induces expression of stress-related genes (Shinozaki and Yamaguchi-Shinozaki, 2007). The link between ROS and ABA signaling has been reported in many reports (Cruz de Carvalho, 2008; Verslues and Zhu, 2005). There is specific genetic evidence that ROS generated by NADPH oxidases act downstream of ABA in mediating stomatal closure (Kwak et al., 2003). On the other hand, ABA-dependent proline accumulation under drought stress highlights further the intricate and complex relation between ABA and ROS since the proposed functions of proline under stress are ROS scavenging and regulation of the redox-status (Hare et al., 1998). It has been shown that ROS production plays a role in ABA synthesis under drought stress in wheat seedling (Zhao et al., 2001). The MAPKs are actively involved in many cellular responses under stressful conditions. They play an important role in signaling in different stresses. MAPK cascades can be activated following ROS accumulation in the cell (Saini et al., 2018). In Arabidopsis protoplasts, MAPKKKs including ANP1 are activated by H$_2$O$_2$ which may cause the downstream activation of MPK3 and MPK6 (Kovtun et al., 2000). H$_2$O$_2$ also increases expression of the Arabidopsis nucleotide diphosphate kinase 2 (NDPK 2) whose overexpression acts as negative feedback for H$_2$O$_2$ accumulation (Moon et al., 2003). In maize, MAP65-1a was reported to enhance the antioxidant enzymes SOD and APX through the brassinosteroid signaling pathway positively to control H$_2$O$_2$ amplification (Zhu et al., 2013). These findings imply that multiple MAPK modules mediate oxidative stress responses and that MAP kinase cascades are not only induced by ROS but may also regulate ROS levels by affecting enzymes activities. ROS signaling is also related to sugars signaling. Some studies suggested that sugars play multiple roles in the regulation of the expression of several photosynthetic related genes as well as some ROS related genes (Couée et al., 2006; Sulmon et al., 2006). Small soluble sugars and their metabolic enzymes are presumed to connect to oxidative stress and ROS signaling, but their effects on gene expression are resulting from sugarspecific signaling cascades (Couée et al., 2006). On the other hand, Cruz de Carvalho, (2008)
suggest that the connection between sugar sensing and ROS signaling seems to also involve ABA signaling, at least in some specific pathways.

Upon perception of stress, a signal is communicated to downstream components resulting in the change of gene expression and thereby of proteins required for the initial damage-repair and physiological re-programming for better adaptation (Melloul et al., 2014). Antioxidant genes are central players in this network and their function has a profound effect in controlling ROS levels and cellular redox balance. Indeed, when the presence of ROS causes only a little change in the cell redox potential, the cell’s antioxidant system is stimulated and defends the plant from the injury caused by ROS (Hasanuzzaman et al., 2018). ROS, on the other hand, can remarkably regulate the level of antioxidant gene expression providing a feedback regulation mechanism of ROS levels, which is a critical component in the modulation of signaling networks (Mylona and Polidoros, 2010).

Transcriptome analysis of drought-inducible antioxidant genes in wheat

Drought tolerance traits are complex, controlled by multiple genes, thus posing a challenge to fully revealing genetic control of functional physiological traits for drought tolerance across variable environments (El-Soda et al., 2014; Gupta et al., 2017). Classical breeding approaches at transferring tolerance to crop plants are limited by the complexity of stress tolerance traits and the lack of effective selection techniques (Patnaik and Khurana, 2001). Differentially expressed gene profiling is an alternative way to identify genes and pathways related to stress responses using modern molecular techniques, such as qRT-PCR and microarrays. These techniques may allow plant breeders to manipulate only a characteristic of interest (Casu et al., 2005). Knowledge of antioxidant genes coding for SOD, POX and CAT could provide valuable information for understanding the drought tolerance related mechanisms in wheat that could potentially lead to better breeding strategies in the future.

SOD genes

SODs are encoded by a small multigene family, and the first plant SOD gene cloned was from Zea mays (Cannon et al., 1987). Although numerous studies on plant SOD have been made, only a few have involved detailed molecular analysis in wheat. As of today, there have been one durum wheat SOD gene: TdMnSOD (Feki et al., 2016) and six bread wheat SOD genes, including one FeSOD with GenBank accession number JX398977, two CuZnSODs: SOD1.1 and SOD1.2 (Wu et al., 1996), three MnSODs: SOD3.1 and SOD3.2 (Wu et al., 1997); and another one with GenBank accession number AF092524 have been isolated and characterized. In wheat, previous studies demonstrated overexpression of SOD genes under drought stress conditions. In a study, the mRNA
level for TdMnSOD was quantified in durum wheat genome (Feki et al., 2016). It was noted that 15 % PEG (6000) increased the expression of TdMnSOD in wheat leaves. The same results were obtained by Bouchemal (2018) where durum wheat plants were treated with 20 % PEG (6000) for 10 days. The TdMnSOD gene encodes for a manganese superoxide dismutase which plays a pivotal role in preventing the over accumulation of ROS in mitochondria and consequently enhanced drought stress tolerance in wheat (Feki et al., 2016). In bread wheat cultivars, the SOD1.1 gene showed transcript up-regulation by drought stress (Wei et al., 2013). Another report showed a significant increase in SOD 1.1 gene expression in durum wheat leaf tissue in response to drought stress (Bouchemal, 2018). This gene mapped on the long arms of the group-7 chromosomes of Triticum aestivum, encodes for a Cu/Zn superoxide dismutase whose main function is catalyzing the dismutation of the superoxide radical to molecular O$_2$ and H$_2$O$_2$ in the cytosol and/or chloroplasts (Wu et al., 1999). Previous transcriptional analysis in bread wheat showed that SOD 1.1 was also induced by heat stress (Kumar et al., 2013), cold (Wu et al., 1999) and ozone (Li et al., 2013), suggesting that the wheat SOD 1.1 gene is a promising candidate gene for the development of crops with multiple stress tolerances. There have been many reports of the production of drought stress tolerant transgenic plants overexpressing different SODs (Van der Mescht et al., 2007; Melchiorre et al., 2009; Feki et al., 2016). These studies indicated that the SOD gene family play a critical role in wheat drought tolerance.

**CAT genes**

Plant catalase is usually encoded by a small gene family. Three CAT genes have been identified in Angiosperm species studied so far, including tobacco, Arabidopsis, maize pumpkin and rice (Willekens et al., 1995; Frugoli et al., 1996). Two CAT isoforms (CAT1 and CAT2) were purified from wheat germ and some biochemical properties were determined (Garcia et al., 2000). However, protein gels have indicated more than three bands in studies of several plant species (Corpas et al., 1999; Zimmermann et al., 2006). Previous reports in bread wheat identified a total of four candidate CAT genes by genome-wide analysis, including wcat1 (Saruyama and Matsumura, 1999) and three genes with GenBank accession numbers: CAT3, CATA and LOC542902. Furthermore, one CAT gene was reported in durum wheat (Feki et al., 2015). CAT genes have a crucial role to mope the hydrogen peroxide (H$_2$O$_2$) resulting in reducing oxidative damage. Little is known about CAT genes from durum wheat and their role in response to drought. A novel TdCAT1 gene isolated from durum wheat showed transcript up-regulation in response to drought stress (Feki et al., 2015). It encodes a putative peroxisomal catalase which may have a physiological function more related to catalase proteins that belong to class I. Researchers showed that TdCAT1 is able to impart drought stress tolerance in yeast and in transgenic Arabidopsis. Another study of TdCAT1 showed that expression
of this gene increased in durum wheat cultivars treated with 20 % PEG (6000) (Bouchemal, 2018). In the same study, the expression level of CATA gene was increased under drought conditions. This gene encodes for a CAT2 enzyme, essential for the removal of H$_2$O$_2$ produced in the peroxisomes by photorespiration. The up-regulation of CATA gene was also reported in bread wheat (Luna et al., 2005; Wei et al., 2013). A transcriptomic analysis using qRT-PCR in durum wheat cultivars showed a significant increase in expression level of CAT3 gene under drought stress conditions (Bouchemal, 2018). Transgenic plants overexpressing CAT genes showed an increase in CAT activity and thus enhanced tolerance to various abiotic stress (Nagamiya et al., 2007; M’Hamdi et al., 2009; Guan et al., 2009).

**POX genes**

Plant POX, designated class III POXs, are members of a large gene family. The automated annotation of the whole genomes of *Arabidopsis* (Arabidopsis Genome Initiative, 2000) and *Oryza sativa* (Goff et al., 2002; Yu et al., 2002), the automated clustering and assembling of EST sequences, and numerous EST projects led to the identification of a large number of sequences coding for class III plant peroxidases. PeroxiBase was originally created for the exhaustive collection of class III POX sequences from plants (Passardi et al., 2007). Currently, the PeroxiBase database (http://peroxibase.toulouse.inra.fr/index.php) lists 115 class III wheat peroxidases. The biological functions of most of them remain unknown. Previous studies have indicated the involvement of POXs genes in response to various environmental factors: salt stress (Fan et al., 2014), aluminum stress (Kumari et al., 2008), air pollutants and UV radiation (Kim et al., 2007), cold (Kim et al., 2012). In a study, plants overexpressing AtPrx3 (POX3) showed an increase in dehydration and salt tolerance, while antisense suppression of AtPrx3 expression gave dehydration and salt sensitive phenotypes (Llorente et al., 2002). Few drought-inducible POX genes have been reported, in wheat (*Triticum aestivum* L.) (Mohammadi et al., 2008; Sečenji et al., 2010; Csiszár et al., 2012). It was shown that peroxidase genes increased the wheat tolerance to drought stress.

**Antioxidant enzyme genes expression : A way to understand drought stress response in durum wheat**

Given the sporadic and varied reports regarding antioxidant genes expression during stress responses, the elucidation of gene expression profiles at the genomic level in durum wheat in a specific drought-tolerance system appears to be evident. RT-qPCR and microarray technologies are a powerful tool for analyzing gene expression profiles of plants exposed to abiotic stresses such as drought (Krebs et al., 2002; Regier and Frey, 2010). Indeed, one promising approach towards understanding the drought stress responses in plants is to identify the candidate genes involved in
various biological processes and stress regulatory networks via genome-wide expression profiling and to control the transcriptional activation or repression of stress-responsive genes via transcriptome profiling (Chen et al. 2002).

Despite similarities among different plants, it must not be forgotten that species such as wheat, with far less characterized genome compared to plants, may offer unique and interesting features (Ali et al., 2011). Their high level of abiotic tolerance and diversity may provide important resources for validation of candidate gene and accelerate important breeding programs (Langridge et al., 2005). In a recent study, a set of 4,422 candidate genes involved in the drought responses including antioxidant metabolism, was identified in a bread wheat cultivar (Poersch-Bortolon et al., 2016). The identification of drought-related genes has a vital role in the modulation of metabolic processes and development of antioxidant defenses to improve drought tolerance of durum wheat. Several transcriptomic studies have identified the antioxidant enzyme genes as candidate genes for drought stress tolerance in different species (Melloul et al., 2014; Jiang et al., 2015; Pan et al., 2016). CAT genes (CATA, CAT3) and SOD genes (TdMnSOD, SOD1.1) were suggested as candidate genes for drought stress tolerance and to develop stress tolerant varieties of durum wheat (Bouchemal, 2018). Analyzing the expression of these genes is critical to further our understanding of the metabolic pathways involved in durum wheat drought response.

**Conclusion**

Drought stress-induced ROS triggers signaling cascades and alters the expression of several genes in plant cells particularly the ones related to antioxidant enzymes. The researches suggest that drought stress always enhance the transcription of antioxidant enzyme genes, and increase enzyme activities subsequently, which controlling redox homeostasis in plant cells. Techniques for transcriptome analysis including qRT-PCR and microarrays have provided powerful tools for discovery of drought stress-responsive antioxidant enzymes genes in various crop plants. Understanding the association of antioxidant enzymes at gene expression levels with genetic variation in wheat tolerance is essential for the identification of the principal antioxidant defense pathways contributing to drought tolerance. Key candidate genes associated with drought tolerance such as POXs, CATs and SODs genes can be used for improving drought tolerance of durum wheat through marker-assisted selection. In the long run, the interesting targets and molecular markers can be used to achieve more sustainable wheat production.
References


Effect of inoculation of cereals by PGPR and mycorrhizae under water deficit conditions.

Wassila NADJI, Nadir Belbekri, Nadia Ykhlef, Abdelhamid Djekoun

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team II Biotechnology and Plant Amelioration’, Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria

Email: wassila.nadji@umc.edu.dz

Introduction

In Algeria, cereal production remains deficient and does not meet growing demand. It is strongly linked on the first hand to climatic conditions, water stress, increased abnormal temperatures, low rainfall and on the other hand to the characteristic of soils which lack nitrogen and phosphorus and all these factors affect and fall yields. Several approaches to improve cereal production are used, among which is the method based on the use of microorganisms such as Plant Growth Promoting Rhizobacteria (PGPR) and arbuscular mycorrhizal fungi which are the main symbiotic components of the soil. These fungal symbiotes are known in the course of the main biogeochemical cycles of soils in the growth of plants by promoting their mineral and hydric nutrients. The aim of this work is to study the diversity of endomycorrhizal spores in durum wheat fields (Triticum durum) in arid, semi-arid and coastal zones. Secondly a comparative study on the morphological and biochemical physiological level of the behavior of plants inoculated by mycrosymbiotes of two genotypes of durum wheat, soft wheat, barley and triticale in the presence or absence of water stress Under greenhouse conditions under controlled conditions. Trials were conducted for three years. These genotypes were inoculated with three Frankia Cci3 inocula, Azospirillum brasilense and mycorrhizal spores. After cultivation, the plants were harvested. The growth parameters were measured as well as the dry weight of the plant and roots and the chlorophyll and nitrogen levels.

Material and methods

Study was taken up in 10 cereal fields of the east part of Algeria, covering a semi-arid area of the high plateau Tellian, an arid area characterized by a cold winter and a very hot summer, and a littoral area characterized by a soft winter and a hot dry summer. The soil samples were collected from 10 different sites of the eastern part of the country of Triticum durum Desf. fields. All the soil samples were collected from a depth of 0-30 cm were taken in March 2015, they were sieved (2 mm mesh), homogenized, and air dried in the shade, they were put in plastic bags and stored in the refrigerator at 4°C until use for physico-chemical analysis of the soil properties and for the isolation and characterization of AMF spores.
**Physico-chemical analysis of the soil**

The granulometric characterization of the soil samples are determined by wet sieving technique (Afnor, 1990), the pH and electrical conductivity (EC) were measured on soil suspensions by a digital pH meter and a digital conductivity meter respectively, total nitrogen and total phosphorus content were determined using the Kjeldahl method (Rinaudo, 1970).

**Isolation and identification of AM spores**

The AM spores were isolated from 100 g of each soil sample by wet sieving and decanting technique of Gerdemann and Nicolson, 1963.

**Control of mycorrhization**

Mycorrhization check was performed to confirm the level of colonization of wheat roots by AMF. The seeds of *Triticum durum* Desf. Boussellem variety were inoculated with the spores isolated from durum wheat fields (5 morphotypes) and put in plastic pots in the greenhouse at a temperature of 25 ± 10 °C and relative humidity of 45 ± 15% for 3 months. The effect of mycorrhization is determined by the staining method described by (Vierheilig et al., 1998). Mycorrhizal structures were observed (magnification 100) and for each root system showing at least one point of infection (penetration of hyphae in the root) is considered as mycorrhized. This technique calculates 5 parameters of infection by the method of (Giovannetti&Mosse,1980) and MYCOCALC software.

**Plant material**

The plant material used in our study is constituted of: Two common wheat varieties: Hidhab, Ain Abid and two durum wheat varieties: Boussellem, Waha, two barley varieties Tichedrett, Saida, two triticale varieties LAMB2, FAHAD5. Supplied by ITGC (Institut Technique des Grandes Cultures) of El Khroub/Constantine, Algeria).

**Bacterial Material**

Pure cultures of bacteria were prepared using *Frankia Cci3* according to the method described by Diem (1983). The stumps of *Azosprillum brasileanse* were firstly grown in NFb N-free semi solid medium (Baldani and Dobereiner, 1980) for 48h at 30°C ; then a loop full of each culture was transferred separately to 100 ml NFb liquid medium After incubation, the cells were centrifuged for 10 min and washed twice with phosphate buffer pH 7. And we proceeded to mycorrhiza spores isolation from soil by wet sieving and sedimentation method "Wet sieving and Decanting."

**Implementation of the essay**

The essay was conducted under a greenhouse. After sterilization, seeds are germinated in Petri dishes on moisturized Wattman paper and then transplanted into plastic pots of 1.5 liters, filled with an
equivalent mixture of earth and sand at the rate of 2 seeds by pot. The inoculation of varieties cultivated under greenhouse, was made by pure cultures of bacteria. The experiment was realized in six repetitions for the statistical analysis. Later, plants were collected in order to compare their root system. The irrigation of plants was regularly made up to the field capacity, until water stress application.

**Studied parameters**

- **Leaves length**: Measures of leaf lengths have been taken to check the effect of bacterial strains and endomycorrhizae on the aerial part developments for both durum wheat and common wheat genotypes.
- **Root length**: After harvesting, measures were taken to see the effect of the bacterial strains and endomycorrhizae on root elongation.
- **Ear length**: For each variety, we measured ear length (beard not included).
- **Rate of total chlorophyll**: The rate of total chlorophyll was measured using a SPAD chlorophyll meter. Devise Calibration was performed by closing the vacuum clamp on itself. Then three test sockets are made on the sheet (top, middle, base) then the average value appears on the screen.
- **Nitrogen determination**: The Kjeldhal method was used to nitrogen determination as described by Rinaudo, (1970).
- **Plants dry weight**: Plants dry weight, expressed in grams, was determined after drying in an oven at 60°C for 96 hours.
- **Roots dry weight**: roots dry weight, expressed in grams, was determined as well after drying in an oven at 60°C for 96 hours.

**Statistical analysis**

The results are statistically interpreted by an analysis of variance (ANOVA) and principal component analysis (PCA) through the XLSTAT software (2014) using XLSTAT 2014 software. The Newman-Keuls test lists the averages with a threshold of 5% meaning.

**Results and discussion**

The results of the physico-chemical analysis of the soil presented show a clay loam, loam clay and loam sandy soil. Significant difference in all the parameters tested: pH, EC, saturation, phosphorus value except the nitrogen content was observed. The pH recorded in different areas was alkaline which might be due to the mother rock of the study area (Djebaili et al., 1984; Halitime et al., 1988). We notice that the total phosphorus levels are low which may be due to drought, erosion or plant uptake (Li et al., 2004; Urosite et al., 2006).
To describe and identify the diversity of AMF spores associated with cereal fields of the east part of Algeria, we used the wet sieving method, microscopic observations and keys of determination based on the color, shape and size as well as the different structures; arbuscules, vesicles, intracellular and extracellular hypha to compared our results to INVAM and BEG sites. Most of the explored sites which are characterized by the low rainfall show the presence of spores. Low mechanization practices that prevent the disruption of these cultivated areas are practiced which allow the development of AMF in the vicinity of the wheat fields. A variety of spores are found in Batna (11 spore/100g of soil) and Khenchela (12 spores/100g of soil) (Table 4), these two regions are present in the arid zone where he analysis of soil type of revealed a clay loam soil that is reported to supports the installation of Glomales populations (Dalpé, 1989; Abe et al., 1994; Bâ et al., 1996). The different mycorrhizal taxa identified on the basic of spores morphological and cellular characteristics belong to *Glomus*, *Acaulospora* and *Scutellospora* belonging to three families (Glomeraceae, Acaulosporaceae and Gigasporaceae) respectively.

In the coastal zone, only *Glomus* was observe, *Glomus* and *Scutellospora* were found in the arid zone and the three genera were observed in the semi-arid zone.In addition, AMF spores were found to be rare in the littoral zone (Annaba and Jijel). This could be explained by the high content of soil salts (EC 2mS/cm$^{-1}$). Thoen, 1987 have shown that salty soils are not favorable for the installation of endomycorrhizal symbioses.

The predominance of *Glomus* and *Acaulospora* in semi arid and arid soils is consistent with previous results obtained in Algeria by Fraga-Beddiar & Le Tacon (1990) on alder, Chafi & Fortas (1999) on truffles, Adjoud-Sadadou & Halli-Harrga, (2000) on eucalyptus, Hamza (2010) on watermelon and Neffar on figs (2012). Both *Acaulospora* and *Scutellospora* are less abundant and poorly represented compared to *Glomus* which might be due to the difficulty encountered in their observation and identification as they become sessile because of the fragility of their related structures (spore sacs and connecting hyphae).

Overall, we found in the 10 prospected regions, six species of yellow, brown, dark brown and orange brown color *Glomus*. These isolated spores are of globose, subglobose and ellipsoid shape, collected in the 100 and 250 µm fractions with a size ranging from (64 to 220 X 66.5 to 150 µm). In all the studied regions, the diversity of AMF was of a maximum 5 species found in the Batna region, which is characterized by a clay loam soil, 4 species of AMF were recorded in the region of Biskra characterized by a dry land. *Glomus* spores are among the most frequently found species. According to several authors, species of *Glomus* are distributed in different parts of the world and dominate the communities in arid, semi-arid and temperate zones (Li & Zhao, 2005; Gay et al., 2006; Uhlmann et
al., 2006). And according to Pande and Trafdar (2004), the AMF dominate the alkaline and neutral soils under various edaphic conditions.

The observation of inoculated wheat roots by the isolated spores in pots and under greenhouse conditions show colonization by AMF. These fungi form characteristic structures such as arbuscules, different shapes of vesicles, intra and intercellular hyphae and spores. Furthermore, the vesicles presence inside cortical tissues partly confirms the predominance of *Glomus* as they are the only kind that produces vesicles inside the host tissue (Smith & Read, 2008). A high intensity of the mycorrhizal colonization of the roots of durum wheat inoculated by the 1, 3 and 4 (*Glomus, Acaulospora*) morphotypes was observed. In addition, the arbuscule abundance (a) was higher in roots inoculated with the morphotype 1 (*Glomus*) with a 60% rate but a very low arbuscule abundance of the mycorrized fragments was noticed (13%). This might be due to the short life of arbuscules (1 to 3 weeks) which degenerate and will be replaced by hyphae and vesicles that persist until the death of the cell. Our results are similar to those of Saad (2009). Thus the level of colonization of the roots by the 1 and 3 (*Glomus*) morphotypes are most significant and would be interesting for the production of inoculums.

About the response of cereal species to water stress and inoculation depends both on the characteristics of the stress and the physiological, morphological and biochemical abilities of the plant. The measured parameters reflect a tolerance in the sense that the rate of nitrogen and chlorophyll, the length of the leaves, ear and roots, the dry weight of the plant and the roots would be significantly higher compared to the witnesses thus indicating a metabolic disturbance. When the plant is stressed, the level of chlorophyll decreases and slows down its growth activities. To resist water stress the plant adopts strategies by establishing symbiotic relationships with telluric microorganisms, such as PGPR and mycorrhizae. Indeed, the mycorrhizal symbiosis can have access to additional resources of water and mineral elements that are then transmitted to the plant at the root level. Therefore, mycorrhizal infection can increase the volume of soil explored through its mycelial network which is said to be responsible for the greater drought tolerance of mycorrhizal plants compared to non-mycorrhizal plants. At the end of this study, according to the species, two groups are distinguished: group (A) represented by the varieties (Hidhab, Ain Abid, Boussellem, Waha) group (B) represented by (Tichederett, Saida, Lamb2, Fahad5) respond differently to water constraints. The Hidhab, Boussellem, and Waha (subgroup A) varieties have the highest chlorophyll content with significant leaf length and dry root weight and the dry weight of the tall plant. Subgroup B) is represented by the varieties Saida, Tichderett and Lamb2 recording a high nitrogen level with a long ear length and root length. This difference for these traits probably results from a different genetic potential between the varieties studied, which is widely observed elsewhere (Shao et al., 2008,
Zerrad et al., 2008, Geravandi et al., 2011). The resistance of wheat species under water stress is better compared to other species. On the other hand, in the absence of water stress, the barley species seems to be however the most suitable compared to other species. Represented by the varieties Tichderett and Saida (subgroup C).

**Conclusion**

This study focused on the diversity of AMF and characterization of the isolated spores from the surveyed areas. The characterization of the spores revealed the predominance of *Glomus* (Glomeraceae) and the presence of the two genera *Acaulospora* and *Scutellospora*. Thus our work revealed the phenotypic description of the main spores found in the soil. It would be interesting to continue this research highlighting the diversity of Glomales in the east of Algeria extracting significant numbers of spores for systematic studies in order to reinforce the role of mycorrhizal symbiosis as a biological agent improving the growth of cereals in the arid and semi-arid areas, to produce adequate inoculums and to produce a bank of AMF.

About second study, it appears that the best tested inoculation treatments for durum and common wheat under both water regimes are the mycorrhiza and *Azospirillum brasilense* (PGPR bacteria). Our tests show that the inoculated varieties are more tolerant to water stress (Boussellem with mycorrhiza and Waha with *Azospirillum brasilense*). The mycorrhizal fungi are among the most important soil organisms to consider. The mycorrhizae are involved in mineral nutrition, absorption of water and protection against abiotic stresses. Thus mycorrhizae can contribute as an alternative to establish and develop adequate agriculture as the use of chemical fertilizers has reached their limits. Today, agriculture must move towards more sustainable practices.

**References**


The interactions between wheat and PGPR: a review

KECHID M, MAOUGAL R.T and DJEKOUN A

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team II Biotechnology and Plant Amelioration’, Department of Biology and Ecology, Faculty of Natural Sciences and Life, University of frères Mentouri Constantine. Algérie.

Email: maya.kechid@umc.edu.dz, rym.maougal@umc.edu.dz

Introduction

The rhizosphere is a thin zone of soil surrounding the root zone that is immensely influenced by the root system (Hartmann et al., 2008). The rhizosphere contained a variety of root associated bacteria commonly referred to as rhizobacteria, such beneficial rhizobacteria that positively influence plant growth are referred to as plant growth promoting rhizobacteria (PGPR), term created by Kloepper and co workers in the late 1970’s (Kloepper and Schroth, 1978). Plant growth promoting rhizobacteria (PGPR) are a multiple genera of soil bacteria that have been studied for their ability to promote plant growth, improve productivity, and development of plants, they remain associated for the major part of their life cycle (Saharan and Nehra, 2011; Pandey et al., 2012). (Chauhan, 2015)

The relationship of PGPR with the host may either be restricted to the rhizosphere (some colonize the rhizosphere, rhizoplane, superficial intercellular spaces or dead root cell layers) or endophytic (while some actually reside within apoplastic spaces inside the host plant with or without forming specialized structures such as nodules) (Vessey, 2003).

PGPR affect plant growth in two different ways, indirectly or directly or a combination of both. Independent of the mechanisms of vegetal growth promotion, PGPRs colonize the rhizosphere, the rhizoplane (root surface), or the root itself (within radicular tissues) (Gray and Smith, 2005). It is well established that only 1 to 2% of bacteria promote plant growth in the rhizosphere (Antoun and Kloepper, 2001).

The objective of this review article is to list the mechanisms of action of PGPR on wheat and describe some utilized successful examples of these rhizobacteria.

Plant responses to PGPR

PGPR improve plant growth by indirect or direct mechanisms although the difference between the two is not always distinct (Lugtenberg and Kamilova, 2009; Ashraf et al., 2013).

Direct mechanisms include the secretion of plant growth promoting metabolites like indole acetic acid (IAA), cytokinins, gibberellins, etc., and the stress regulating hormone 1-aminocyclopropane -1-
carboxylate (ACC) deaminase. Direct mechanisms also facilitating the improvement of nutrient availability to the plant by uptake of essential nutrients (N, P, Fe, Zn, etc.) from the atmospheric air and soil, production of iron chelating siderophores, organic matter mineralization and solubilization of insoluble phosphates.

The indirect promotion of plant growth occurs when PGPR lessens or prevents the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host (Cartieaux et al. 2003). Indirect promotion of the plant growth include inhibition of microorganisms that have a negative effect on the plant (by niche exclusion) viz. hydrolysis of molecules released by pathogens, synthesis of enzymes that hydrolyze fungal cell walls, synthesis of HCN, improvement of symbiotic relationships with rhizobia and mycorrhizal fungi, and insect pest control (Das et al., 2013).

**PGPR and WHEAT**

Many studies and reviews showed the effect of certain bacterial genus on the plants: A series of laboratory experiments with rhizobial inoculation of wheat conducted by Zahir et al. (2004) also demonstrated increases in root elongation (up to 20%), root dry weight (up to 13%), shoot elongation (up to 38%) and SDW (up to 36%). Azoarcus strain BH 72 colonized the interior of wheat (*Triticum aestivum*) (Wieland and Fendrik 1998). A few strains of Exiguobacterium are reported to increase the growth and nutrient uptake of wheat seedlings under glass house conditions (Selvakumar et al., 2010). *Paenibacillus* spp. are metabolically diverse and can inhabit different habitats such as soils, roots, and rhizosphere of various crop plants including wheat (*Triticum aestivum*), maize (*Zea mays*), sorghum (*Sorghum vulgare*), sugarcane (*Saccharum officinarum*) and barley (*Hordeum vulgare*) (von der Weid et al., 2000; Guemouri-Athmani et al., 2000; Ravi et al., 2007. Guemouri-Athmani et al. (2000) reported that the long-term cultivation of wheat in Algerian soils (>70 years) seems to modify rhizospheric populations of *P. polymyxa* by increasing their size, reducing their diversity, selecting a dominant genotype, and increasing the proportion of nitrogen fixers. (Chauhan, 2015). *Pantoea agglomerans* is a diazotroph, and is able to fix molecular N\(_2\) both in pure culture and in association with wheat (Merbach et al., 1998). Ruppel et al., (1992) reported *Pantoea agglomerans* to be the most effective bacterial strain associated with winter wheat. It was also able to colonize the rhizosphere and the phyllosphere of different cereals. It colonized the roots of wheat to a greater extent than those barley (*Hordeum vulgare*), whereas the colonization of shoots was higher in barley compared to wheat. In field experiments, inoculation with *P. agglomerans* led to an increase in the grain yield of different wheat cultivars (Verma et al., 2004) (Chauhan, 2015). A strain of *Pantoea dispersa* was able to positively influence and promote the growth and nutrient parameters of wheat under greenhouse conditions Selvakumar et al. (2008). Some strains such as *B. pumilus* 8N-4 can be used as a
biofertilizer to increase the wheat harvest (Hafeez et al., 2006). Inoculation of wheat with Rhizobium leguminosarum bv trifolii strains isolated from the rhizosphere of wheat plants grown in Morocco increases the dry weight of the leaves by 16 to 19% and the grain yield by 23 to 25%. (Hilali et al., 2001), Okon and Labandera-Gonzalez (1994) by studying data from 20 years of global work on inoculation of grasses with strains of Azospirillum sp. in different soils and environmental conditions, have concluded that these bacteria are able to increase the wheat harvest by 15 to 30%.

The inoculation of wheat with strains of *Pseudomonas cepacia*, *P. fluorescens* and *putida* induces resistance against the pathogens *Rhizoctonia solani* and *Leptosphaera maculans* (de Freitas and Germida, 1990).

**Production of phytohormones**

Among the direct growth promoting mechanisms we can cite the production of phytohormones such as auxins (indole acetic acid (iaa)), cytokinins, gibberellins and lowering of ethylene concentration (kloepper et al. 1989; glick et al. 1999). Bacterial production of phytohormones in the rhizosphere is known to be an important but poorly understood process. the appearance of stimulation and/or inhibition of plant development by phytohormone-like compounds has been shown especially for oil crops (George et al. 1989), tobacco tissue cultures (Helgeson, Leonard 1966), bean crop (Temple et al. 1989) and wheat (Zimmer et al. 1988a, b). (c. Scholz-Seidel and s. Ruppel 1992)

Cytokinin is known to promote seed germination, *de novo* bud formation and release of buds from apical dominance, stimulation of leaf expansion and reproductive development and retardation of senescence (Mok, 1994), some of which were reported in wheat by Lindberg and Granhall (1986) when inoculated with *P. polymyxa*.

*P. polymyxa* strains isolated from different proximities to wheat roots produced auxins and other indolic and phenolic compounds viz. indole-3- ethanol, indole-3- lactic, carboxylic and benzoic acid (Lebuhn et al., 1997). The effect of inoculation with *P. polymyxa* on growth parameters of wheat plants and the activities of enzymes present in the leaves of these plants such as glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase and glutathione S-transferase have been observed (Cakmakci et al., 2007).

It is generally well known and accepted that auxins (IAA) stimulates cell growth and elongation and auxin action may be regulated through the prolinelinked pentose-phosphate pathway through the regulation of phenolic synthesis for lignin and phenolic antioxidants (McCue et al., 2000)
Nutrition:

The PGPR can play a role as biofertilizers. The primary effect of PGPR is on the control of development which should lead to an increase in mineral nutrition to ensure the synthesis of additional biomass. PGPR could improve nutrition in macronutrients such as phosphorus and nitrogen, and also nutrition in trace elements such as iron.

Phosphorus

A phosphate solubilizing P. agglomerans was isolated by Kim (1997) from wheat rhizosphere. The bacterium was reported to strongly solubilize hydroxyapatite in culture medium (Kim et al., 1997). The effect of organic energy sources on the survival of P. agglomerans and soil microbial activity was studied by Kim et al. (1998) by introducing it into unsterilized soil containing 1% hydroxyapatite and either 1% glucose, phytic acid dodecasodium salt, glycerol-2-phosphate disodium salt, soluble starch or no addition (Chauhan, 2015).

Nitrogen

Several PGPR are diazotrophic. In addition, bacteria involved in symbiotic nitrogen fixation with legumes can stimulate the growth of non-legumes. Thus, Rhizobium growth stimulation has been reported for radish (Antoun et al., 1998) and rice (Mirza et al., 2006). The binding of N2 by rhizospheric bacteria, if it is quantitatively important, could lead to sufficiently enrich the rhizosphere in NH4 + ions to increase the nitrogen nutrition of the plant. Dal cortivo et al. (2017) demonstrate that the inoculation of wheat by Azospirillum spp., Azoarcus spp. and Azorhizobium spp. can increased the concentration of Nitrogen and grain yield, the same observation was demonstrated on wheat inoculated with Bacillus amyloliquefaciens GB03 (BamGB03), B. megaterium SNji (BmeSNji), and Azospirillum brasilense 65B, this strains are able to increase biomass and nutrient accumulation of N and P (Minh Luan Nguyen et al., 2018).

Siderophore

The iron is the fourth most abundant element in Earth (Ma, 2005), it is very little available for plant nutrition. It is nevertheless an essential trace element for plants where it acts as a cofactor for several enzymes. Sequestration of most of the soil iron in the insoluble form of iron hydroxide makes it a limiting factor in plant growth (Podile and Kishore, 2006). The activity of soil bacteria, and particularly PGPR, can improve plant iron nutrition by releasing siderophores (Whipps, 2001). Many studies confirm the ability of several plants to absorb the complex formed between a bacterial siderophore and Fe3 + ions, and that this process is vital in the mechanism of iron absorption by plants (Wang et al., 1993; Masalha et al., 2000). Several siderophore producing bacteria have shown their ability to enhance plant growth such as Bacillus megaterium on tea (Chakraborty et al., 2006).
The use of specific strains of Pseudomonas fluorescens and P. putida on different crops leads to improved growth and increased yield by solubilization of iron (Saharan and Nehra, 2011). Siderophores produced by rhizosphere bacteria may enhance plant growth by increasing the availability of Fe near the root or by inhibiting the colonization of roots by plant pathogens or other harmful bacteria. (B. Alexander* and D.A. Zuberer, 1990)

Références


PGPR, paranodules, growth stimulation and water deficit tolerance in durum wheat

(Triticum durum Desf.)

Benmati Mahbouba¹*, Le Roux Christine², Belbekri Nadir¹, Ykhlef Nadia¹ and Djekoun Abdelhamid¹

¹Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team II Biotechnology and Plant Amelioration’, Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria

²CIRAD, UMR LSTM, F-34398 Montpellier Cedex 5, France.

Email: benmati.m@gmail.com

Abstract

The development of new methods and approaches to improve the quality and the quantity of wheat production in Algeria is now a major issue and a national priority. One of the alternatives to the use of chemical fertilizers is the use of rhizosphere microorganisms as bio-fertilizer. The most recent research in this field emphasizes the use of PGPR inoculation as a sustainable organic alternative in the improvement of crop and agricultural production.

Our study consisted in the evaluation of the interaction between rhizosphere microorganisms (Azospirillum brasilense, Bacillus sp., And Frankia CcI3) previously isolated from soils of different regions of eastern Algeria, and two varieties of durum wheat (GTA / DUR and WAHA). The evaluation of the effect of PGPR in the formation of paranodules, root growth and the aerial part of durum wheat demonstrated a significant effect in nitrogen fixation and root growth compared to control conditions. In addition, the presence of bacteria in the formed paranodules has been clearly demonstrated.

Other studies have also been carried out on the same durum wheat varieties under water deficit condition for the evaluation of the capacities of these PGPR in the restoration of the growth and in the increase of the production. The obtained results confirm the significant abilities of PGPR under these stress conditions for maintaining growth and plant survival.

Keywords: PGPR, Durum wheat, Paranodules, Water deficit.

Introduction

Wheat, rice and maize are the most important cereals in the world. Of these three cereals, wheat is the most grown in the world. It provides more calories and protein in the diet than any other cereal. In Algeria, wheat is one of the most important crops; several hectares are used for its plantation.
Durum wheat (*Triticum durum* Desf.) is the first cereal grown in Algeria and occupies about 2 million hectares.

The wheat production in Algeria for the 2004/2005 season was around 2,600,000 t while the annual demand is well over 6,000,000 t. According to the latest figures given by the ITGC in 2011/2012, cereal production was 51.3 million quintals, of which more than 58% was durum wheat.

Most of the research work done on durum wheat has for many years had the main objective of increasing productivity, an approach based essentially on agronomic performance. In recent years, there has been a growing interest in studies that relate to the genetic improvement of water stress tolerance in wheat breeding programs.

However, domestic production does not meet the needs of the population given the low yield, which ranks Algeria as one of the most important grain importing countries. This is mainly due to soil degradation, which poses a threat to the long-term survival of agricultural production. In addition, the cultivation of wheat requires significant inputs of nitrogen fertilizer, which promotes the contamination of groundwater with nitrate.

Currently, several studies have used other methods to improve wheat production, including an approach based on the use of microorganisms mainly in programs to improve wheat production. These rhizobacteria can stimulate the development of the root system and nodulation and promote the mineral nutrition of the plant including the biological fixation of atmospheric nitrogen (Dilfusa and Gisela, 2002).

These so-called Plant Growth-Promoting Rhizobacteria (PGPR) rhizobacteria can reduce fertilizer doses, hence the importance of understanding the ability of wheat varieties to interact with PGPR and benefit from their roles (Niranjan et al. 2005).

Several studies have shown that PGPR have beneficial effects on plants, these rhizobacteria can provide the plant nitrogen as ammonium by their associations with wheat (Kennedy et al., 2006, Ficher et al., 2007). In addition, these PGPR can also synthesize growth hormones (phytohormones) to stimulate growth of the root and also the plant (Baca and Elmerich, 2007).

Many studies have shown that these rhizobacteria are involved in the biocontrol of the plant by reducing the deleterious effects of phytopathogens by synthesizing specific antibiotics (Fischer et al., 2009). On the other hand, Arzanech et al. (2011) showed the importance of *Azospirillum* spp. in the tolerance of soft wheat to moisture stress, as well as their ability to increase biomass and root growth.

With this in mind, the work carried out consisted in characterizing at the phenotypic and molecular level rhizobacteria to select from this one the most promising strain (*Azospirillum*
and to inoculate it with durum wheat in order to measure their capacity to act as a PGPR in order to meet the needs of increased production and tolerance to the moisture deficit of durum wheat.

At the end of this bibliographic synthesis, we could see that the PGPR are bacteria with multiple roles: On the one hand, these bacteria have classic, documented benefits for many years, these nitrogen-fixing bacteria par excellence are also involved in the synthesis of hormones and solubilization of phosphate and also in iron chelation.

PGPRs have proven to be beneficial bacteria for many processes: STIs and ISRs. Thus, since the discovery of their skills and their positive implication to acquire plants tolerance to environmental stresses. As a result, there are several questions regarding the roles played by PGPR in improving durum wheat growth. Knowing that many studies described above have placed the importance of interactions between PGPR and cereals at the center of their functions.

Nevertheless, the interactions between PGPR and wheat remain the least studied. Thus, in the case of durum wheat no high affinity study has been carried out. In the same way for the nodulation of durum wheat. Hence the need to develop these interactions to meet these needs.

The data set led us, during this thesis work, to focus on four areas of research targeting:

- The first line of research concerns the phenotypic and molecular characterization of PGPR. As this biological tool was lacking, it was necessary to develop it to continue the study on durum wheat. Thus the strains characterize as being *Azospirillum brasilense*, we tested them from a functional point of view to evaluate their beneficial effects by inoculation of durum wheat.

- The second line of research aims to characterize the interactions between *Azospirillum brasilense* and durum wheat and also Frankia CcI3 and durum wheat for the formation of root paranodules. These interactions should make it possible to nodulate durum wheat with or without induction of the growth hormone. For this purpose, a series of analyzes was carried out to characterize the nodulation and the role of nodules during nitrogen fixation, followed by a microscopic study for the localization of the bacteria *Azospirillum brasilense* inside the paranodules by the use of a fluorescent agent.

- The third line of research aims to highlight the role of PGPR to acquire durum wheat tolerance against the water deficit. Thus, several parameters have been considered to study the beneficial effect of these PGPR.
Results

1 - Phenotypic and molecular characterizations of rhizobacteria promoting plant growth isolated from the durum wheat rhizosphere (*Triticum durum* Desf.) In Algeria.

This part presents data on the phenotypic and molecular characterizations of PGPR isolated in Algeria. These rhizobacteria have demonstrated their ability to promote plant growth. Phenotypic characterization of rhizobacteria was performed by several Gram stain approaches which revealed that rhizobacteria were all Gram negative, then grown in several culture media with different concentrations of NaCl and showed that most of these bacteria have the ability to grow in saline environments with high concentrations (700 mM NaCl).

Then these cultures were cultured in media of different pH so an optimal pH of 6.8 was demonstrated. A study with several sugars to use as a source of carbon was carried out to see the possibility of growth of its strains with most of its sugars and therefore it was noticed that its isolated strains have a selectivity for the majority of sugars.

The study of PGPR properties shows that rhizobacteria behave like PGPR. Interestingly, these bacteria can synthesize AIA and ammonium but also solubilize phosphate. These properties therefore confer the characteristic of PGPR on these rhizobacteria.

The technique of 16S rDNA used allowed a molecular identification of these bacteria through the revelation of the gels which show that these strains all have a 16S rDNA of 680 bp which corresponds to the genus *Azospirillum*. Subsequently, the sequencing of these strains was able to confirm that these rhizobacteria are indeed *Azospirillum brasilense* with an identity rate of 98 to 100%. Finally, we tested the efficacy of *Azospirillum brasilense* on durum wheat growth by inoculation under controlled conditions, which showed an increase in root and air growth accompanied by a biological fixation of nitrogen. atmospheric measured by the Kjeldahl method.

2 - Effect of 2,4 Dichlorophenoxyacetate in the formation of root paranodules in durum wheat (*Triticum durum* Desf.) Inoculate with *Azospirillum brasilense* isolated in the rhizosphere of durum wheat in Algeria

The study in this section required the use of several physiological and biochemical techniques, thus confirming the biological fixation of nitrogen in durum wheat inoculated with *Azospirillum brasilense*.

Many tools have been used to locate *A. brasilense* in cereal roots (paranodules) (Gough et al., 1997, Nie et al., 1992). However, no durum wheat study has been conducted to induce paranodule formation.
This part of the thesis will be devoted to present the formation of these paranodules at the root level of durum wheat by the induction of 2,4-D. These paranodules were obtained by inoculating durum wheat with *A. brasilense*.

3 -Study of the formation of root paranodules in durum wheat (*Triticum durum* Desf.) Inoculate with Frankia Cc3 and treat with 2,4-D

After having demonstrated the ability of *Azospirillum brasilense* to induce root nodulation in durum wheat, it seemed interesting to carry out the same experiments using the actinomycete Frankia on durum wheat. Indeed, it appears to have important beneficial effects on trees and shrubs (Perrine-Walker et al., 2010). What is it really with durum wheat? And which of these strains between *Azospirillum brasilense* and Frankia will be the most effective? The determination of the presence of paranodules in this study consisted in checking their formation following the inoculation of durum wheat with actinomycete Frankia with or without a 2,4-D treatment and this through microscopic observation Roots.

4 -Effect of inoculation of durum wheat (*Triticum durum* Desf.) With PGPR (*Azospirillum brasilense*, *Bacillus* sp and Frankia CcI3) and tolerance to water deficit

This work concerns the effect of inoculation of durum wheat (*Triticum durum* Desf.) With PGPR (*Azospirillum brasilense*, *Bacillus* sp and Frankia CcI3) and tolerance to water deficit.

It involves the inoculation of two durum wheat varieties (GTA / DUR and WAHA) by the three PGPRs (*Azospirillum brasilense*, *Bacillus* sp and Frankia CcI3) and perform several physiological and biochemical parameters in order to determine the effect of rhizobacteria to the water deficit.

Although there are already documented results concerning the effects of *Azospirillum* on plant growth under water deficit conditions, but in the case of durum wheat there are few or no results that show it and so in this study we will test the effect of the strains *Azospirillum brasilense*, already identify and test for their beneficial effects on durum wheat development beforehand, on the growth of durum wheat under severe water stress.

For this purpose, the vegetative parameters including the water content and the effects on the growth of durum wheat inoculated and not inoculated under stress, were studied during this experiment. The objectives of this study were: (1) to evaluate the inoculation effects of durum wheat under stress and (2) to test if the inoculation of durum wheat with PGPR strains under stress effect can restore its growth.
General Discussion and Perspectives

The results obtained in this thesis concerning the population of rhizobacteria isolated from the same soil as the genus *Azospirillum* is the most dominant. Thus, many strains of this genus are able to grow in a saline environment with high concentrations of NaCl. These PGPRs can also synthesize AIA, ammonia and solubilize phosphate.

In addition, the molecular study allowed us to obtain a phylogenetic tree after alignment of the 16S rDNA gene sequences, so a cluster of the *A. brasilense* species is formed of the 6 isolated strains. In plants treated with 2,4D, a variation in the number of para-nodules per plant was found, these results are similar to those reported by Zeman et al., (1992).

This phenomenon of formation of para-nodules has been described in the literature (Sriskandarajah et al., 1993, Katupitiya et al., 1995, Fischer et al., 2009). These were found that in a medium containing 2.4D low concentration after wheat inoculation with *A. brasilense* Sp7 and an *A. brasilense* mutant Sp7.S which is deprived of polysaccharide synthesis, they observed that *A. brasilense* Sp7 colonizes all the surface of the roots, while treatment with the mutant strains shows a bacterial accumulation only on the lateral parts of the roots.

Inoculation with *Azospirillum brasilense* and actinomycete Frankia CcI3 with or without 2,4 D treatment, allowed the formation of root paranodules essential for nitrogen fixation. However, other studies on these interactions will promote the practical application of paranodules for improving nitrogen nutrition in cereals. In addition, intensification of microorganism plant interaction studies using labeled bacteria is required to locate these bacteria within the paranodules.

The positive results of this work, particularly those of the interaction between Frankia and wheat, open the door to several perspectives, such as the combination between Frankia, who has expressed great potential on root elongation, and a promoter rhizobacteria growth, and the exploitation of sandy soils with inoculation with PGPR as biofertilizing agents.

In addition, para-nodule formation through 2,4-D treatment is a physiological process independent of bacterial action. It is highly possible that the para-nodules obtained in our study without 2,4-D were induced by the Frankia effect, by comparing with the work of Saatovich (2006) who obtained para-nodules without adding phytohormones on the wheat roots inoculated with *Azospirillum* using two strains A1-3 and A13-6.

Another point was addressed in this work, it is the study of the tolerance and the increase of the yield of durum wheat, under greenhouse conditions, with the rhizobacteria and the actinomycete Frankia CcI3 in the face of stress, we have shown the importance of inoculation in the restoration of durum wheat. This could provide a solution to the water stress problem that is hitting the wheat crop in Algeria.


Dilfusa Egamberdiyeva and Gisela Höflich (2002). Root colonization and growth promotion of winter wheat and pea by Cellulomonas spp. at different temperatures.


Kennedy Ivan R., Lily L. Pereg-Grek, Graig Wood, Rosalind Deaker, Kate Glichrist and Sunietha Katupitiya (2006). Biological nitrogen fixation in non-leguminous field crops: facilitating the evolution of an effective association between Azospirillum and wheat. SUNFix Centre for Nitrogen Fixation, Department of Agricultural Chemistry and soil Science, University of Sydney, NSW, Australia.

Nie, Y; E, Vesk, M. Kennedy, et al., 1992. Structure of 2,4-D induced Paranodules with Rhizobium on wheat. Phytochemistry, no. 11, pp. 67–73.


The bioinformatic tools for the creation of wheat specific databases

HAMIDECHI Mohamed Abdelhafid and DJEKOUN Abdelhamid

Department of Applied Biology. Faculty of Nature and Life Sciences, University Frères Mentouri Constantine. Algeria
Email: Hamidechi.abdelhafid@umc.edu.dz

Introduction

In 1995, the genomes of the first two bacteria, *Haemophilus influenzae* and *Mycoplasma genitalium*, were reported (Fleischmann *et al.*, 1995; Fraser *et al.*, 1995). By the beginning of 2000, genomes of 23 different unicellular organisms (5 archaeal, 17 bacterial, and 1 eukaryotic) had been completely sequenced. Updated lists of both finished and unfinished publicly funded genome sequencing projects are available in the GenBank Entrez Genomes division and at the site maintained by The Institute for Genome Research (TIGR) and at Integrated Genomics. A complete list of sequencing centers world-wide can be found at the NHGRI Web site (e.g. The Whitehead Institute/MIT Center for Genome Research, Genome Sequencing Center, The Wellcome Trust Sanger Institute).

Seven years later, Johns Hopkins scientists\(^1\) report they have successfully used two separate gene technologies to assemble the most complete genome sequence to date of *Triticum aestivum* (Aleksey V Zimin *et al.*, 2017). The newly assembled bread wheat genome, took a year for the Johns Hopkins researchers to assemble 1.5 trillion bases of raw data into a final assembly of 15.34 billion base pairs.

Sequencing a genome of this size requires not only genetic expertise, but also very large computing resources (*Bioinformatics*) available at relatively few research institutions around the world.

The project relied heavily on the Maryland Advanced Research Computing Center, a computing center shared by Hopkins and the University of Maryland, which has over 20,000 computer cores (CPUs) and over 20 petabytes of data storage. It used approximately 100 CPU years to put this genome together.

Because the World Wide Web makes genome sequences available to anyone with Internet access, there exists a variety of databases that offer more or less convenient access to basically the same sequence data. However, several research groups, specializing in genome analysis, maintain

---

\(^1\) November 20, 2017, Johns Hopkins University School of Medicine
databases that provide important additional information, such as operon organization, functional predictions, three-dimensional structure, and metabolic reconstructions.

In addition to general genomics databases, there exists a variety of databases that center around a particular organism or a group of organisms.

With the spectacular advancement of bioinformatics and sequencing, we are witnessing the birth of more and more specific databases and research projects relating to plants and wheat specifically.

For example, **PRGdb**\(^2\) is a web accessible open-source (http://www.prgdb.org) database that represents the first bioinformatic resource providing a comprehensive overview of resistance genes (R-genes) in plants. PRGdb holds more than 16 000 known and putative R-genes belonging to 192 plant species challenged by 115 different pathogens and linked with useful biological information. The complete database includes a set of 73 manually curated reference R-genes, 6308 putative R-genes collected from NCBI and 10463 computationally predicted putative R-genes.

In order to manage all the genomic data and integrate them in order to make them accessible and even exploitable to the general user public and alongside the generalist databases, multi-species, redundant and variable quality databases, a large number of databases specialized in the genomes of cereals are developed:

1- **GENOSCOPE Project:**

In one of its activities, Genoscope undertakes the comparative sequencing of regions carrying genes and sequences of interest that have played a key role in the domestication of. About 100 BAC clones with an average size of 130 kb will be needed for this project (6 BAC clones per region), which leads to a sequencing volume of about 20,000 reads.

The project aims to obtain about 300 polymorphic markers for each of the three species (*Triticum urartu, Triticum speltoides* and *Triticum tauschii*) ancestral of *Triticum aestivum*. This number of markers, added to those already available, will allow coverage of about one polymorphic marker every 10 cM on average.

2- **ANET-GB Plateforme of Agroscope:**

The aim is to provide Agroscope's projects with the necessary infrastructure for data processing and to optimally coordinate the planning, execution and analysis of data in the context of collaborative projects.

---

The ANET-GB platform is creating new key skills in the field of Next Generation Sequencing (NGS) data analysis, especially with respect to genome and metagenome sequencing data as well as to transcriptome. The access to software solutions for the analysis of NGS data (eg CLCBio, Geneious) will be made using centralized servers (Molecular Diagnostics, Genomics and Bioinformatics Group).

I- WHEAT SPECIFIC DATABASES AND GENOMES PROJECTS

I- The Bristol Wheat Genomics site is divided, according to the target audience, into three distinct areas

The CerealsDB consists in three axes:

1. SNPs Databases: whose information is divided by platform: Axiom® 820K and 35K SNP Arrays; iSelect Array; KASP probes and TaqMan® Probes (In an ongoing effort to support wheat breeders and the wheat research community, CerealsDB partnered with Life Technologies to develop a collection of 4,800 TaqMan® SNP assays for variety markers in the wheat genome).

2. Draft Genome Assembly: A BLAST search on a wheat genome project based on 5x coverage of 454 reads (sequences) can be performed. Alternatively, the 454 raw reads can be searched using the same link.

3. Downloadable genome sequence reads: Over 85 Gigabases of 454 genomic sequences (equivalent to 5x coverage) can be downloaded.

**Figure 1:** nX coverage of four reads.
History of the database:

2003 - 2008: Originally built by Gary Barker, to store a set of 26,382 EST sequences.

2008 - 2013: The database expands to become an online resource, hosting a wide range of genomic data related to *Triticum estivum*. A developed platform containing DArT markers in addition to EST sequences. The database contains 11 SNP tables and one contig table with 111,442 records.

2014 - present: During Dec2013-Jan2014, the website of the database has been moved to a new server with 32 cores and 256 Gb of RAM. Major expansion of the database with the addition of iSelect and Axiom SNP data. The following table shows the iSelect SNPs of *Triticum estivum* mapped for each of the 21 chromosomes for genotypes A, B, and D:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2306 (1974)</td>
<td>2059 (2224)</td>
<td>1920 (2045)</td>
<td>1769 (2117)</td>
<td>2556 (2045)</td>
<td>2135 (1779)</td>
<td>2597 (2010)</td>
<td>15342 (14194)</td>
</tr>
<tr>
<td>B</td>
<td>3697 (2099)</td>
<td>5865 (2294)</td>
<td>2548 (2455)</td>
<td>1438 (2028)</td>
<td>2862 (2152)</td>
<td>2438 (2241)</td>
<td>2318 (2224)</td>
<td>21166 (15493)</td>
</tr>
<tr>
<td>D</td>
<td>553 (1494)</td>
<td>1310 (1797)</td>
<td>272 (1903)</td>
<td>320 (1601)</td>
<td>1056 (1850)</td>
<td>511 (1565)</td>
<td>1174 (1797)</td>
<td>5196 (12007)</td>
</tr>
<tr>
<td>Total</td>
<td>6556</td>
<td>9234</td>
<td>4740</td>
<td>3527</td>
<td>6474</td>
<td>5084</td>
<td>6089</td>
<td>41704</td>
</tr>
</tbody>
</table>

II- The database Wheat Genetics, Diversity and Genome Biology

The project uses multi-omic approaches, bioinformatics and functional genomics, to understand the genetic control of important agronomic traits of wheat and its wild relatives. The projects conducted on this database are:

- Genomics and genetics of wheat adaptation to diverse environments
- Genetics and systems biology of wheat-pathogen interaction
- Genomic resources and assays
- Wheat genome editing using CRISPR/CAS9
- Wheat nested association mapping population:
Example, the link TableS11 provides the genetic maps (file size excel 56.5 MB).
The database has links to some projects:

1. http://wheatgenomics.plantpath.ksu.edu/snp/

2. http://wheatgenomics.plantpath.ksu.edu/hapmap/

Lines whose total exome has been sequenced (WEC: whole exome capture) are presented in the form of a dendrogram built according to the NJ algorithm using a R ape package. V3.0.6 and showing the place of three Algerian varieties.
III- Oxford Journals Databases publishes plant-specific databases in its "NAR Database Summary Paper Category List" section:

- Chloroplast Genome Database (http://chloroplast.cbio.psu.edu/)

- GRAMENE

- PRGdb

1- For the Chloroplast Genome Database project, work in developmental genetics and genomics has identified more than 100 genes that play a specific role in the development of Angiosperms (including cereals) and other model organisms. This database is the result of a multi-institutional, multi-collaborator collaboration, funded through NSF's Plant Genome Research Project (National Science Fundation); it was launched to address the issues of evolutionary genetics by studying the evolutionary diversification of genes and regulatory pathways in major plant lineages.

The CREST Project: Center for Research Excellence in Bioinformatics and Computational Biology. New Mexico State University (NMSU) has created a Center for Bioinformatics and Computational Biology (BCB). The center combines the complementary skills of a team of researchers in computer science, biology, chemistry and agriculture. Its research mission is to develop computer models
integrating the use of multiple and complex genomic data sources to improve the understanding of biological systems. The center pursues three areas of research:

(1) development of computer models and tools for extracting knowledge from complex biological data sources;

(2) development of new computer methods to improve the prediction of protein structure;

(3) build computer tools to improve the determination of genomically important parts and understand the various biological mechanisms.

2- Gramene: Three main components make up this database:

i- Genome Assembly (IWGSC);

ii- Comparative genomics;

iii- Regulation.

The Details about each chromosome of *Triticum aestivum* are provided by simple click:

IV- Groups and Individual Bioinformatic databases projects

In his thesis project *Creation of an integrated application for the management and analysis of proteomic data*, BOUTTES C. (2005), takes part of the development of one of the modules of the GpiIS information system (Genoplante-info Information system). The great features planned for the application of ProteomIs / GnpProt are:
A second version of ProteomIs / GnpProt was delivered on December 12, 2004 to Génomplante. In this version, the novelties mainly concerned technical complements allowing in particular to improve the scalability of the application:

**Table 1:** GANTT chart of tasks / achievements and distribution of human resources around the ProteomIs / GnpProt project

<table>
<thead>
<tr>
<th>Tasks / achievement</th>
<th>Apr</th>
<th>May</th>
<th>June</th>
<th>Jul</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automatic waking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specifications/Analyze</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Design and Implementation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interface Model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exchange Format</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data import script</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gels Visualization Interface</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conception of the bioinformatic analysis of the data

The main tasks of this project of creation of bioinformatics databases are:

- creating non-redundant groups of sequences (Clustering, Perl)
- Sequences Alignment (BLAST Interface)
- Pattern search (MSDiest and NetPhos).
REFERENCES


Genetic Diversity of High and Low Molecular Weight Glutenin Subunits in Saharan Bread and Durum Wheats from Algerian Oases

Ines BELLIL, Mohammed CHEKARA BOUZIANI and Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: ies.bellil@umc.edu.dz

Abstract

Saharan wheats have been studied particularly from a botanical viewpoint. Genotypic identification, classification and genetic diversity studies to date were essentially based on the morphology of the spike and grain. For this, the allelic variation at the glutenin loci was studied in a set of Saharan bread and durum wheats from Algerian oases where this crop has been traditionally cultivated. The high molecular weight and low molecular weight glutenin subunit composition of 40 Saharan bread and 30 durum wheats was determined by SDS-PAGE. In Saharan bread wheats 32 alleles at the six glutenin loci were detected, which in combination resulted in 36 different patterns including 17 for HMW and 23 for LMW glutenin subunits. For the Saharan durum wheats, 29 different alleles were identified for the five glutenin loci studied. Altogether, 29 glutenin patterns were detected, including 13 for HMW-GS and 20 for LMW-GS. Three new alleles were found in Saharan wheats, two in durum wheat at the Glu-B1 and Glu-B3 loci, and one in bread wheat at the Glu-B1 locus. The mean indices of genetic variation at the six loci in bread wheat and at the five loci in durum wheat were 0.59 and 0.63, respectively, showing that Saharan wheats were more diverse. This information could be useful to select Saharan varieties with improved quality and also as a source of genes to develop new lines when breeding for quality.

Keywords: allelic variation; genetic diversity; glutenin subunits; polymorphism; Saharan wheats.
Genetic Variation and Geographical Diversity for Seed Storage Proteins of Seventeen Durum Wheat Populations Collected in Algeria

Wahiba HAMDI, Ines BELLIL, Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Abstract

Variation in the HMW and LMW glutenin subunits of a collection of 856 accessions of botanic durum wheat collected in Algeria and divided a priori according to their agronomical and morphological traits in 17 populations were analyzed using SDS-PAGE. Among the 16 alleles identified at the Glu-1 loci, two were new. The first named Glu-B1i1 encoding for two bands located between 17 and 18 which was assigned the nomenclature 17i and 18i. The other named Glu-B1e1 codes two bands similar to 20x and 20y but with faster mobility, which were named 20x1-20y1. At Glu-3 and Glu-2 loci, 19 alleles were identified, where the allele named Glu-B3ab (encoding for subunits 2-8-9-13-16) was considered as new. Global genetic diversity index (H) was relatively high (0.34). A final core collection of 21 accessions was selected. All the different geographical areas and the allelic diversity at the Glu-1, Glu 2 and Glu-3 polymorphic loci were represented in this core which represents a minimum of wheat Algerian genetic resources that could be used for dedicated breeding programs.

Keywords: Algerian botanic durum wheat, core collection, glutenin subunits, SDS-PAGE, variability
Allelic Variation in Glu-1 and Glu-3 Loci of Bread Wheat (Triticum aestivum ssp. aestivum L. em. Thell.) Germplasm Cultivated in Algeria

Ines BELLIL, Ouahiba HAMDI and Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: ies.bellil@umc.edu.dz

Abstract

Wheat endosperm storage proteins are the major components of gluten. They play an important role in dough properties and in bread making quality in various wheat varieties. In the present study, the different alleles encoded at the 6 glutenin loci were identified from a set of 71 hexaploid wheat germplasm cultivated in Algeria using SDS-PAGE. AtGlu-A1, Glu-B1 and Glu-D1, encoding high molecular weight glutenin subunits (HMW-GS), 3, 6 and 5 alleles were observed, respectively. Low molecular weight glutenin subunits (LMW-GS) displayed similar polymorphism, as 4, 9 and 3 alleles were identified at loci Glu-A3, Glu-B3 and Glu-D3, respectively. A total of 52 patterns resulted from the genetic combination of the alleles encoding at the six glutenin loci. This led to a significantly higher Nei coefficient of genetic variation in Glu-1and Glu-3 loci (0.54). The Algerian hexaploid wheats exhibited allelic variation in HMW and LMW glutenin subunit composition and the variation differed from that of hexaploid wheats of other countries. The presence of high quality alleles in glutenin loci have led the Algerian wheat cultivars to be considered as an asset in breeding programs aimed for wheat quality.

Key words: allelic variation, genetic diversity, glutenin subunits, polymorphism, Triticum Aestivum.
Diversity of five glutenin loci within durum wheat (*Triticum turgidum* L. ssp. Durum (Desf.) Husn.) germplasm grown in Algeria

Ines BELLIL, Ouahiba HAMDI and Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: ies.bellil@umc.edu.dz

Abstract

The HMW and B-LMW glutenin subunits composition of 120 durum wheat germplasm grown in Algeria was examined using SDS-PAGE. All together, 39 glutenin patterns were detected, including eight for HMW and 21 for B-LMW glutenin subunits. Twenty-six different alleles were identified for the five glutenin loci studied, that is, Glu-A1(3), Glu-B1 (7), Glu-A3(5), Glu-B3(9) and Glu-B2(2). Two new alleles were found at Glu-B3 locus: Glu-B3new1 encodes for five subunits (7 +8+14+16+18) and Glu-B3new2 codes for five subunits (4 +6*+12+15+15*), of which subunit 15*with mobility between bands 15–16 was not described previously. At theGlu-1loci, the Glu-A1c/Glu-B1e allelic composition was predominant. For the B-LMW glutenins, the most common allelic composition wasGlu-A3a/Glu-B3a/ Glu-B2a. The collection analysed shows glutenin alleles and allele com-binations related to high gluten strength. This information could be use-ful to select varieties with improved quality and also as a source of genes to develop new lines when breeding for quality.

Key words: durum wheat, allelic composition, glutenins, polymorphism.
The genetic potential of a germplasm of interspecific crosses between durum wheats
(\textit{Triticum turgidum} L. ssp. \textit{durum} (Desf.) Husn.) and their relatives (\textit{T. dicoccum}
Sch\"{u}bl. And \textit{T. polonicum} L. ) in five glutenin loci

\textbf{BELLIL Ines, HAMDI Ouahiba, BENBELKACEM Abdelkader, KHELIFI Douadi}

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri
Constantine University, Constantine, Algeria.

\textit{Email: ies.bellil@umc.edu.dz}

\textbf{Abstract}

Wheat endosperm storage proteins are the major components of gluten. They play an important role in dough properties and in bread making quality in various wheat varieties. In the present study, the different alleles encoded at the 5 glutenin loci were identified from a set of 38 tetraploid wheat germplasm obtained from interspecific crosses between durum wheats (\textit{Triticum turgidum} L. ssp. \textit{durum} (Desf.) Husn.) and their relatives (\textit{T. dicoccum} Sch\"{u}bl. And \textit{T. polonicum} L. ) using SDS-PAGE. At \textit{Glu-A1} and \textit{Glu-B1}, encoding high molecular weight glutenin subunits (HMW-GS), 2 and 4 alleles were observed, respectively. Low molecular weight glutenin subunits (LMW-GS) displayed similar polymorphism, as 3, 5 and 3 alleles were identified at loci \textit{Glu-A3}, \textit{Glu-B3} and \textit{Glu-B2}, respectively. One new allele was detected at \textit{Glu-B3} locus and appeared in nine accessions obtained from five crosses. This allele codes for five subunits (2 + 8 + 9 + 13 + 18), encoded by the \textit{Glu-B3b} without subunit 16 plus subunits 2 and 18. A total of 38 patterns resulted from the genetic combination of the alleles encoding at the five glutenin loci. This led to a significantly higher Nei coefficient of genetic variation in \textit{Glu-1}, \textit{Glu-3} and \textit{Glu-B2} loci (0.54). The germplasm analyzed exhibited allelic variation in HMW and LMW glutenin subunit composition and the variation differed from that of tetraploid wheats of other countries. The presence of high quality alleles in glutenin loci have led the accessions to be considered as an asset in breeding programs aimed for wheat quality.

\textbf{Keywords:} genetic diversity, allelic variation, glutenin subunits, polymorphism, durum wheats and relatives, interspecific crosses.
Diversity of seven glutenin and secalin loci within triticale cultivars grown in Europe

Nardjes Amiour and Douadi Khelifi

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri
Constantine University, Constantine, Algeria.

Abstract

Analysis by SDS-PAGE of the majority of hexapoid triticales (×Triticosecale) (134 cultivars) grown in Europe allowed to identify 40 alleles at seven loci: Glu-A1, Glu-B1, Glu-R1, Gli-R2, Glu-B2, Glu-A3 and Glu-B3. Glu-B1 and Glu-B3 loci were the most polymorphic with 9 alleles at each locus. 95 allelic combinations were observed including 71 specific for one cultivar each. On the basis of allelic frequencies at the seven loci, genetic distances between hexaploid triticale grouped according to their origins revealed two clusters: winter triticale mostly originating from European germplasm and spring triticale essentially of CIMMYT origin. Comparison of allele frequencies between hexaploid triticale cultivars and a world collection of bread (Triticum aestivum) and durum (Triticum durum) wheat was investigated at Glu-A1 and Glu-B1: only a significant association was found for Glu-A1 alleles (χ² = 2.26, p=0.36) between triticale and bread wheat.

Key words: polymorphism, rye, storage proteins, triticale, wheat
Allelic variation of HMW and LMW glutenin subunits, HMW secalin subunits and 75K gamma-secalins of hexaploid triticale

Nardjes Amiour and Douadi Khelifi

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Abstract
Although the endosperm storage protein of hexaploid triticale have already been analysed, the allelic diversity of glutenins and secalins remains to be described. Analysis, by SDS-PAGE, of about one thousand F2 seeds from ten triticale crosses allowed the inheritance of these storage proteins to be studied in order to determine their allelic forms and to assign them to particular chromosomes. Two new alleles encoding HMW subunits of glutenin and five new alleles encoding HMW subunits of secalin were determined at Glu-B1 and Glu-R1 loci respectively. In addition to the three allelic forms of 75K gamma-secalins encoded at Gli-R2 and previously reported, a null form was found. A nomenclature for these proteins and their corresponding alleles was suggested. The composition of B-LMW glutenin subunits of hexaploid triticale was described and allelic forms were determined: Five alleles were encoded at Glu-A3 and seven at Glu-B3 including one and two new allelic forms respectively.

Key words: polymorphism, rye, storage proteins, triticale, wheat.
Diversity of Seven Glutenin and Secalin Loci within Triticale Cultivars grown in France

Ines BELLIL, Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: ies.bellil@umc.edu.dz

Abstract

Although the endosperm storage protein of hexaploid triticale have already been analysed, the allelic diversity of glutenins and secalins remains to be described. Analysis by SDS-PAGE of the majority of hexaploid triticales (69 cultivars) grown in France allowed to identify 36 alleles at seven loci: Glu-A1, Glu-B1, Glu-R1, Gli-R2, Glu-B2, Glu-A3and Glu-B3. Glu-B1and Glu-B3loci were the most polymorphic with 9 alleles each. On the basis of allelic frequencies at the seven loci, genetic distances between hexaploid triticales grouped according to their origins revealed two groups: winter triticales mostly originating from European germplasm and spring triticales essentially of CIMMYT origin. Comparison of allele frequencies between hexaploid triticale cultivars and a world collection of bread (Triticum aestivum) and durum (Triticum durum) wheat was investigated at Glu-A1 and Glu B1: only a significant association was found for Glu-A1 alleles ($\chi^2= 2.36$, p=0.26) between triticale and bread wheat.

Keywords: polymorphism, rye, storage proteins, triticale, wheat.
Evaluation of two diploid Aegilops species variation in terms of HMW, LMW glutenin subunits and gliadins

MEDOURI Asma and KHELIFI Douadi

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: asmabioch@yahoo.fr

Abstract

A collection of 31 accessions of Aegilops comosa (14) and Aegilops umbellulata (17) from different origins was examined for high molecular weight glutenin subunits (HMW-GS), low molecular weight glutenin subunits (LMW-GS) and gliadin composition. For A. comosa, results showed an extensive polymorphism; eight alleles were detected at the Glu-M1 locus including 5 new alleles and 14 alleles at the Glu-M3 locus. For gliadins, a high genetic diversity index (0.93) was found for both Gli-M1 and Gli-M2 loci. In A. umbellulata, lower but important variation was found; 3 alleles including a new one were counted for Glu-U1 locus, 7 alleles at Glu-U3 locus, a high variation was determined for gliadins; H=0.92 and H=0.90 for Gli-U1 and Gli-U2 respectively. Altogether, variation found in A. comosa was slightly higher than A. umbellulata for all the loci studied, furthermore, gliadins variation was the greatest, followed by LMW then HMW glutenin subunits. The important storage proteins variation found in this work suggests their possible utilization in breeding for wheat quality.

Key words: Aegilops comosa, Aegilops umbellulata, storage proteins, loci, patterns; polymorphism
Polymorphism at High Molecular Weight Glutenin Subunits and Morphological Diversity of Aegilops geniculata Roth Collected in Algeria

Asma MEDOURI, Ines BELLIL and Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: asmabioch@yahoo.fr

Abstract

A collection of 35 accessions of the tetraploid wild wheat Aegilops geniculata Roth (MM, UU) sampled in northern Algeria was evaluated for morphological and biochemical variability. Morphological and ecological analyses based on morphological traits and bioclimatic parameters, respectively, were assessed using principal component analysis (PCA). Accessions were differentiated by width characters, namely spike’s width, and a weak relationship between morphological traits and ecological parameters was found. Polymorphism of high molecular weight (HMW) glutenin subunits was carried on by sodium dodecyl sulphate-poly-acrylamide gel electrophoresis (SDS-PAGE). Among accessions analyzed, 27 alleles were identified at the two loci Glu-M1 and Glu-U1: resulting in twenty-nine patterns and a nomenclature was proposed. Two alleles at the Glu-U1 locus expressed a new subunit with a slightly slower mobility than subunit 8. These results provide new information regarding the genetic variability of HMW glutenin subunits, as well as their usefulness in cultivated wheat quality improvement.

Keywords: Aegilops geniculata, Algeria, morphology, HMW glutenin subunits
Genetic Diversity of High and Low Molecular Weight Glutenin Subunits in Algerian
*Aegilops geniculata*

Asma MEDOURI, Inès BELLIL, Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri
Constantine University, Constantine, Algeria.

Email: asmabioch@yahoo.fr

Abstract

*Aegilops geniculata* Roth is an annual grass relative to cultivated wheat and is widely distributed in
North Algeria. Endosperm storage proteins of wheat and its relatives, namely glutenins and gliadins,
play an important role in dough properties and bread making quality. In the present study, the different
alleles encoded at the four glutenin loci (Glu-M1, Glu-U1, Glu-M3 and Glu-U3) were identified from
thirty five accessions of the tetraploid wild wheat *A. geniculata* collected in Algeria using Sodium
dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE). At Glu-M1 and Glu-U1 loci,
encoding high molecular weight glutenin subunits (HMW-GS) or A-subunits, 15 and 12 alleles were
observed respectively, including one new subunit. B-Low molecular weight glutenin subunits zone
(B-LMW-GS) displayed a far greater variation, as 28 and 25 alleles were identified at loci Glu-M3 and
Glu-U3 respectively. Thirty two subunits patterns were revealed at the C subunits- zone and a total of
thirty four patterns resulted from the genetic combination of the two zones (B- and C-zone). The wide
range of glutenin subunits variation (high molecular weight glutenin subunits and low molecular
weight glutenin subunits) in this species has the potential to enhance the genetic variability for
improving the quality of wheat.

Keywords: alleles, electrophoresis, goatgrass, glutenins, polymorphism
Abstract

The gliadins of the wild wheat Aegilops geniculata represent a valuable gene pool in breeding for bread making quality. The genetic diversity of gliadins in A. geniculata was studied among 36 of its accessions, collected in the north of Algeria, using acid polyacrylamide-gel electrophoresis (Acid-PAGE). In total, sixty-one polymorphic bands and 35 gliadin patterns were identified. Twenty-eight different bands and 34 patterns were found in the ω-gliadin region, 13 polymorphic bands and 33 patterns for γ-gliadins, 12 bands and 34 different patterns for β-gliadins and eight bands in combination resulted in 25 different patterns in the α-gliadin zone. Thirty-five patterns were found for each of the Gli-1(γ/ω region) and Gli-2(α/β region) loci. The genetic diversity index (H) was higher for ω-gliadins (0.968), followed by γ- and β-gliadins (0.964 and 0.961, respectively), and the lowest value was detected in α gliadin patterns (0.944). Cluster analysis based on Ward’s method divided the analysed collection into five separated groups in which genetic diversity did not follow the geographical distribution. The polymorphism observed in the electrophoretic patterns highlights close correlations between bioclimatic features and some ω-gliadin proteins.

Keywords: Acid-PAGE; gliadin patterns; polymorphism; wild wheat
Genetic diversity of *Aegilops geniculata* Roth from Algeria as revealed by RAPD and morphological markers

Asma MEDOURI, Mohammed CHEKARA BOUZIANI and Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: asmabioch@yahoo.fr

Abstract

Random amplified polymorphic DNA (RAPD) and morphological markers were used to estimate genetic variability and relationship between 34 accessions of *A. geniculata* Roth collected in Algeria. In the RAPD analysis, seven selected decamer primers yielded 145 bands ranging from 112 to 2513 bp. Based on the percentage of polymorphism (79.31%) and the dissimilarity coefficient of Pearson (1-r) which ranked from 0.25 to 0.91, high genetic diversity was detected at the species level. In parallel, the study of fifteen morphological traits related to spike, spikelet, glume, lemma and awns have shown that Euclidean distances ranged between 0.92 and 6.41 indicating the high intraspecific morphological variation. Cluster method of RAPD separated the accessions into four groups whereas the collection was divided into three clusters using morphological characters. There was no significant correlation between RAPD and morphology. RAPD was not related to evident geographic origin however this latter was significantly associated with morphology. This study shows that data derived from RAPD markers and morphological traits work in different way to determine the relationships among accessions nonetheless they could be useful for breeding.

Key words: *A. geniculata* - RAPD - Morphology - Polymorphism – Relationships.
Assessment of the allergenic potential of wheat and identification of some allergens in the soluble fraction

Ines BELLIL, Douadi KHELIFI

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Email: ies.bellil@umc.edu.dz

Abstract

Wheat is indisputably an important part of the daily diet of millions of people. Unfortunately, this popular and cultivated crop is also one of the six major foods involved in food allergy. The aims of this work was to characterize the major allergens of wheat and to break down the specific IgE response to evaluate the role of different proteins present in the soluble fraction (albumins/globulins) involved in wheat allergies. The analysis included 10 varieties and 14 sera from allergic Algerian and French patients to wheat. Reactivity of IgE towards albumins/globulins extracts from these varieties was analyzed by immunoblotting after SDS/PAGE separation. The results showed that IgE of all sera recognize more than one protein whatever the observed clinical class. Behavioral differences in terms of response intensity were observed with respect to the origin of patients allergic to wheat, it was significantly greater for French patients. The soluble fraction can be described as a major allergen. The results allow observing that the immunoreactivity varies with genotypes. Several protein bands recognized by IgE from allergic patients were observed, which shows a large number of potential allergens in this fraction. Four protein bands were identified by LC-MS/MS, thirteen wheat allergens were found in the four bands analyzed. The approaches used allowed to increase the knowledge on proteins involved in wheat allergies especially those present in albumins/globulins fraction, that have been less intensively studied with respect to the insoluble gluten proteins.

Key words: wheat allergy, IgE antibodies, allergens, immunoblotting, ELISA, proteomic analysis
Enzymatic degradation of gliadin by nigella sativa seeds protease: implications for new treatment of celiac disease

Bellir Nousseiba, Bellil Ines, Khelifif Douadi et Rouabah Leila

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team III Genetics Biochemistry and Proteomic’
Department of Biochemistry and Molecular and Cellular biology, Faculty of Natural Sciences and Life, Frères Mentouri Constantine University, Constantine, Algeria.

Abstract

The protease was extracted from Nigella sativa seeds with 0.1 M citrate/phosphate buffer (pH 7.5), the crude enzyme extract showed maximum protease activity at pH 1.5, and optimal temperature at 50°C. After the partially purification of enzyme and analyses of RP-HPLC and SDS-PAGE results it appeared that Nigella sativa seeds protease degrade Triticum aestivum gliadin more efficiently than Triticum durum gliadin after 24h of incubation. The activity of Nigella sativa seeds protease with gliadin as substrate, in pH 7.5 at 37°C after 2h of incubation, before and after partial enzymatic purification proved that the crude enzyme extract have a low activity with Triticum durum gliadin however it was important with Triticum aestivum gliadin, this protease activity was increased in the same conditions using partially purified enzyme and it persist always higher with Triticum durum gliadin comparing with Triticum aestivum gliadin. On the bases of these results, Nigella sativa seeds protease represent the alternative means of treating celiac disease in the future using the detoxification of gliadin to eliminate the immunogenicity of gluten.

Key Words: Gliadin, Celiac disease, Nigella sativa, Protease.
Identification of association between phenotypic and genotypic traits using Simple sequence repeat (SSR) markers in *Durum wheat*

R. Bousba, M. Baum, A. Jighly, A. Djekoune, S. Lababidi, A. Benbelkacem, M. Labhilili, F. Gaboun and N. Ykhlef

1 Laboratory of Genetics, Biochemistry and Plant Biotechnology 'Team IV Molecular Physiology and Plant Biodiversity' Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria
2 Laboratory of Biotechnology International Centre for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.
3 INRA Constantine Algeria
4 Biotechnology Unit, Institut National de la Recherche Agronomique (INRA), P.O. Box 415, Rabat, Morocco

Email: ratiba.bousba@umc.edu.dz

Abstract

Association mapping has been used in various crops to detect molecular markers associated with a variety of complex traits. Simple sequence repeat (SSR) markers were significantly associated with various agronomic traits. The objectives of the present work were to characterize a collection of durum wheat genotypes (landraces and improved varieties) with a number of simple-sequence repeat (SSR) microsatellites and to identify associations between specific alleles and variation in the expression of traits important for rainfed agriculture. Such associations would be essential prerequisites for marker-assisted selection (MAS) of traits in a breeding programme for improved drought tolerance. For this study forty *durum wheat* genotypes were used. The cultivars are characterized by contrasting agricultural productivity, collected from various sources, obtained from the Technical institute of the Field Crops El khroub CONSTANTINE (Algeria) (TIFC) and wheat New Partnership for Africa's Development (NEPAD) project, comprising 13 old local varieties/populations. These varieties have been commonly cultivated in different locations of Algerian climate (arid and semi arid), differing in annual temperatures and precipitations. Passing those yield trials successfully indicates the high genetic potential of these varieties for adaptation to different stresses like drought, heat, cold and salinity, these old varieties/population are more cultivated and the most appreciated (Ducellier, 1930) such as Béliouni, Rahouia, Bidi and Hedba3. The collection comprises also; five ‘founder genotypes’ widely used as parents in breeding programmers throughout the Mediterranean Basin and at International Centers (CIMMYT and ICARDA) like Waha, Gta dur, Djenah khotifa, Vitron and Sahel (ITGC, 1995), modern varieties released between 1980 and 1996 and nine new varieties and elite lines were provided by the International Center for Agricultural Research in the Dry Areas (ICARDA). All varieties were grown in a randomized block design with three replications at the experimental field of TIFC. Our results shows that phenotypic variation are highly significant, as revealed by a principal component analysis,
describing 74% of total variation introduced by two axes; CP1 and CP2. The hierarchical classification (cluster) shows the distribution of genotypes according to their phenotypic variation, based on their origins. For association analysis, the results show that the markers used are significantly associated with the traits studied. The majority of these studied markers were significantly associated with the traits studied, located on chromosome 4B. For productivity, three significant SSR markers were detected respectively associated: (WMS149 (p <0.01), WMS30_2) (p <0.05), WMC177_2 (p<0.05) respectively with 68.94%, 99.46% and 49.74% of phenotypic variation, located on chromosome 4B, 3A and 2A. Also, the hypothesis of association of SSR with heading date (HD) was tested by the GLM (General Linear Model), two SSR markers were found significantly associated with HD they are: (WMS6 (p <0.01) located on chromosome 4B, with 96.84% of phenotypic variation, and WMC445 (p <0.05) on chromosome 5A with 32.46% of phenotypic variation. Our results also reveal a significant allelic diversity is characterized by high values of polymorphism information content (PIC), the mean value obtained for all primers was 74%, the highest ones were obtained for WMC177 and WMC78 markers with (94% and 93%), respectively. Furthermore, the molecular variance analysis (AMOVA) showed that the proportion of variance explained by within and among geographical groups diversity was 83% and 17%, respectively. Thus, our study showed significant variation in morphological traits and microsatellite DNA polymorphisms among wheat varieties. These results reinforce and justify the choice to use these primers association analysis in our Durum wheat varieties.

**Key words:** Triticum durum Desf, association analysis, SSR marker, PIC, AMOVA.

**Introduction**

Molecular markers associated with a quantitative trait in plants are traditionally identified using a population derived from a biparental cross. This requires substantial time for the development of a recombinant inbred line populations. The complementary method of association mapping has been proven to be useful and powerful for genetic dissection of complex traits. Historically originating from human genetics (Templeton and al., 1995), association mapping is emerging as a novel tool in plant genomics (Myles and al., 2009) and utilizes diverse natural plant populations in detecting the correlations between genes/markers and traits of interest (Yu and al., 2006). The QTL mapping and analysis provides unprecedented opportunities to identify and locate chromosome regions controlling adaptive traits such as time to heading, plant height (Wang and al., 2010; Wu and al, 2010; Zhang and al., 2011) and yield (Quarrie and al., 2006).

The advantages of population-based association study (Ibrokhim and al., 2008), utilizing a sample of individuals from the germplasm collections or a natural population over traditional QTL-
mapping in biparental crosses primarily, are due to (1) availability of broader genetic variations with wider background for marker-trait correlations (i.e., many alleles evaluated simultaneously), (2) likelihood for a higher resolution mapping because of the utilization of majority recombination events from a large number of meiosis throughout the germplasm development history, (3) possibility of exploiting historically measured trait data for association, and (4) no need for the development of expensive and time-consuming biparental populations (Kraakman and al, 2004; Hansen and al., 2001).

In this study, SSR markers were applied to investigate the genetic variation within and between Forty wheat genotypes (Triticum durum Desf) cultivated at the experimental field of TIFC Algeria.

All varieties were grown in a randomized block design with three replications. The annual rainfall and its monthly distribution differed from year to year at TIFC station, Algeria. The total annual rainfall was 363 mm. In general, rainfall was lower than the long-term average (487 mm) in 25 preceding years, rainfall in the months of March and April was low and much less than the crop requirements. This low rainfall and its poor distribution affected crop performance; it subjected the crop to severe drought stress, particularly during the grain filling period. However, the months of March and April were warmer, drier, with more evaporative demand than in the last 25 years.

Phenotypic data of these varieties, were measured for plant height PH (cm), yield and its compounds (Number of grains per main spike (NGS), thousand kernel weight (g) TKW, Number of spike per meter square (NS/m²) and Grain yield (q/ha) GY) and phenological traits (Days to heading (days) DH.

For molecular analysis, the total genomic DNA was isolated from fresh leaf material (at ICARDA), by a modification of the method described by (Saghai- Maroof and al., 1984).

SSR Analysis

Twenty five primer pairs, were chosen among the publicly available sets catalogued in the GrainGenes database (http://wheat.pw.usda.gov) for wmc (Xwmc) and as described by Röder et al. (1998) for wms (Xgwm). PCR amplification was prepared in a volume of 10μL using 50 ng genomic DNA, 0.2mM dNTP, 1.5mM MgCl₂, 10 pmol of each primer (forward and reverse) and 0.5U Taq polymerase. For multiplexing, sets of 1–3 SSRs with different fluorescent dyes such as blue (FAM) (5-carboxy fluorescein), green (VIC) (tétrachloro_fluorescein), or yellow 2'-chboro-5'-fluoro-7',8'-fused phenyl-1,4-dichloro-6-carboxyfluorescein (NED) for the forward primers has been prepared. After PCR amplifications, fragments were electrophoretically, separated on an ABI Prism 3100 Genetic Analyzer Applied Biosystems/HITACHI, Foster city, CA, USA). Before multiplexing markers, each PCR product was optimized for genotyping on ABI 3100. For submission of samples
into ABI 3100, 1μL of this PCR mix was added to 5μL ROX (formamide) containing the Genescan G350 standard and then heated to 95 °C for 5 min.

**Statistical analysis and data scoring**

Phenotypic data were analyzed by the MIXED procedure of the SAS version 9.1 (SAS Institute, 2000, Cary, NC, USA). Agronomical traits were used in multivariate analysis with the major goals to distinguish between varieties and to determine the main characters that allow differentiation between the varieties based on their geographical origin by Factorial discriminant analysis (FDA) using Genetix version 4.04 (Belkhir and al., 1999). The distance between individuals, was calculated with similarity coefficient of “Manhattan" complete linkage", than regroupment was performed with method of UPGMA (Unweighted Pair Groups Method of Analysis), These analyses were carried out using the DARwin 5.0.148 software program available at [http://darwin.cirad.fr/darwin/Home.php](http://darwin.cirad.fr/darwin/Home.php).

**Molecular data**

After extracting microsatellite data from the ABI 3100 sequencer, they were analyzed for allele calls with GeneMapper software version 3.7 (Applied Biosystems). The genetic structure of the population was analyzed using Structure 2.1. (Evanno and al., 2005). When an inflexion emerges in the LnP(D) curve, the corresponding K value is adopted as the optimal group number. The Q values were calculated to serve as covariates in the association analysis that was carried out using Tassel 2.0 adopting a general linear model (GLM) (Bradbury et al. 2004). Allelic variation and polymorphism information content (PIC) were analyzed using Powermarker 3.25, and cluster trees were drawn using DARwin 5.0 by Jaccard distance (Jaccard, 1908). The relationships between the similarity matrix based on phenotypic and genetic similarity matrix obtained with microsatellite was analyzed according to Mantel (1967) using the NTSYS pc ver. 2.01 program (Sneath and Sokal,(1973). An analysis of molecular variance (AMOVA) (Excoffier and al., 1992) across all studied material using gene stat software, AMOVA was used to partition the total SSR variation into within geographical regions and among geographical regions components (Excoffier and al., 1992).

**Results and discussion**

**Phenotypic traits**

High significant difference was identified between genotypes for all phenotypic traits According to genotypes geographical origin, the genetic distance between CIMMYT (Gta dur, Yavros-79, Cocorit C 71 and Chen 'S' ) and Syria ( Korifla), ICARDA(Waha and Beltagy) Varieties was the lowest 0.091, however the distance between Spain and ICARDA/Algeria was the highest (47.052 ) (Fig. 1). The UPGMA dendrogram clearly shows the relationships among 11 geographical regions.
Figure 1. Unweighted Pair Group Method using Arithmetic Means (UPGMA), based on Euclidean distance of genotypes origin geographic

A. Algeria, IT. Italy, IC. ICARDA, T. Tunisia, E. Egypt, C. CIMMYT S. Syria CH. Cyprus, SJ. Syria Jordan, IA. ICARDA/Algeria and ES. Spain

Using the 25 SSR loci, six groups were most reasonably identified for the population of 40 varieties. This division of the population was supported by statistical probability and could ensure the accuracy of association analysis with a minimum of false association. Based on the population structure at \( K=6 \), association analysis was conducted, which could avoid the influence of artificial division (Gupta and al., 2005 ). The TASSEL (Trait Analysis by aSSociation, Evolution, and Linkage) program was used to detect associations between markers and phenotypic data by using a general linear model. A total of 14 SSR markers showed significant associations with six agronomic traits on chromosomes of durum wheat. Most of the significantly associated markers with the examined traits were mapped on chromosome 4A and 3B. The results indicate that these region of chromosome 4A and 3B are important for drought tolerance in durum wheat. These SSR markers, significantly associated with phenotypic traits in GLM, explained between 32.46% and 99.46% phenotypic variation. (Maccaferri and al., 2008 and Diab and al., 2008), reported that a genomic region on chromosome 3B and 4A were associated with drought tolerance traits such as stomatal resistance, leaf canopy, time to heading, yield components and harvest index in wheat genotypes. Drought tolerance genes are located throughout the genome and are genotype dependent.

In this study, we found that for TKW eight significantly associated markers were detected in tolerant varieties to drought and show high TKW in rain fed conditions. However, some of the markers were also detected in drought sensitive varieties like \( WMS \ 177 \) 2A for PH. (Börner and al., 2002) and (Huang and al., 2003) found similar results for other traits of interest. These findings further
confirm that drought tolerance is a quantitative trait and that apparently sensitive varieties may contain alleles for tolerance, which may not be found in the tolerant varieties.

Conclusion

In summary, our data showed significant variation in morphological traits and microsatellite DNA polymorphisms among wheat varieties. The wms and wmc data can be used in selecting diverse varieties in breeding programs for improvement of traits needed for adaptation to various stress conditions. These results may be helpful in wheat breeding programs aimed at improving drought tolerance.

References


Chourouk Hamla1*, Faical Brini2, Malika Ayadi2, Khaled Masmoudi3, Abdelhamid Djekoun1, Nadia Ykhlef1

1. Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team IV Molecular Physiology and Plant Biodiversity’ Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria
2. Plant Protection and Improvement Laboratory, Centre of Biotechnology of Sfax, Tunisia.
3. International Center for Biosaline Agriculture (ICBA), P.O.Box 14660, Dubai-United Arab Emirates.

Email: chourouk.hamla@umc.edu.dz

The impact of the climate change resulting from: temperature rise, atmospheric CO₂ elevation, a decrease in rainfall as well as an elevation in number and intensity of extreme weather events, is already affecting the agricultural sector (Pachauri et Reisinger., 2007; Zandalinas., 2018). This scenario will subject crops to a greater range and number of environmental stresses that could occur simultaneously with severe consequences (Mittler and Blumwald., 2010). Cereals provide the majority of calories for human consumption, making this stress scenario particularly threatening for global food security (Lawas et al., 2018). Investigating the molecular basis of drought tolerance mechanisms in cereals especially in wheat remain essential in order to cope with stress damages. Plant response relies on the expression of several genes which unleashes a variety of mechanisms, including the production of proteins and enzymes directly involved in stress metabolism, contributing to the specificity of the acclimation response to a given stress stimuli (Casaretto et al. 2016). There are few problems in investigating the genomics of drought tolerance in species such as wheat. However, recent technological advances and the imperative to ensure sustainable food production has driven research programs to improve this crop genetically despite the size and complexity of the genome (Fleury et al., 2010). The researcher’s interest in physiological, biochemical and molecular aspects of the plant response to stress is quite justifiable. Bring in new knowledge which can be used to develop new drought tolerant species. In this field a lot has been done already, covering different aspects, with a main purpose: understanding plant’s ability to grow and survive under limiting conditions. In spite of the tremendous efforts done for now, there is still a lot to do. The genetic complexity of the mechanisms used to tolerate the stress has made this task even more difficult. This is why, biotechnologies should be complemented with conventional plant physiology and breeding procedures (Vinocur and Altman., 2005, Gupta et al., 2014). The present work summarizes a study in which the main goal was to perform a comparative analysis of durum wheat genotype responses to drought at physiological, biochemical and molecular levels by focusing on two stress responsive...
genes. Tree experimentations have been done on seven Algerian durum wheat genotypes (DK, OZ, W, B, Bou, GGR and B17), two of them under controlled conditions in a growth chamber and the remaining one under semi-controlled conditions in a greenhouse. The used genotypes were generously supplied by ITGC (Institut Technique des Grandes Cultures (Station El-khroub Algeria). Under controlled conditions (growth chamber), the water stress was applied by adding polyethylene glycol (PEG-6000) to the media and under semi-controlled conditions (greenhouse); it was applied by withholding water. At physiological and biochemical level, the response of these genotypes to water stress was analyzed through several indices: germination rate, leaf and root length, root number, Root to Shoot ratio, relative water content, leaf temperature, chlorophyll content, stomatal conductance and electrolyte leakage. Also a 1D SDS-PAGE electrophoresis was performed on leaf proteins.

Drought tolerance during the germination phase can be used as criteria to evaluate the ability of durum wheat varieties to tolerate water stress during this early phase of development. The germination processes start with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley et al., 1997). According to our results the germination percentage was influenced by osmotic stress. The greater PEG-6000 concentration increases the more germination ability decreases. The observed differences could be explained by the fact that PEG-6000 may act as an osmotic agent generating low water potential. It seems that lowering the osmotic potential with PEG-6000, decreases water availability for the seeds and then causes low germination. The physical process of water uptake leads to activation of metabolic process, as the dormancy of the seed is broken following hydration. Elevated drought stress slows down water uptake by seeds, thereby inhibiting their germination and root elongation (Pirdashi et al., 2003). As an average for the seven studied varieties the germination rate decreased by -2,091% for each PEG-6000 concentration. Root length can be considered as an important factor for drought tolerance. In fact tolerant varieties tend to develop an important root system and by consequence a better capacity to access deeper water. Shoot and root lengths of the studied genotypes declined in all of the treatment solutions relative to the control. However, the interaction among treatments and genotypes was not statistically significant. In addition to root and shoot lengths, root to shoot ratio also plays a major role in selecting drought tolerant lines. Genotypes with high root/shoot ratio under drought are much preferred. Our findings revealed significant variations for the root/shoot ratio among the genotypes studied. In fact under drought conditions the plasticity of the plants allow increased allocation of several primary metabolites to roots while decreasing allocation to shoot (Joshi et al., 2011). We also noticed a diminution in root number due to osmotic stress.
Stomatal closure constitutes one of the used mechanisms to cope with drought and is considered as an early response that takes place within minutes (Assman et al., 2000; Khan et al., 2009). A decrease in stomatal conductance is a commonly observed response among different plant species (Djekoun., 1991; Liang et al., 2002; Parwata et al., 2012). Plants under optimum watering showed higher values than those under stress conditions. The registered data varied from a minimum of 19.85 mmole m\(^{-2}\)s\(^{-1}\) for GGR and a maximum of 33 mmole m\(^{-2}\)s\(^{-1}\) for OZ. Plants respond to drought by closing their stomata, which reduces leaf transpiration and prevents the development of excessive water deficits in their tissues (Cochard., 2002). As stomata close in response to water deficit stress, transpirational cooling ceases, leading to a rise in leaf temperature (Luquet et al., 2003; Jones, 2004). The genotype showing a relatively lower leaf temperature may be able to preserve a more favorable leaf water status (Silva et al., 2007). Leaf temperatures in drought stressed plant were higher than in control plants despite the fact that the stress was applied by withholding water or using PEG-6000; but the differences were not significant when submitted to PEG-6000 regardless concentration or time exposure. In addition to the previous parameters, leaf chlorophyll content was also affected by water deficit. Leaf chlorophyll can be used as an indicator for plant physiological performance (Neufeld et al., 2006). Drought affects Chl content in many crops by imposing adverse effects on Chl synthesis or accelerating its degradation, which reduces the photosynthetic capacity. The reduction of chlorophyll may be a consequence of chlorophyll degradation resulting from a prolonged photo-inhibition (Silva et al., 2007). The recorded data showed reduced leaf chlorophyll content with an average value of 40.28 SPAD units for controls and 35.89 SPAD units under water shortage. For PEG-6000 treatments, the decrease is even more observed when the time of exposure is extended. Under 1,2MPa the values go from 35.9 SPAD units for 3 days of exposure to 34.6 SPAD units for 5 days (average values). Studies have shown that, the most tolerant wheat varieties tend to keep the highest chlorophyll and relative water content (Atteya., 2003, Parwata et al., 2012). Significant decrease in relative water content was observed under PEG-6000 and when withholding water. This decrease is accentuated as the time of exposure is extended. The observed differences between varieties could be attributed to their ability to assimilate water through the root system, their capacity to control water loss via evaporative surfaces (Bayoumi et al., 2008), their osmoregulation mechanisms (Sassi et al., 2012) and a better water membrane permeability (Jones et al., 2006). Cell membrane could also be targeted by drought and its stability is of a great importance (Rana et al., 2013). The range of membrane damage was assessed indirectly by measuring of cell solute leakage which is inversely proportional to cell membrane stability (CMS) (Farooq and Azam., 2001). Our results revealed that membrane integrity was more conserved for B and DK after two weeks of drought stress. The rate of injury to plasma membrane can be evaluated through electrolyte leakage
from cells (Rana et al., 2013), and the proportion of leakage is relative to cell membrane damages (Sayar et al., 2008).

In an attempt to understand the molecular basis of drought tolerance, proteomics using SDS-PAGE were analyzed to identify protein patterns involved in drought stress response in the studied Wheat genotypes. Detection of proteins which levels are altered by PEG-6000 stress was done by comparing patterns of control and PEG-6000 treated plants (two concentrations 10% and 20% applied for 3 and 5 days). A set of control plants was grown without adding PEG-6000. The observed differences regarding protein bands revealed by 1D SDS-PAGE electrophoresis affected their intensity and their molecular weight. A total of 114 bands were revealed and they range in size from 14 to 106.8KDa. Higher plant exposed to drought conditions exhibit a characteristic set of cellular and metabolic response, including a decrease or increase in the synthesis of proteins (Bayoumi et al., 2008).

There is a strong connection between the modifications occurring on the molecular and the physiological level. These modifications lead to an up or down regulation of some proteins or a synthesis of a new set of proteins. Among the numerous transcripts and proteins which accumulate during drying, dehydrins are assumed to play a protecting role. Also, another group of proteins called aquaporins turns out to be involved in drought tolerance mechanisms. The expression levels of two stress responsive genes (a plasma membrane aquaporin and a dehydrin gene) were also investigated in two varieties selected from the previous group of genotypes after being exposed to PEG-6000 (Hamla et al., 2014). RT-PCR analysis of the AQP gene TdPIP2,1 in two cultivars W and B exposed to 10% and 20% PEG showed higher expression levels in the sheaths and the leaves of the two genotypes in comparison to the roots under stress. In control plants the mRNA levels of the TdPIP2,1 were maintained at low levels (Hamla et al., 2014). For DHN-5, the expression levels were close to TdPIP2,1 except for the control tissues where no transcripts has been detected (Hamla et al., 2014). It seemed that B accumulates more transcript than W for both genes (Hamla et al., 2014). Our finding suggest that durum wheat is able to adjust the expression of DHN5 and TdPIP2,1 according to environmental conditions. The upper expression of these two stress responsive genes may be valuable by setting up stress tolerance mechanisms in different tissues and especially in leaves. Thus conduct to an appropriate cellular response and prevent plants from dehydration damages. This investigation could be a starting point for more elaborate analysis of the regulatory network DHN-stress responsive genes. Identifying possible connection between activated stress responsive genes can be extremely useful for further elaborate biotechnology-based approaches.
References


**TaSTRG** gene expression under abiotic stress in interspecific lines durum wheat × *Aegilops geniculata* Roth.

KELLOU K., DJEKOUN A. and YKHLEF N.

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team IV Molecular Physiology and Plant Biodiversity’
Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria

e-mail: kamel.kellou@umc.edu.dz

**Abstract**

Wheat is one of the most important crops worldwide. Many efforts have been undertaken to generate some genes of tolerance, by manipulating their expressions facing environmental constraints such as drought, salt and cold. In 2009, a study conducted by a Chinese team a candidate gene (**TaSTRG**) was found involved in tolerance to drought and salt stress. The objective of this work was to study the effect of abiotic stresses (salinity and drought) on expression of the **TaSTRG** gene at recombinant lines (**RIL**) of interspecific cross from durum wheat (variety Oued Zenati 368) and *Aegilops geniculata* compared with the *Chinese spring* variety of common wheat. The analysis of the expression of the **STRG** gene was performed by a semi quantitative RT-PCR on the roots and the aerial parts of seedlings (three-leaf stage) stressed or not. Water stress was induced by polyethylene glycol (PEG6000) (15%) for 48 h and salt stress with NaCl (170 mM) also for 48 h. Our results indicate that **STRG** gene of *Triticum aestivum* has distinct expression profiles among different genotypes studied, allowing us to suggest that it is involved in improving the resistance of these individuals under difficult environmental conditions.

**Introduction**

The *Aegilops* genus related genus of *Triticum* has great genetic variability and is a valuable source of important economic traits for wheat improvement, including tolerance to drought and salinity.

Researchers have developed breeding programs aimed primarily at selecting varieties that are well adapted to climatic conditions. The breeder must choose an action strategy that can maximize his chances of creating a good variety by using, genetic resources, classical breeding methods and molecular biology techniques by integrating the tools of biotechnology such as marker assisted selection.
Favors to advances in the field of genomics, scientists are able to know the genes involved in the tolerance of plants to biotic and abiotic constraints. But the distribution of genes and the impact of genome structure on their expression are still poorly understood.

In this context, we focused on demonstrating TaSTRG gene expression in recombinant cross-species between durum wheat and Aegilops geniculata, under water stress conditions induced by PEG and salt stress induced by NaCl with a semi-quantitative Rt-PCR method.

**Material and methods**

The plant material used in this study is 08 lines from an auto-fertilization of the interspecific crosses between durum wheat (Triticum durum Desf) «variety Oued Zenati 368» and Aegilops geniculata Roth. These lines were compared with the Chinese Spring variety of soft wheat. The hybrids are obtained by the team of biotechnology and plant breeding, laboratory of genetics, biochemistry and plant biotechnology, university of the brothers MENTOURI Constantine 1.

The roots and leaves of each genotype treated with PEG, NaCl and the controls are collected separately in 2 ml Ependorf tubes. The tubes are then rapidly frozen in liquid nitrogen and stored at -80°C for RNA extraction.

Total RNA extraction is provided by TRIzol according to Chomczynski and Mackey, 1995, it is a general method developed to deproteinize the total RNA of plants.

Next, a complementary DNA strand (cDNA) is synthesized from mRNA template using an oligo-dT. The reaction of polymerization is catalyzed by retrovirus reverse transcriptase using the MMLV kit (5U / μl) reverse transcriptase (Invitrogen).

The PCR amplification is carried out from a reaction volume of 20 μl, with primers: TaSTRG F 5'TCGTGTTTCTCGTGAAAGGG 3' and TaSTRG R 5'CAGGGTCTTGAGGAAGTTGTTG3'.

The wheat expression of β-Actin gene is analyzed as a reference and the sequences of the primers used are β-Act F 5'TGCTATCCTTCGTTGACCTT 3' and β-Act R 5'AGCGGTTTGGAGGGGT 3'.

The PCR program suitable for the genes studied is: 94 °C. for 5 min, 94 °C. for 15 sec, 60 °C. for 30 sec, 72 °C. for 30 sec and 72 °C. for 7 min.

**Results and discussion**

This study was carried out on the interspecific material resulting from hybridization between hard wheat (var. Oued Zenati 368) and Aegilops geniculata in comparison with the Chinese spring variety of soft wheat based on the work Zhou et al., (2009) that were able to highlight a candidate gene for salinity tolerance by identifying its function.
This gene was published on the Genbank database under the name *Triticum aestivum* salt tolerance-related gene (TaSTRG) and the accession number EF599631, with a mRNA size of 879 bp. The activation of this gene led to the synthesis of the TaSTRP protein, with 293 amino acids, and carries accession number AB48810. NCBI, 2014.

It is recorded that the amount of RNA from the leaves is greater than that of the roots. However, the 2 lines of the *Chinese spring* variety showed high mRNA levels for roots relative to leaves.

The primer pairs used to amplify TaSTRG sequences and the constitutive β-Actin gene of 101 and 92 bp, respectively, gave amplifications with the expected size.

In order to verify that the amplicons obtained have the expected size, the PCR products are deposited on a 1.8% agarose gel. A control without DNA is added to check for contamination during handling.

Indeed, the bands obtained after migration of the different PCR products are of different intensity regardless of the stress treatment performed for both TaSTRG and Actin genes in roots and leaves. Thus, we have not verified that the expression of the Actin gene is constant or not in untreated seedlings and in seedlings treated with stress. On the other hand, the results of the electrophoresis gels are exploitable.

After treatment with NaCl and PEG on the different genotypes studied, the TaSTRG gene expression was evaluated by a semi-quantitative RT-PCR, compared with the expression of the β-Actin reference gene.

The results obtained by RT-PCR indicate that the TaSTRG gene is expressed in all genotypes studied and under the different stress treatments, which is consistent with the size of the partial sequence of the gene (101pb) obtained despite their low intensities.

Therefore, the TaSTRG gene does not show an expression dependent on the degree of abiotic stress alone but also on its nature.

No variation in actin expression, while expressed in all genotypes, in root and leaf tissues, and with or without stress, generally, the level of expression of the β-Actin is not influenced by stress.

The variability obtained in the level of gene expression can also be due to the contamination of the roots of our seedlings by mold during the cultivation, the latter probably influenced the transcriptome of the plants before the application of the different stresses.

We can also propose to characterize the expression profile of this gene of tolerance to biotic and abiotic stress by the quantitative RT-PCR technique.
Conclusion:

The semi-quantitative RT-PCR technique demonstrated the expression of the TaSTRG gene, but it did not allow a good understanding at the functional level. Better knowledge through functional genomic approaches to this gene should help the development of new varieties better adapted to environmental constraints.

The introduction of Chinese spring the reference variety of wheat in this study has clearly shown that this gene is carried on one of the chromosomes genomes A, B, U or M but we are sure that it does not come chromosomes of the D genome of wheat.

The TaSTRG gene is possibly also involved in the response to biotic stress.

Bibliographic reference:


Morpho-physiologicals, biochemicals and transcriptomics markers of drought tolerance in durum wheat (*Triticum durum* Desf.)

**MOUELLEF Adra, YKHLEF Nadia**

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team IV Molecular Physiology and Plant Biodiversity’

Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria

*Email: adra.mouellef@umc.edu.dz*

**Abstract**

In Algeria, wheat is an important component of nutritious foods as a source of energy. Its national production does not respond to the needs of population, which ranks Algeria as one of the most important wheat grain importing countries in the world. This is mainly due to environmental change. Drought stress is one of the main causes of crop loss, reducing average yields for durum wheat. Plants have developed coping strategies to respond to environmental changes, controlling and adjusting their metabolic systems. The present study aims to characterize and express the diversity in the response of some durum wheat genotype to water stress. In the first part, the water stress was applied in a greenhouse by stopping irrigation at different stages of development of the plant, in order to understand and characterize the morpho-physiological and biochemical aspects of the water stress response in the genotypes tested in order to select tolerant genotypes that can be exploited to improve this cereal. The results obtained show that the different genotypes studied showed different behaviors and modes of tolerance and sensitivity towards water stress. Water stress decreased the plant's water status parameters, reducing leaf area and plant size. This response presents the structural and functional regulation that result in metabolic changes in plants. Molecular responses translate mainly through the translation of mRNAs, whose products will allow the plant to cope with water deficiency. Most often osmolytes; proline plays a major role in regulating osmotic cellular potential. This second part of our study is devoted to identifying and quantifying the expression of the candidate genes (P5CS1, GS1 and GS2) responsible for the synthesis of this amino acid in durum wheat by RT-PCR in real time. The results of the transcripts tested showed no correlation between GS1, GS2 and proline accumulation. On the contrary, P5CS1 expression is correlated positively with the accumulation of proline and can be considered a good stress indicator in an important role in drought tolerance in durum wheat. In conclusion, durum wheat actually displays morpho-physiological, biochemical and transcriptomic adaptation traits under water stress conditions. They are markers and indicators of tolerance to this type of water stress. These adaptation indicators can be used as parameters for selection and improvement of durum yield in Mediterranean regions.
Keywords: durum wheat, tolerance, drought, morph-physiological, biochemical, transcriptomic, $P5SC1$, gene.

Summary

Cereals appear since the origin of agriculture, several millennia before our era. They are closely related to the history and development of civilizations, which they contribute to characterize through diets marked by the cultivation and consumption of a given cereal. They are considered as a major source of human and animal nutrition (Slama et al., 2005). Among these cereals, Wheat occupies the first place for world production and the second after rice, as a food source for human populations, it provides 15% of its energy needs (Bajji, 1999). Wheat is an important cereal in terms of domestic consumption in many countries of the world. It is used mainly for the manufacture of semolina, raw material of pasta alimentary (Feillet, 2000), breads, pancakes and couscous in the Maghreb countries. This species is cultivated mainly in arid and semi-arid Mediterranean countries where agriculture is in the worst case. It is characterized by increased temperature coupled with lower rainfall, and desertification and drought kill the agricultural soils (Abeledo and al., 2008). This makes the production of this cereal weak and irregular; it does not meet the needs of the population. Algeria is one of the largest importers of wheat in the world. It buys more than 5% of the world's cereal production annually, and this situation is likely to last for several years due to the lack of sufficient yields and ever-increasing consumption needs in the face of strong demographic change. In fact, a insufficient production of 2.7 Mt to cover the needs of the national market and to feed the stocks pushes to make a systematic recourse to imports. This weakness in wheat production was still linked to the effects of drought, which has been felt very dramatically over the past two decades.

The ability to quantitatively evaluate the performance of cultivates plants sustaining water stress is very important in research programs aimed at the rehabilitation and improvement of production in semi-arid regions (INRA, 2000).

Several studies have shown that, in a water deficit, plants adopt coping strategies that differ from one species to another and involve a broad combination of morphological, physiological and biochemical factors (Wang and al. 2003). The analysis of this situation shows that it would be urgent to develop strategies to regulate the yields of these regions where water stress affects crops considerably. Improving tolerance remains a priority selection goal in areas with high climate variability. In cereal zones characterized by this type of stress, tolerance could play a major role in improving yields. This improvement uses the techniques of genetics, genetic engineering, biochemistry, physiology and biotechnology ( Dubos, 2001). Knowing that the genetics of the characters related to water stress is complex and difficult to explain hence the need to identify and analyse the different mechanisms (morphological, physiological and biochemical) involved in the...
adaptation to water deficit. Genetic study by the search for molecular markers of the mode of inheritance transmission and heritability, as good indicators of tolerance to water stress is necessary to facilitate the use of these characters in breeding programs for improvement genetic.

Water stress causes the establishment of a state of water regulation of the plant that manifests itself by stomatal closure and by a regulation of the osmotic potential (Brisson and Delecolle, 1992). Osmotic adjustment is generally considered an important element in plant tolerance to water stress (Bajji and al., 2001). This adjustment involves the accumulation, at the cellular level, of sugars, amino acids, ions or other compatible solutes (Wang and al., 2003, Cai and al., 2007, Zhou and Yu, 2009).

The accumulation of osmolites makes it possible to create an influx of water into the cell or at least to avoid a flow, by increasing the retention strength of the water molecules. The maintenance of this quantity of water thus makes it possible to preserve the necessary turgescence for cell growth (Maury and al., 2011). It appears that this accumulation of osmolites is related to maintaining the integrity of proteins and membranes. For example, Kumar and Dubey (1999) have shown that during water stress the accumulation of osmolites also seems to be related to the protection of cells against activated species of oxygen. However, an increase in osmolites is not always related to an increase in tolerance (Maggio and al., 1997). The accumulation of constitute proline is also a real mechanism of tolerance to water stress. The existence in cereals of an intraspecific variation for proline accumulation under the effect of water stress suggests the possibility of selection, on the basis of this character, of genotypes that will have a good ability to survive and a stable grain yield in limiting water conditions. For this reason, some authors, Bellinger and al., (1991) have proposed the accumulation of proline as a selection technique. Tahri and al., (1997) show that several breeders and physiologists have used the ability of its accumulation in screening for genotypes resistant to water deficit. On the other hand, sugars are considered by many authors to be good, compatible osmoregulators that can play an important role in osmotic adjustment and plant adaptation to drought (Slama, 2002). In conditions of water deficit, the sugars participate to a large extent in the lowering of the osmotic potential. Indeed, sugars represent osmoticums much less powerful than proline. They also participate in maintaining the balance of osmotic force.

Tolerance to water stress is a complex phenomenon involving many genes. Molecular analysis of the dehydration response has therefore arrived at a stage where many stress-variant gene sequences are available. It is now essential to combine physiological, genetic and biochemical approaches as well as molecular biology techniques in order to have an integrated approach to the phenomena that govern the stress response and to be able to identify the genes most strongly implicated in tolerance. The changes that occur in the primary metabolism of the plant are part of the general response to stress. Among genes that vary during stress, genes involved in the biosynthesis of proline. This
nitrogen metabolism is affected by water stress. Transcript accumulation coded for cytosolic glutamine synthase (GS1) in plant during water stress related to leaf protein rearrangement following stomatal closure.

Selection for adaptation to abiotic stresses follows several pathways between the use of phenology, morphology, physiology and biochemistry, as well as the overall behavior of the plant with respect to environmental variation. Analytical approaches, consisting of individually isolating and studying a given resistance mechanism, through the observation of a particular parameter (selection criterion) have been proposed. Several physiological and biochemical criteria were thus identified in order to distinguish the susceptible varieties from the varieties resistant to water stress: the hydraulic state of plants, proline accumulation, specific protein induction, stomatal resistance, chlorophyll fluorescence. This research has generated a thorough knowledge of the physiological processes related to the plant's response to water stress.

The work carried out in this study has focused on the adaptation of some genotypes of durum wheat seedlings to the different levels of water stress applied at the three different stages of growth. The response of plants to variations in water stress varies according to the type of stress, the stage of development of the plant and the characteristics of the plant. Water stress has affected the physiology of the body by altering its metabolism, growth and development. A common response to this type of stress is the accumulation of osmotic components, these components that serve as osmoprotectives and in some cases, they serve to stabilize biomolecules under conditions of water deficit. The varieties studied in this experiment have shown behaviors and different modes of resistance to the stresses to which they have been exposed. As a result, resistant varieties have developed through the presence of several tolerance mechanisms. The morphological and physiological study of the leaves shows that the water deficit caused a reduction in the morphological parameters and more or less affected the water status of the plants studied; Stomatal resistance increases as water stress increases. To interpret the essential traits involved in the drought tolerance process such as osmotic accumulation, more in-depth studies have been conducted on three biochemical parameters; the determination of proline, soluble sugars and the analysis of the electrophoretic profile of the total leaf proteins. A strong accumulation of sugars and proline has been recorded, variation in proline accumulation between durum genotypes reflects biological diversity between these genotypes. In addition, water stress induces metabolic changes related to protein levels, this is probably related to the alteration of protein synthesis, and the maintenance at the same protein level or the degradation of proteins. Significant and positive correlations are obtained between the relative tolerance and the majority of the variables. The expression of the P5CS1 gene is regulated under water stress, which proves that this amino acid has a role in adapting to water stress.
In conclusion, durum wheat actually shows morphophysiological and biochemical adaptation traits under water stress conditions. These are markers of stress indicators of adaptation to this type of water stress. These adaptation indicators can be used as parameters for selection and improvement of durum yield in Mediterranean regions.

References


INRA. 2000. La résistance des plantes à la sécheresse. Centre de Monpellier.


Aquaporins gene expression and the control of water status in durum wheat

BENTAHAR Soumia, DJEKOUN Abdelhamid and YKHLEF Nadia

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team IV Molecular Physiology and Plant Biodiversity’
Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria
Email: soumia.bentahar@umc.edu.dz

ABSTRACT

Plant growth and development are dependent on tight regulation of water movement. Water diffusion across cell membranes is facilitated by aquaporins that provide plants with the means to rapidly and reversibly modify water permeability. As an integral regulator of plant–water relations, aquaporins are presumed to play an important role in plant defense responses against biotic and abiotic stressors. This review highlights the crucial role of aquaporins in plant–water relations as well as their expression under drought conditions focusing on the relationship between the expression patterns of AQP genes, water use efficiency (WUE) and the tolerance capacity of two Algerian wheat cultivars.

Key words: Aquaporin expression, durum wheat, water relation, drought tolerance, Water Use Efficiency (WUE).

INTRODUCTION

Plant growth and development are dependent on the tight regulation of water uptake and transport across cellular membranes and tissues. Environmental stresses such as water shortage, high salinity and extreme temperatures negatively impact plant survivorship, growth and crop yield. Among environmental stresses, water shortage is the most serious challenge to plant survivorship (Zhao et al., 2010).

As wheat is one of the most important crops in the world, it is desirable to have a wheat plant that has high water use efficiency (WUE). High WUE would lead to a wheat plant that is able to produce more biomass and higher grain yield, with equal or less water uptake (Zhang et al., 2002; Boogaard, et al 196). Although a large number of drought induced genes have been identified in wheat, a molecular basis for water stress tolerance remains far from being completely understood (Houde et al,2006). One of the most important genes related to water use in plants is aquaporin (AQPs) gene, which belongs to the membrane intrinsic protein (MIP) gene family. Many AQPs have been identified in plants (Forrest, et al 2008).
Aquaporin proteins play an important role. AQPs are known to transport water and other small molecules through biological membranes and many AQP genes have been identified from different plant species (Sade et al., 2001) including 35 from Arabidopsis (Johanson., et al, 2001) 36 from maize (Chaumont et al., 2001 ) and 33 from rice (Sakurai et al 2005) Compared to other species, little is known about the AQPs in wheat because of the unavailability of its complete genome sequence and the allohexaploid nature of its genome.

Production of wheat, a global staple crop is constrained by multiple environmental stress factors, such as drought, salinity and extreme temperature. An understanding of the molecular mechanisms underlying the response to abiotic stress responses is necessary for genetic improvement of stress tolerance in wheat. Although AQP genes respond to various stresses, their exact role in abiotic stress tolerance remains unclear. (Zhou et al., 2012). Plant water use efficiency (WUE) and drought tolerance show a positive relationship with the amount of plasma membrane intrinsic proteins, i.e., PIP1 and PIP2 (Tsuchihira et al., 2010). In the present review, we highlights the crucial role of aquaporins in plant–water relations as well as their expression under drought conditions focusing on the relationship between the expression patterns of AQP genes, water use efficiency (WUE) and the tolerance capacity of two Algerian wheat cultivars.

**ROLES OF AQUAPORINS IN PLANT–WATER RELATIONS**

Water is a key element of all physiological processes, the plant, for its growth and metabolism, uses only a small fraction. The remaining 99.5% is lost during transpiration (Freeman et al 2007) .In order to fix one kilogram of carbon during photosynthesis, plants transport several hundred kilograms of water (Tyerman et al., 2002). This bulk flow of water through plants can take three different routes: the apoplastic route along cell wall structure, the symplastic route from cell to cell through the plasmodesmata, and the transcellular path across the cellular membranes (Steudle et al.,1998).Aquaporins as transmembrane water and solute transporter channels could be speculated as potential regulators of plant cell water relations that reflect their key roles in plant cell osmoregulation, root hydraulic conductivity (Lpr), leaf hydraulic conductivity, transpiration and cell elongation (Zunaira et al.,2016).Maintenance of the cell’s osmotic potential under stress conditions such as pathogen infection, drought, flooding, salinity, high or low temperatures, and biotic stresses is a major challenge for plant growth and development.

The majority of abiotic stress conditions directly impact plant water relations and stimulate an array of complicated cellular and physiological responses that lead to turning on plant water-saving strategies such as stomatal closure to cut off water loss during transpiration. Aquaporins, as vital regulators of plant–water relations, are potential targets in developing stress resistant crop plants.
Their significance in all facets of plant growth and development is well-established, but the mechanistic pathways behind their roles under plant defense responses remains to be elucidated. Numerous comparative transcriptome studies under various abiotic stress conditions have shown a differential response of different aquaporins homolog’s in diverse plant tissues. (Zunaira et al., 2016). Reverse genetic approaches have also been extensively used to fine-tune the role of different aquaporin encoding genes in response to diverse environmental stresses. However, the presence of a large number of diverse aquaporins, integrated complex expression patterns, and technique limitations for measuring accurate solute and water movement across transmembrane aquaporin channels are major hurdles in establishing their conclusive roles in plant growth and survival under abiotic and biotic stresses (Kaldenhoff et al. 2008).

**AQUAPORIN EXPRESSION**

The first clues about aquaporin function in plants come from the study of the level of expression in different organs, tissues, or cell types according to the developmental stages and in response to different environmental conditions. The mRNA abundance is nowadays measured by reverse transcription quantitative PCR approaches, a widely used technique that however requires a strict design of the experimental conditions (primer specificity and efficiency, housekeeping genes for normalization, and analysis method; Bustin et al., 2009).

Many studies comparing PIP and TIP aquaporin expression in different organs and conditions in various plant species have been published and have highlighted their involvement in the control of radial transcellular water transport but also in cell osmoregulation. It is interesting to mention that, in general, PIP and TIP aquaporin expression seems to be more abundant in roots than in leaves (Alexandersson et al., 2005; Heinen et al., 2009; Besse et al., 2011), but several isoforms are highly or exclusively expressed in leaf tissues (Sakurai et al., 2005; Azad et al., 2008).

For instance, expression of PIP aquaporins in roots and leaves has been correlated to the presence of apoplastic barriers, the exodermis and endodermis in roots or in suberized bundle sheath cells in leaves, suggesting an essential role in the transmembrane water diffusion when its movement is hindered. Interpretation of global aquaporin mRNA or protein level detected in an organ has however to be done with caution, as an isoform can be expressed, and hence plays an important role, in specific but lowly abundant cell types, such as guard cells or bundle sheath cells. These “gatekeeper” cells are positioned in the flow pathway to have relatively large impacts on plant water relations (Chaumont and Tyerman, 2014).
AQUAPORINS IN WATER STRESS

Based on conclusive evidence, it is widely accepted that, in most plant species, water uptake and transcellular water flow in roots are largely mediated by PIPs and TIPs. These are the most abundant aquaporins in the plasma membrane and tonoplast of the plant cells, respectively (Zunaira et al., 2016). Comparative transcriptome studies revealed differential expression of multiple aquaporin homologs in response to drought stress suggesting definite roles in stress responses. In 2005, Alexandersson et al. monitored the expression of all 35 aquaporin homologs in Arabidopsis in response to drought stress alone and found that most PIP and some TIP genes have high levels of expression, while NIP genes have very low expression (Alexandersson et al., 2005). The authors also showed that all PIP genes are down-regulated in drought stress response in leaves except AtPIP1;4 and AtPIP2;5, which are up-regulated. Moreover, AtPIP2;6 and AtSIP1;1 are constitutively expressed and are not significantly affected by the drought stress (Alexandersson et al., 2005). Consistent with these results, several other studies in Arabidopsis have shown that among all subfamilies of aquaporins PIPs are most responsive to drought stress and most of them undergo a transcriptional down-regulation. Only a few genes were found to be up-regulated. (Zunaira et al., 2016). All of these PIP genes that are down-regulated in response to drought are highly expressed in the roots. Strong down-regulation of PIP genes transcription under drought stress was also observed in the roots and twigs of olive plants, in the roots of tobacco and in the fruits of peach. It can be concluded that drought stress response of aquaporins is highly variable depending on stress levels, aquaporin isoform, tissue, species, presence of symbionts, and the nature of stimuli causing dehydration similar to drought stress. However, a general down-regulation of most of the PIP genes is thought to reduce water loss and to help prevent backflow of water to drying soil (Zunaira et al., 2016).

AQUAPORIN GENES IN DROUGHT-TOLERANT AND SUSCEPTIBLE WHEAT GENOTYPES

To investigate in more depth the relationship between the expression patterns of AQP genes and the tolerance capacity of wheat, we performed an analysis of relative expression of two AQP genes TdPIP1;1 and TdPIP2;1 previously mentioned by qPCR in leaves and roots between two wheat genotypes (accessions) with contrasting tolerance responses to water deficit. In this sense, we observed important differences at the transcript level between the drought-tolerant (BOUSSELAM) and the drought-susceptible genotype (CIRTA).

Research results led by (Bentahar, 2017) indicated that the two genes studied TdPIP1;1 and TdPIP2;1 have exhibited variations in their expression under stress conditions, they are regulated differently in the roots and leaves of wheat, which is in line with the results of (Ayadi, et al 2011).
Under severe water stress 20% field capacity, negative regulation was detected in TdPIP1.1 transcripts in leaves but with high accumulation in (CIRTA) roots, which is consistent with numerous studies of various species. Zunaira et al (2016) report that most of the PIP genes that are negatively regulated under stress conditions are strongly expressed in the roots, a strong downregulation of PIP gene transcripts was observed under water stress in roots and twigs of olive plants (Secchi, et al., 2007); in tobacco roots (Mahdieh et al., 2008); and in the fruits of the fishery (Sugaya et al., 2002). In wheat leaves, Elseehy et al (2015) report that three PIP isoforms (PIP1, PIP2, PIP3) showed down-regulation under PEG-induced stress. The genotype (CIRTA) could be suggested as susceptible genotype due to the down-regulation of PIP genes in roots under sever water stress conditions.

In contrast, results of (Bentahar, 2017) revealed that the TdPIP2.1 gene was positively regulated (up regulated) under severe stress conditions. The abundance of TdPIP2.1 mRNA transcripts is detected slightly in the leaves; the expression of this gene is significant in the roots of the variety (BOUSSELAM). This genotype may be suggested as tolerant genotype showing high values of water use efficiency under water stress conditions (Bentahar et al., 2015) which is a result of the positive regulation of the aquaporin genes in the roots.

Many studies report the same observations on the same isoform PIP2.1 on different species. In durum wheat, the work carried out by (Hamla et al., 2014) on the expression of the PIP2.1 gene from different tissues (roots, leaves, seeds) under PEG-induced water stress revealed that this gene is positively regulated with a strong expression in the leaves. By applying another stress mode (water regime at 20% of the field capacity) results of Bentahar (2017) indicated that the abundance of TdPIP2.1 transcripts was more prominent in the roots.

In conclusion, tolerant genotype tends to have a positive regulation (up-regulation) of PIP genes roots in response to severe water stress conditions. Whereas in the genotype susceptible the expression levels were down-regulated. The same results were reported by (Espinoza et al 2018) in \textit{aestivum} wheat.

Conclusions

Water stress often triggers a down-regulation of AQPs transcript levels, but this is not a general rule. Indeed, positive regulation of specific AQPs genes resistant to drought has been reported. Whatever the regulation of AQPs expression (positive or negative) and the corresponding changes in membrane permeability have actually been suggested to be beneficial for plants under water stress. Zunaira et al (2016) conclude that the response of Aquaporins to drought conditions is highly variable depending on stress levels, isoforms, tissues, species, and the nature of the stimuli causing dehydration similar to water stress.
The breeding of wheat cultivars with augmented tolerance to water deficit requires, among other subjects, an exhaustive characterization of the molecular mechanisms that underlie physiological processes in the plant. The characterization of proteins related to the control of water status in tissues and organs, from a structural and functional genomics point of view, could contribute to this goal.

References


Forrest, K. L. and Bhave, M. (2008). The PIP and TIP aquaporins in wheat form a large and diverse family with unique gene structures and functionally important features. Functional and Integrative Genomics 8:115-133.


Genetic diversity and molecular characterization of Algerian Durum Wheat (*Triticum durum* Desf) Using molecular marker

KHENAOUI Amina, YKHLEF Nadia

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team IV Molecular Physiology and Plant Biodiversity’
Department of Biology and Ecology, Faculty of Natural Sciences and Life, Frères Mentouri University, Constantine, Algeria

Email: amina.khenaoui@umc.edu.dz

Introduction

In Algeria cereals in general, durum wheat in particular is the backbone of the food system. Occupies a vital place in socioeconomic terms. On the world market Algeria is among the major importers of cereals with 10 million ton import and wheat represents half of those imports (Ammar; 2014).

According to the Algerian Ministry of Agriculture statistics, the production of durum wheat has not exceeded 20 million quintals / hectare from the campaign 2010/2011 until the 2014/2015 campaign. There are multiple causes of:

- The areas under this crop have not changed for a long time.
- Production areas are characterized by high climatic variability.
- The rainfall > 450 mm / year (Ziza; 2007).

Faced with this situation several strategies can be applied from:

The study and characterization of genetic resources to create new varieties with good quality, high yield, adapted to climatic variations and resistant to diseases.

Currently the use of existing resources seems insufficient in relation to the ecological diversity of the Algeria and the requirements of modern agriculture. For adequate resolution: Our contribution is the study of the genetic variability of a collection of durum wheat varieties grown in Algeria by the use of SSR and RAPD markers.

Microsatellite (SSRs) were used for genetic diversity studies and localization of genes in wheat (Röder et al, 2004) and determining quantitative trait loci (Ganal et al, 2007). Microsatellites are one of the most promising types of molecular markers (Fahima et al, 2002, Singh et al, 2010). These properties make SSR popular for studies of genetic diversity (Yildirim et al, 2011). RAPD are another group of molecular markers simple, cheaper and more efficient over time (Kafeel, 2014, Cui Hou et al, 2005).
The importance of analyzing this genetic variability materializes through various ways to use these data for economic or scientific purposes. Economically, the exploitation of this database to meet the needs and interests of farmers will cope with biotic and abiotic stresses. From the scientific point of view, these data will also be used for breeders as a source of genes for creating new and better varieties.

**Experimentation**

**Plant materials:**

The plant material studied is composed of 26 varieties of durum wheat (*Triticum durum* Desf.) from various origins (CIMMYT; ICARDA; ITALY, Algerian varieties).

The seeds of 26 varieties were grown in a greenhouse under semi-controlled conditions with a temperature of 25-32 °C and a relative humidity of 40-55%. After 21 days of culture (at the 3 leaf stage), the leaves are harvested for DNA extraction.

**Isolation and purification of genomic DNA from wheat cultivars:**

The plant material was crushed in liquid nitrogen and reduced to a fine powder from the young leaf samples. Using ionic detergent cetethyltrimethyl ammonium bromide (CTAB); following the protocol of Saghai-Maroof; (1984) with a slight modification.

**SSR analysis**

Out of 19 pair of primers were tested to detect the polymorphism, only 10 pairs (Barc 100, Barc 142, Wmc 50, Wmc 177, Barc 119, Wmc 44, Wmc 105, Wmc 09, Gwm 386, Wmc 307) have recorded clear reproducible amplifications among the 26 wheat cultivars. The amplification was performed in a 25 μl final volume of a reaction mixture, which included 10-50 ng of genomic DNA, 0.2 mM dNTPs, 0.25 μM primers and 1 unit of Taq polymerase (GoTaq, Promega) and its once-concentrated buffer, containing 1.5 mM MgCl₂. The PCR reaction was performed using a thermocycler programmed for: 3 min initial denaturation at 95 °C, followed by 35 cycles, each consisting of: 1 min at 94 °C, 1 minute of hybridization at a specific temperature for each primer, 2 min at 72°C and a final extension of 10 min at 72 °C.

PCR products were evaluated by electrophoresis, on 3% agarose gels. Two specific size markers are used to compare the sizes of the resulting bands (100 bp size marker and a second 50 bp size marker) and photographed with E-BOX VX 2 system.
RAPD analysis

PCR reaction and condition:

A total of 05 random primers were used to detect the polymorphism among the 26 wheat cultivars. The sequence of the 05 primers that produce a clear sociable and reproducible banding pattern (OPG 09, OPF 20, OPA 17, OPE 13, OPC 05). The amplification performed in a 25 µL reaction volume containing: 12.5µl Taq Gold 360 Master Mix Amplifier (Applied biosystems) + 1µl primer + 1µl GC enhancer (360, GC Enhancer Applied Biosystems) + 1µl DNA + 9.5µl H2O.

The thermal cycler was programmed with an initial step of 5 min at 94 °C; the amplification reaction was carried out using 40 cycles of 60 sec at 94 °C, an annealing step of 1 min at 37 °C and an elongation step of 1 min at 72 °C and finally a 7 min extension at 72 °C (El Assal et Gabber; 2010). The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg mL−1) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using E-BOX VX2 system.

Data scoring and statistical analysis:

Clear and distinct amplification products were scored as ‘1’ for presence and ‘0’ for absence of bands. Bands of the same mobility were scored as identical. The Genetic Similarity coefficient (GS) between two genotypes was estimated according to coefficients "Jaccard for RAPD markers and simple matching coefficient (Sokal and Michener, 1958) for SSR markers". The similarity matrix was used in the cluster analysis.

Then the similarity dendrogram of two types of markers was established using the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) method. These analyzes were performed through the NTSYSpc version 2.02 analytics program.

Polymorphism information content (PIC) of each SSR was computed according to Botstein et al. (1980). The PIC values calculated from the RAPD markers are obtained according to the following formula: PIC = 2 × fi × (1-fi) (Roldán-Ruiz et al, 2000, Soengas et al, 2006), where, fi is the frequency of the amplified allele (present band) and (1-fi) is the frequency of the null allele (band absent).

The following variables were estimated from the RAPD marker data using the GenAlEx software version 6.502) (Peakall et Smouse ; 2006); Shannon Diversity Index and Analysis of molecular variance (AMOVA) was used to assess the variance between and within varieties from different geographical origins.
Results and Discussions

The analysis of 26 varieties of durum wheat by SSR markers significantly helped their differentiation. Ten primers used to amplify the DNA of each variety was registered in total, 291 polymorphic bands, the results revealed a variability within varieties. The total number of allele revealed by the primers is about 44 alleles varies between 01 observed in WMC 09 primers, Wmc 307 and 09 for primers WMC 50 and BARC 142 and BARC 100, with an average of 4.4 primer. The size of the bands generated by the primers varies between 50bp and 319Pb. Algerian varieties have higher values of number of alleles that modern variety. For this reason the old varieties that are very important as germplasm for breeders. The maximum PIC value is (0.85) and (0.73) in the primer WMC 307 and Barc 142 respectively. However, the minimum PIC value (0.21) obtained by WMC44 and Barc100. An average was recorded (0.45) for all the primers examined.

The classification of varieties by the method UPGMA showed a wide genetic diversity can be used in the selection of parents in the breeding program, and in the maintenance of genetic variation. Modern wheat cultivars have diverged from Algerian and Italian durum wheat in two distinct main groups. This genetic similarity between modern varieties (CIMMYT and ICARDA) can be explained by the common origin of allelic sources, as well as the imposition of a similar selection for specific traits. Geographic origin was also an important factor in the grouping. This result, which is in agreement with those of other authors, links the agronomic characters to the molecular ones.

The use of 05 RAPD primers generated 30 alleles. The results estimated by the RAPD markers also reveal a genetic polymorphism expressed by PIC values ranging between 0.22 to 0.40 with an average the 0.31, the lowest and the highest obtained respectively by OPC 05 and OPF 20, and an important value of Shannon Diversity Index (0.410). In addition, AMOVA (Analysis of Molecular Variance) showed that the genetic variation within the groups represents 97% (ΦPT = 0.173 p <0.05).

Six private alleles were revealed by the microsatellites present in the varieties (Beliouni and Djennah-khetifa, GTA / DUR69 and Capeiti 8, Beni Mestima), and three by the RAPD markers in the varieties (Beni Mestina, Djnnah khetifa and Tassili). These varieties are a very good material for starting an improvement program. Some studies associate the high number of private alleles with the high rate of mutations but also with a possible selection of these specific alleles associated with the morphology and the area of adaptation of the accessions.

The dendrogram, based on the statistical analysis of RAPD markers using the UPGMA method, the UPGMA method has made some exploitable discriminations possible. However, the interpretation of some cases of separation and / or genetic approximation remains highly controversial. The RAPD markers used also made it possible to make a good estimate of the genetic
diversity through the different parameters studied. The results obtained are promising, given the level of genetic diversity recorded.

Indeed, SSR and RAPD markers sometimes reveal groups of varieties partially isolated from each other who may have fixed distinct alleles for useful characters, under the action of different selection pressures.

Therefore, we do not assume that the differences in genetic relationships revealed by the RAPD and SSR markers can be attributed solely to differences in the level of polymorphism detected by each marker system; they rather reflect the complexity in heredity of adaptability traits. Discrepancy between different marker systems can be very instructive in understanding genetic relationships within the study group (Mardi et al., 2011). The results obtained in this study will provide evidence for decision making in the selection of markers for future work for the characterization, selection and management of durum wheat germplasm.

Conclusion

In the context of future work, it would be desirable to produce dense genetic maps that make it possible, on the one hand, to position loci with a qualitative or quantitative effect and, on the other hand, to estimate the effects of these loci. on the different characters relating to productivity and technological quality, through the exploitation of complementarities between phenotypic characterization and molecular markers, in order to establish a molecular marker assisted selection strategy. This objective implies real research efforts to make the best use of the available genetic resources and meet the new challenges of human nutrition in terms of innovation and diversification of cereal products.

References


**In vitro selection for saline and thermal stress tolerance in bread wheat**  
*(Triticum aestivum L.)*: Morpho-physiological, biochemical and molecular aspects

**BENDERRADJI Laid and YKHLEF Nadia**

Laboratory of Genetics, Biochemistry and Plant Biotechnology ‘Team IV Molecular Physiology and Plant Biodiversity’ Frères Mentouri University, Constantine, Algeria

SNV Department, Faculty of Sciences, UMB, M’sila - Msila 28000, Algeria

Email: benderradjilaid@yahoo.fr

Introduction

The scientific research that has been carried out within plant biotechnology team at the Laboratory of Genetics, Biochemistry and Plant Biotechnology (GBPBL) focuses on a serious problem affecting cereals and more specifically the cultivation of wheat and especially bread wheat *(Triticum aestivum L.)*, in view of their importance in human food and their permanent uses in bread making. To do this, the proposed work was started, firstly, by determining the appropriate methodology to achieve well-targeted objectives. For this, several axes including different aspects were set in motion, such as the effect of salt stress on morpho-physiological characters and the expression of candidate genes in two varieties of bread wheat *(T. aestivum L.)*, Mahon-Demias (MD) and Hidhab (HD); the effect of saline and thermal stress on the proliferation of callus and the regeneration of seedlings from mature embryos of the two varieties previously used.

Aims the work carried is to define the methods of selection and improvement of bread wheat in the high plains in the Sétif and Constantine regions in Eastern Algeria. As a result, the first part concerning the morpho-physiological aspect which was carried out in the laboratory (GBPBL), with emphasis on the tolerance of bread wheat to saline and thermal stress. These two types of abiotic stress diminish production and affect the grain yield and consequently the cereal production whose bread wheat cultivation is taken as a priority by the Algerian state which continues to provide this type of food, even if the import bill is up from one year to another. It should be noted that average yields in favorable environments reached 7 t/ha and increased by 1% per year (Hervieu *et al.*, 2006). Genetic progress accounts for 50% of this improvement (Slaffer *et al.*, 2005). The cultivation of environments more favorable to the expression of the production potential of the new varieties, as well as the improvement of the technical itinerary, which became possible, greatly helped to increase the productivity per unit of soil surface. (Slaffer et Araus, 2007). In variable environments, the contribution of new accessions was, on the other hand, hardly perceptible or even nil (Sinebo, 2005). Negative effects have even been reported (Royo *et al.*, 2007). Cereal production in dry rainfed areas...
remained low and very irregular in space and in time too (Annichiarico et al., 2005). The search for a better adaptation to environmental variation has become a necessity to stabilize the yields of these regions. The variation in yields is caused by the sensitivity of new cultivars to the various biotic and abiotic stresses that characterize the production environment.

Richard et al., (1997) suggest, in this context, that physiological approaches are the most recommended selecting in such environments. The difficulties associated with this research are, however, the large number of factors that seem to condition these characteristics (Reynolds et al., 2007). These factors have effects on several organizational levels, from the cell to the scale of the whole plant. It is difficult to identify who is the most effective and who can make the best possible progress in a given environment. Reynolds et al., (2007) explain that the inefficiency of the analytic approach is in relation with the variability of linkages between targeted traits and production in constraining environments. This approach is neither reliable enough nor simple enough to be applicable to hundreds or even thousands of plants under selection. Breeders therefore remain fairly convinced that physiological approaches cannot produce better results than the empirical methods they have used so far. These methods are, in fact, more effective insofar as appreciable gains in yields are obtained in favorable environments. They, therefore, show little enthusiasm or enthusiasm for using physiological approaches in selection. The character most used in selection is the performance itself. This direct selection has, in most cases, been ineffective in identifying suitable and stress-tolerant genotypes (Rodrigues et al., 2008). The response exhibited by plants to various abiotic stresses is a characteristic under complex genetic control. It is determined in part by the cellular properties. It is therefore possible to select tolerant cell lines and to generate interesting varieties for inclusion in the breeding program as pool genes. Plant biotechnology, by allowing the precise control of environmental conditions and the rapid screening of a large number of plant cells

A very small space, represent in this sense a privileged approach for the implementation of screening tests applicable at the cellular level (Eleuch et al., 2008). The use of these plant biotechnology techniques has proven effective in making progress in understanding the mechanisms involved in stress tolerance (Baum et Johnson, 2007). In addition, the identification of genes induced under stress conditions is a very promising approach in an improvement program. Indeed, the isolation of candidate genes and their characterization should allow in the short term their transfer to other high yielding plants, generally sensitive to abiotic stresses. Therefore, our scientific research proposes as objectives the evaluation of the tolerance of two bread wheat varieties (T. aestivum L.), previously mentioned, namely (MD) and (HD), with abiotic stresses. through the study of the morphological and physiological behavior of these two varieties under different abiotic stress conditions and the implementation of a robust experimental protocol for callus induction and
maintenance to facilitate the callogenesis followed by a caulogenesis and then the regeneration of whole seedlings from stressed callus and other non-stressed, by exploiting the possibilities offered by in vitro culture to select salt and heat stress tolerant cell lines and to identify the morphological identification of somaclonal variation and finally, the study of the level of expression of a certain number of candidate genes such as ion transporters and the biochemical characterization of enzymatic activity (oxidant and antioxidant) in both varieties under different stress conditions. Results obtained during this scientific research and over the years of realization of experiments at the laboratory of genetics, biochemistry and plant biotechnologies of Constantine university in Algeria and also at the laboratory of Protection and Plant Improvement at Centre of Biotechnology of Sfax (CBS) in Tunisia) have resulted in three aspects cited below.

**Morpho-physiological aspect**

A first part of this work done in the laboratory of Constantine University was focused on the in vitro regeneration capacity of mature embryos of two varieties (MD) and (HD), to determine the genotypic capacities concerning the different stages of plant cloning, and at the same time the use of growth regulators, such as, the calogenesis auxin namely (2.4, D.) for callus formation, cytokinins (Ken and BAP) for aerial part formation and other auxins (AIA and ANA) for root formation. All these steps were carried out under controlled conditions of light, temperature and hygrometry under the effect of salt and heat stress. This part was sanctioned by a publication (Benderradji *et al.*, 2007) in the journal (PTC & B): Plant Tissue Cult. & Biotech. 17 (1): 19-27, 2007 (June), with the title "Effects of NaCl stress on callus proliferation and plant regeneration from mature embryos of wheat bread (Triticum aestivum L.) Cultivars: Mahon-Demis and Hidhab". A second part of the work also took place in the laboratory of Constantine University, from where all manipulations are done in a greenhouse and the results obtained are the subject of another publication (Benderradji *et al.*, 2010), appeared in the journal “Sciences & Technology” of Mentouri Constantine University in 2010, (N ° 32-December, 2010. PP 23-30), under title: Study of the mechanisms of tolerance to salinity in two varieties of bread wheat (Triticum aestivum L.), subjected to salt stress. A third part of the work concerns the regenerative ability of whole seedlings by applying one of the in vitro culture techniques namely culture of mature embryos. This manipulation was also carried at the laboratory of Constantine University and was the subject to a third publication (Benderradji *et al.*, 2012), published in the journal ISRN Agronomy (2012), Article ID 367851, 8 pages with the title: "Callus induction, proliferation, and plantlets regeneration of two bread wheat (Triticum aestivum L.), Genotypes under salt and heat stress conditions".
Molecular aspect

This part was performed at the laboratory of Protection and Plant Improvement CBS, Sfax-Tunisia), where it was found that the genes HKT: 1; 5 and HKT: 2; 2 linked to ion transporters in two varieties (MD) and (HD) of bread wheat (T. aestivum L.), subjected to salt stress, are expressed only in the roots, with a better expression of HKT: 1; 5 at (HD) variety. These two genes are involved in the transport of Na^+ & K^+ through the plasma membrane of the cortical cells of the roots, with however a more active role of the HKT1 gene in the tolerant variety (HD). Levels of Na^+/H^+ TNHX-1 vacuolar anti-carrier transcripts in the roots, sheath and lamina are higher in (HD) variety. This difference in expression is explained by the difference in accumulation of the Na^+ ion in the vacuoles. The level of expression of vacuolar pyro-phosphatase H^+-PPase, TVP1 is comparable to that observed for transcripts of the TNHX1 gene. The roots and sheath of both varieties accumulate more TVP1 transcripts than leaf blade. The similarity of the expression type of the genes TNHX1 and TVP1 is noted in (MD) and (HD) varieties, suggests that vacuolar compartmentalization acts with equal effectiveness. More transcripts of the Ta-SOS-1 gene (anti Na^+/H^+ plasma membrane carrier) that accumulates in the roots and sheath of (MD) compared to (HD). This type of expression suggests that in addition to a better efficiency of retention of the Na^+ ion in the sheaths, the (HD) variety avoids the accumulation of the Na^+ ion in the limb by activating its efflux via a high expression of SOS1 gene. Results indicate that salinity tolerance in bread wheat appears to be related to the ability to avoid the accumulation at toxic levels of the Na^+ ion, associated with a high capacity for osmoregulation and/or maintenance of acceptable level of K^+, especially in leaf blade. It should be noted that the work done at CBS, Sfax-Tunisia is oriented towards the molecular genetics of plants based on the techniques of one-dimensional electrophoresis and PCR. The manipulations of RT-PCR (reverse transcriptase-poly chain reaction) - Trizol method was the most used method to extract the RNA from the different organs of the plant after stressing, then synthesizing the cDNA (Complementary Brin DNA) and subsequently the amplification of a number of genes set in motion for distinction, evaluation and introduction into plants as gene of interest (candidate gene). These very interesting results obtained were published (Benderradji et al., 2011), in an international newspaper (March 2011, AJCS - [5 (3) 2011]: Pages 233-241. The article that appeared in 2011 was titled: "Sodium transport in the seedlings of two bread wheat (Triticum aestivum L.), genotypes showing contrasting salt stress tolerance".

Biochemical aspect

The biochemical aspect was studied by measuring the activity of many antioxidant enzymes using enzymatic extract, through the extraction of plant material composed of fresh material leaves of seedlings of the two varieties (MD) and (HD) subjected to the different levels of salt stress, by quickly
grinding this fresh material with the help of liquid nitrogen until a fine powder is obtained. The enzymatic activity of superoxide dismutase (SOD) is then measured, which is determined by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the experimental protocol described by Velikova et al., (2000). The activity of Catalase (CAT) is determined by measuring the initial rate of hydrogen peroxide dis-appearance according to the method of Velikova et al., (2000). The activity of ascorbate peroxidase (APX) is determined according to the description of Yamaguchi et al., (1995). The levels of hydrogen peroxide (H$_2$O$_2$) are determined according to the method of Velikova et al., (2000). The study of the biochemical aspect was completed by the determination of the chlorophyll content, the soluble protein assay and the proline estimation. The method used for the extraction of chlorophyll is that established by Holden (1975), and then for the determination of soluble proteins, the method used is that of (Bradford, 1976), however the estimation of proline, the method used is that of (Monneveux et Nemmar, 1986).

References


## Index

<table>
<thead>
<tr>
<th>B</th>
<th>Université Frères Mentouri Constantine 1</th>
<th><a href="mailto:ies.bellil@umc.edu.dz">ies.bellil@umc.edu.dz</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>BELLIL Inès</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BENABDELHAFID Zoheira</td>
<td>Ecole Nationale Supérieure (ENS) Constantine</td>
<td><a href="mailto:b.zoheira_28@yahoo.fr">b.zoheira_28@yahoo.fr</a></td>
</tr>
<tr>
<td>BENABDELHAFID Zoheira</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BENDERRADJI Laid</td>
<td>Université Mohamed Boudiaf, M'sila</td>
<td><a href="mailto:benderradjilaid@yahoo.fr">benderradjilaid@yahoo.fr</a></td>
</tr>
<tr>
<td>BENMATI Mahbouba</td>
<td>Ecole Nationale Supérieure de Biotechnologie</td>
<td><a href="mailto:benmati.m@gmail.com">benmati.m@gmail.com</a></td>
</tr>
<tr>
<td>BENTAHAR Soumia</td>
<td>Centre Universitaire Abdelhafi Boussouf, Mila</td>
<td><a href="mailto:soumia_bentahar@yahoo.fr">soumia_bentahar@yahoo.fr</a></td>
</tr>
<tr>
<td>BOUHCHELMA Karima</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:karima.bouchemal@umc.edu.dz">karima.bouchemal@umc.edu.dz</a></td>
</tr>
<tr>
<td>BOUSBA Ratiba</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:ratiba.bousba@.umc.edu.dz">ratiba.bousba@.umc.edu.dz</a></td>
</tr>
<tr>
<td>BOUCAKAMAL Karima</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:kerim.bouchemal@umc.edu.dz">kerim.bouchemal@umc.edu.dz</a></td>
</tr>
<tr>
<td>DJEKOUN Abdelhamid</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:djekoun@umc.edu.dz">djekoun@umc.edu.dz</a></td>
</tr>
<tr>
<td>HAMIDECHI Mohamed Abdelhafid</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:Hamidechi.abdelhafid@umc.edu.dz">Hamidechi.abdelhafid@umc.edu.dz</a></td>
</tr>
<tr>
<td>HAMLA Chourouk</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:chourouk.hamla@umc.edu.dz">chourouk.hamla@umc.edu.dz</a></td>
</tr>
<tr>
<td>HAYOUNE Houda</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:hayoune.houda@yahoo.fr">hayoune.houda@yahoo.fr</a></td>
</tr>
<tr>
<td>K</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:kacem.nadia@umc.edu.dz">kacem.nadia@umc.edu.dz</a></td>
</tr>
<tr>
<td>KACEM Nadia Sandra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KECHID Maya</td>
<td>I.N.A.A.T.A - Université des Frère Mentouri Constantine 1</td>
<td><a href="mailto:maya.kechid@umc.edu.dz">maya.kechid@umc.edu.dz</a></td>
</tr>
<tr>
<td>KELLOU Kamel</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:kamel.kellou@umc.edu.dz">kamel.kellou@umc.edu.dz</a></td>
</tr>
<tr>
<td>KENNAOUI Amina</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:mina_kh87@hotmail.fr">mina_kh87@hotmail.fr</a></td>
</tr>
<tr>
<td>KHELIFI Douadi</td>
<td>Ecole Nationale Supérieure de Biotechnologie Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:dkhelifi@yahoo.fr">dkhelifi@yahoo.fr</a></td>
</tr>
<tr>
<td>L</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:louali.yamouna@gmail.com">louali.yamouna@gmail.com</a></td>
</tr>
<tr>
<td>LOUALI Yamouna</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:rym.maougal@umc.edu.dz">rym.maougal@umc.edu.dz</a></td>
</tr>
<tr>
<td>MAOUGAL Rym</td>
<td>I.N.A.A.T.A - Université des Frère Mentouri Constantine 1</td>
<td><a href="mailto:rym.maougal@umc.edu.dz">rym.maougal@umc.edu.dz</a></td>
</tr>
<tr>
<td>MEDOURI Asma</td>
<td>Université Mohamed Essedik Ben Yahia, Jijel</td>
<td><a href="mailto:asmabioch@yahoo.fr">asmabioch@yahoo.fr</a></td>
</tr>
<tr>
<td>MOUELLEF Adra</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:adra.mouellef@umc.edu.dz">adra.mouellef@umc.edu.dz</a></td>
</tr>
<tr>
<td>N</td>
<td>Ecole Nationale Supérieure de Biotechnologie</td>
<td><a href="mailto:wassila.nadji@yahoo.fr">wassila.nadji@yahoo.fr</a></td>
</tr>
<tr>
<td>NADJI Wassila</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>Université Frères Mentouri Constantine 1</td>
<td><a href="mailto:nykhlef@yahoo.fr">nykhlef@yahoo.fr</a></td>
</tr>
<tr>
<td>YKHLEF Nadia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Université Frères Mentouri Constantine - Algérie
Faculté des Sciences de la nature et de la vie
Laboratoire de Génétique Biochimie, Biotechnologies végétales

www.umc.edu.dz

INTERNATIONAL SEMINAR
GENOME AND WHEAT SEQUENCING
28-29 Janvier 2019