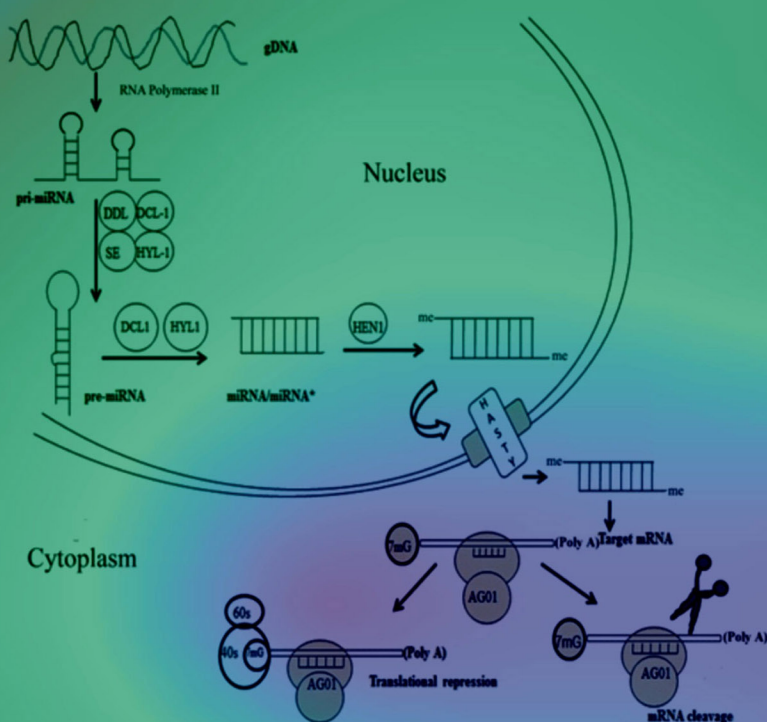


Molecular Approaches in Plant Abiotic Stress

Editors

R.K. Gaur and Pradeep Sharma



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Editors

R.K. Gaur

Department of Science
Faculty of Arts, Science and Commerce
Mody Institute of Technology & Science
Sikar, India

Pradeep Sharma

Directorate of Wheat Research
Division of Crop Improvement
Karnal, India



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Preface

Abiotic stresses including drought are serious threats to the sustainability of crop yield accounting for more crop productivity losses than any other factor, i.e., virus, bacterial or fungal in rainfed agriculture. Studies on low temperature and plant are expected to contribute on understanding of agriculturally important traits such as low temperature-induced male sterility, germinability under low temperature, and precocious sprouting. In this connection our proposed book will be key for the researchers and scientists working in the abiotic research.

The main objective of this book is the demonstration of a clear synergistic effect of stresses. This book will add to our current knowledge of abiotic stress response in plants and will provide groundwork necessary to build future strategies for crop enhancement by using regulatory genes. The fundamental principles that underpin all biotechnology are explained and a full range of examples are discussed to show how these principles are applied; from starting substrate to final product. This book is expected to provide the most recent information regarding advances in genetics and physiology of abiotic stress response and crop improvement.

The proposed book will be beneficial to both plant breeders and molecular biologists, because it combines the topics of mathematical modelling, physiology, tolerance genes, and breeding methods. When these topics are presented together, it is easy to compare all aspects of tolerance mechanisms and breeding methods for abiotic stresses. These comparisons are useful to understand which pathways or which genes are important for rendering more tolerance to a certain abiotic stress, and to bring forward new ideas for improving the tolerance.

R.K. Gaur
Pradeep Sharma

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List of Contributors

Murilo Siqueira Alves

Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.

O.A. Avksentyeva

Department of Plant Physiology and Biochemistry, Kharkov V.N. Karazin National University, 4, sq. Svoboda, Kharkov, 61022, Ukraine.

Ana Maria Benko-Iseppon

Department of Genetics, Federal University of Pernambuco, Avenue Professor Moares Rêgo, 1235, CEP 50670-420, Recife, PE, Brazil.

Borja Belda-Palazón

Institute for Plant Molecular and Cellular Biology, CSIC-Polytechnic University of Valencia, 46022 Valencia, Spain.

Marco Betti

Department of Plant Biochemistry and Molecular Biology, Faculty of Chemistry, University of Seville, C/Profesor García González, 1; 41012-Sevilla, Spain.

Siddanagouda S. Biradar

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy and Yangling Branch of China Wheat Improvement Center, Northwest A&F University, Yangling 712100, Shaanxi, China.

Margarita García Calderón

Department of Plant Biochemistry and Molecular Biology, Faculty of Chemistry, University of Seville, C/Profesor García González, 1; 41012-Sevilla, Spain.

Daniela Andrea Capiati

Genetic Engineering and Molecular Biology Research Institute “Dr. Hector N. Torres”, INGEBI, CONICET and Faculty of Exact and Natural Sciences, University of Buenos Aires. Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina.

Email: dcapiati@dna.uba.ar; dcapiati@gmail.com

M. Mar Castellano

Centre for Plant Biotechnology and Genomics, INIA-UPM, Campus de Montegancedo, 28223 Madrid, Spain.

Sharmila Chattopadhyay

Plant Biology Laboratory Drug Development/Diagnostics & Biotechnology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India.
Email: sharmila@iicb.res.in

Anastasis Christou

Department of Environmental Science and Technology, Cyprus University of Technology, 3603 Lemesos, Cyprus.

Mélanie Cordier

University of Neuchâtel, Department of Sciences, Institute of Biology, Laboratory of Molecular and Cell Biology, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.

Pingchuan Deng

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy and Yangling Branch of China Wheat Improvement Center, Northwest A&F University, Yangling 712100, Shaanxi, China.

Pedro Díaz

Biochemistry Laboratory, Department of Plant Biology, Faculty of Agronomy, Av. Garzón. 780; CP12900, Montevideo, Uruguay.

Veronika Doubnerová

Charles University in Prague, Faculty of Natural Science, Department of Biochemistry, Hlavova 8, 128 43 Prague 2, Czech Republic.
Email: Veronika.Doubnerova@natur.cuni.cz

Alejandro Ferrando

Institute for Plant Molecular and Cellular Biology, CSIC-Polytechnic University of Valencia, 46022 Valencia, Spain.
Email: aferrando@ibmcp.upv.es

Luciano Gomes Fietto

Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.
Email: lgfietto@ufv.br; murilobqi@gmail.com

Vasileios Fotopoulos

Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603 Lemesos, Cyprus.
Email: vassilis.fotopoulos@cut.ac.cy

María Noelia Muñiz García

Genetic Engineering and Molecular Biology Research Institute “Dr. Hector N. Torres”, INGEBI, CONICET and Faculty of Exact and Natural Sciences, University of Buenos Aires. Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina.

Rajarshi Kumar Gaur

Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Lakshmangarh, Sikar-332311, Rajasthan, India.

Email: gaurrajarshi@hotmail.com

Divya Gupta

Department of Biotechnology, G.B. Pant Engineering College, Pauri Garhwal, Uttarakhand, India.

Om Prakash Gupta

Quality and Basic Science, Directorate of Wheat Research, Karnal-132001, Haryana, India.

Saeed Sadeghzade Hemayati

Sugar Beet Seed Institute (SBSI), Karaj, Iran.

Arnd G. Heyer

University of Stuttgart, Institute of Biology, Dept. of Plant Biotechnology, Pfaffenwaldring 57, 70569 Stuttgart, Germany.

Email: Arnd.Heyer@bio.uni-stuttgart.de

Rohit Joshi

School of Plant, Environmental and Soil Sciences, Louisiana State University Agricultural Center, Baton Rouge, Louisiana, USA

Ratna Karan

Agronomy Department, University of Florida, IFAS, Gainesville, Florida, USA.

Email: rkaran@ufl.edu

Éderson Akio Kido

Department of Genetics, Federal University of Pernambuco, Avenue Professor Moares Rêgo, 1235, CEP 50670-420, Recife, PE, Brazil.

Suzana de Aragão Britto Kido

Department of Genetics, Federal University of Pernambuco, Avenue Professor Moares Rêgo, 1235, CEP 50670-420, Recife, PE, Brazil.

Aline Medeiros Lima

Institute of Biological Sciences, Federal University of Pará, Guamá, Belém, PA 66075-110, Brazil.

Email: alinemedeiros14@hotmail.com

Mamrutha H.M.

Directorate of Wheat Research, Karnal, Haryana, India.

George Manganaris

Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603 Lemesos, Cyprus.

Antonio J. Márquez

Department of Plant Biochemistry and Molecular Biology, Faculty of Chemistry, University of Seville, C/Profesor García González, 1; 41012-Sevilla, Spain.

Email: cabeza@us.es

Avinash Marwal

Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Lakshmangarh, Sikar-332311, Rajasthan, India.

Brigitte Mauch-Mani

University of Neuchâtel, Department of Sciences, Institute of Biology, Laboratory of Molecular and Cell Biology, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.

Email: brigitte.mauch@unine.ch

Anita Meena

Directorate of Wheat Research, Karnal, Haryana, India.

Livia Atauri Miranda

University of Neuchâtel, Department of Sciences, Institute of Biology, Laboratory of Molecular and Cell Biology, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.

Jorge Monza

Biochemistry Laboratory, Department of Plant Biology, Faculty of Agronomy, Av. Garzón. 780; CP12900, Montevideo, Uruguay.

Thomas Nägele

University of Vienna, Dept. of Molecular Systems Biology, Althanstr. 14, 1090 Vienna, Austria.

José Ribamar Ferreira Neto

Department of Genetics, Federal University of Pernambuco, Avenue Professor Moares Rêgo, 1235, CEP 50670-420, Recife, PE, Brazil.

María Noelia

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular “Dr. Hector N. Torres”, INGEBI, CONICET and Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina.

Peyman Norouzi

Sugar Beet Seed Institute (SBSI), Karaj, Iran.

Eric Ober

Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK.

Peter Pal’ove-Balang

Institute of Biology and Ecology, P.J. Šafárik University, Mánesova 23, SK-04001 Košice, Slovak Republic.

Valesca Pandolfi

Department of Genetics, Federal University of Pernambuco, Avenue Professor Moares Rêgo, 1235, CEP 50670-420, Recife, PE, Brazil.

Carmen M. Pérez-Delgado

Department of Plant Biochemistry and Molecular Biology, Faculty of Chemistry, University of Seville, C/Profesor García González, 1; 41012-Sevilla, Spain.

Abazar Rajabi

Sugar Beet Seed Institute (SBSI), Karaj, Iran.
Email: rajabi@sbsi.ir

Khadije Razavi

National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

Sávio Pinho dos Reis

Institute of Biological Sciences, Federal University of Pará, Guamá, Belém, PA 66075-110, Brazil.
Email: saviopr@yahoo.com

Ines Ben Rejeb

University of Neuchâtel, Department of Sciences, Institute of Biology, Laboratory of Molecular and Cell Biology, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.

Ansuman Roy

Department of Biology and Microbiology, South Dakota State University, Brookings, USA.
Email: ansumanroy@gmail.com

Helena Ryšlavá

Charles University in Prague, Faculty of Natural Science, Department of Biochemistry, Hlavova 8, 128 43 Prague 2, Czech Republic.

Email: Helena.Ryslava@natur.cuni.cz

Seyed Yaghoub Sadeghian

Azad University of Mianeh, Mianeh, Iran.

Anurag Kumar Sahu

Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Lakshmangarh, Sikar-332311, Rajasthan, India.

Pradeep Sharma

Crop improvement, Directorate of Wheat Research, Karnal-132001, Haryana, India.

Email: neprads@gmail.com

Sonia Sheoran

Directorate of Wheat Research, Karnal, Haryana, India.

Allan T. Showler

USDA-ARS, 2700 Fredericksburg Road, Kerrville, Texas 78028, USA.

Email: Allan.showler@ars.usda.gov

Ajeet Singh

Department of Biotechnology, G.B. Pant Engineering College, Pauri Garhwal, Uttarakhand, India.

Email: ajeetsoniyal@gmail.com

Virender Singh

Directorate of Wheat Research, Karnal, Haryana, India

Cláudia Regina Batista de Souza

Institute of Biological Sciences, Federal University of Pará, Guamá, Belém, PA 66075-110, Brazil.

Email: bsouza@ufpa.br

Song Weining

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy and Yangling Branch of China Wheat Improvement Center, Northwest A&F University, Yangling 712100, Shaanxi, China.

Email: sweining2002@yahoo.com

Deying Xie

College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China.

Guosheng Xie

College of Plant Science and Technology, Huazhong Agricultural University,
Wuhan 430070, China.

V.V. Zhmurko

Department of Plant Physiology and Biochemistry, Kharkov V.N. Karazin
National University, 4, sq. Svoboda, Kharkov, 61022, Ukraine.

Email: zhmurko@univer.kharkov.ua

Sira Echevarría Zomeño

Centre for Plant Biotechnology and Genomics, INIA-UPM, Campus de
Montegancedo, 28223 Madrid, Spain.

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1

Genes *Ppd* and *Vrn* as Components of Molecular Genetic System of Wheat Regulation Resistance (*Triticum aestivum* L.) to Abiotic Stress

O.A. Avksentyeva and V.V. Zhmurko*

ABSTRACT

The level of abiotic stress impact on plants has significantly increased in recent times. This has led to an increase in interest to study plant resistance mechanisms at different levels of its organization. This chapter provides an overview of published data on physiological-biochemical and molecular-genetic mechanisms of plant resistance to heat and drought, which shows the significant progress in this direction. However, it is clear that there is insufficient research on the role of specific genes in the formation of plant resistance to these environmental factors. This largely relates to the genes of photoperiodic sensitivity (*Ppd*) and vernalization requirements (*Vrn*), determining the development of one of the most important food crops of the

Department of Plant Physiology and Biochemistry, Kharkov V.N. Karazin National University,
4, sq. Svoboda, Kharkov, 61022, Ukraine.

* Corresponding author: zhmurko@univer.kharkov.ua

world—soft wheat *Triticum aestivum* L. The research conducted by the authors has shown that heat resistance and drought tolerance of this crop is associated with the conditions (dominant and/or recessive) of gene-specific loci Ppd and Vrn.

Key words: *Triticum aestivum* L., genes Ppd and Vrn, rate development, heat tolerance, drought, resistance mechanisms

Introduction

In the course of evolution, plants have formed mechanisms of resistance to action of stressors. Stability refers to the ability of plants to preserve the constancy of the internal environment and to implement life cycle in conditions of the stressors.

The most common manifestation of the stressors is suppression of plant growth and development. Stressors lead to a decrease in growth rate to a level lower than the level conditioned by genetic potential of plants.

Stress reactions at the cellular level of organization of living matter to various adverse effects are similar: increased permeability of the membrane, marked changes in the nucleus, denatured proteins, coagulated cytoplasm, etc. (Kolupaev and Karpets 2010). These responses are called non-specific as they are accompanied by various injuries and are observed in cells of any tissues. Specific responses are qualitatively different depending on the factors and genotype. Formation of non-specific sustainability elements (synthesis of heat shock proteins, polyamines) takes much less time than for the passage of specific adaptive reactions (synthesis of antifreeze proteins, switching to CAM photosynthesis path and so on). The specific response to the action of extreme factors is controlled by genetic mechanisms through the work of protein-synthesizing apparatus. The basis of non-specific response is physiological plasticity (changes in structure and activity of cellular proteins, the plasticity of membrane components, etc.) (Kordum et al. 2003).

Sometimes the plant becomes resistant to multiple types of adverse conditions. These manifestations are called conjugate stability of plants (cross-resistance) when one of the properties, which in its turn almost always causes a change in a number of other traits conjugated with the first one in an organism, is changing as a result of adaptation (Kordum et al. 2003). The most striking example of the stability of the conjugate resistance is pre-sowing hardening of plants against drought. It has been found out that this hardening leads to an increase in ability to better tolerate not only dehydration, but overheating as well. The similarity in reactions of plants to a number of unfavorable factors, presence of positive stability of the conjugate indicates that resistance to different extreme effects can be

controlled by the same internal factors. Here, both non-specific reactions and specific responses to different external influences carried by a single type of genetic regulation of physiological processes, are of importance.

Genetic Regulation of Resistance

It is known that after the plant cell has perceived a stress signal it is passed on to the nucleus. The resulting mRNAs determine the synthesis of protein products of early genes which become new transcription factors. The latter stimulates later genes whose activity is realized within a few hours or days (Amtmann et al. 2005).

Perception and transmission of stress signals to the nucleus are carried out as follows. Receptor localized on the plasma membrane receives the signal and sends it through the system of intermediates—signal transducers (Kolupaev and Karpets 2010). Proteinkinases and phosphatases either phosphorylate transcription factors themselves in a nucleus, or their phosphorylated proteins penetrating into the nucleus interact with transcription factors. This leads to activation of stress-inducible genes and, consequently, synthesis of mRNA and stress proteins that increase plant resistance (Kosakovskaya 2008). Induction of HSP under heat shock (HS) is a universal model for studying the molecular mechanism of genes switching, regulation of their activity and the restructuring of protein-synthesizing system under the action of the stressor (Feder and Hofmann 1999).

Switching one of the normal life of a cell to stress program includes reprogramming of the genome—expression of genes becomes inhibited whose activity is characteristic of living cells under normal conditions and heat shock genes are activated. Heat shock causes not only reprogramming of the genome and, consequently, changes in the composition of newly synthesized mRNA but reprogramming of ribosomes—disintegration of polysomes synthesizing proteins typical for normal living conditions and the formation of polysomes synthesizing PHS. Heat shock (HS) causes changes in mRNA synthesized in the cell before the shock, modification of the protein translation factors and ribosomal proteins occurs. In addition, the PHS mRNA are different from the normal protein mRNA. All this leads to the weakening and then cessation of normal proteins synthesis in cells and switching apparatus of protein synthesis to the synthesis of HSP. The inclusion of HSP genes at high temperature is determined by the HSP gene regulatory elements, i.e., specific nucleotide sequences in the promoter (regulatory) zone of these genes. Protein denaturation occurs under temperature stress which in turn causes a stress response switching on the intracellular mechanisms, among them increase of heat shock proteins number, whose primary function is to correct installation of newly synthesized polypeptides and re-installation of incorrectly packed

and damaged polypeptide chains. HSPs are one of the most common and conservative elements of the stress protection among different types of organisms (Kosakovskaya 2008). A number of HSPs are synthesized in the cell under normal conditions and, constitutively, their expression is further induced by various physiological disorders and stress. The main function of HSPs is to provide the proper nature of folding polypeptide chains in the native structure. These proteins by binding to unfolded or partially unfolded polypeptide chain do not allow it to “get lost”, to form irregular conformation. They hold the partially unfolded protein, promote its transfer to different subcellular formations and create conditions for its efficient folding. Many so-called heatshock proteins have chaperone activity.

Thanks to chaperone activity the following happens: (1) maintaining the HSP partners in a folded or unfolded conditions, (2) localization of proteins in organelles, their import and/or export, (3) minimization of non-native protein aggregation, and (4) direction of non-native or aggregated proteins for degradation and removal from the cell (Feder and Hofmann 1999). HSPs are involved in the regulation of protein homeostasis; they interact with other cellular proteins and are necessary for the synthesis, maturation and degradation of proteins in all parts of the cell.

Heat and Drought Resistance of Wheat

Wheat is the most valuable food crop which occupies a leading position in the grain balance of Ukraine. Wheat is grown in a variety of conditions that can often be adverse (low and high temperatures, lack of water, oxygen, excess salts, etc.), which leads to a drastic reduction of yield of crops, and even its destruction (Morgun et al. 2010). Wheat is the main cereal food crop in Ukraine, demanding heat and soil fertility. The effects of drought and heat on wheat plants can be significant and inevitably would lead to destruction if they did not have specific morphological, physiological and biochemical mechanisms of resistance. At present special attention is paid to breeding of highly resistant varieties. The work is carried out in different directions, including through the identification of genes that determine wheat resistance (Reynolds et al. 2007). The productivity of wheat depends on the implementation of genetically inherent yield potentials and the influence of the specific climatic conditions existing at a particular stage of plant development (Cattivelli et al. 2009; Morgun et al. 2010).

Under the influence of heat, the proteins contained in the cytoplasm of plant cells coagulate, losing their biological activity but the temperature threshold of coagulation in different wheat varieties differ. In addition, heat-resistant wheat genotypes may differ from the less resistant in more efficient use of water at high temperature (Mason et al. 2011). Critical period in water consumption is the phase of stem elongation and heading, i.e., during

formation of reproductive organs. For almost all plants, heat is especially dangerous during flowering because it causes sterility of pollen and falling of ovaries. Thus, the effect of high temperature and low humidity during the formation of wheat pollen in the anthers, when the process of pollination and seed formation takes place, leads to incomplete grains (not quite full ear) and empty ears. Extremely high temperatures during the period of milky ripeness of summer wheat causes grain hollowness—"fuse". Winter wheat is quite heat-resistant crop. However, at extremely high temperatures (above 40°C), with little humidity and dry winds, the normal process of plant photosynthesis disrupts, transpiration increases, and plant growth is inhibited, which prevents a good grain ripening (Morgun et al. 2010).

Heat and drought resistance of wheat are quantitative traits and are determined polygenically (Aprile et al. 2009). Using QTL analysis in labeling of resistance genes in cereals has made a significant contribution to the study of the problem of resistance to abiotic stress but many questions to this problem still remain unsolved (Collins et al. 2008; Tuberosa and Salvi 2005).

Genetic Regulation of the Rate of Wheat Development

Transition of wheat plants to flowering (or heading) is the most important stage of ontogeny determining such economically valuable characteristics as adaptation and productivity of plants (Khotyl'ov et al. 2002). The key genes that determine the soft wheat transition from vegetative growth to generative are the loci *Vrn* (vernalization response) and *Ppd* (photoperiod response), determining the plant response to vernalization and day length (Jill et al. 2008). Genetic control systems of the type and wheat pace of development—*Vrn* and *Ppd*—affect the rate of plant development (Stelmah et al. 2000), the structure of the harvest, and cold-hardiness (Dhillon et al. 2010; Kosova et al. 2008). These genetic systems are regarded as regulators (Danyluk et al. 2003).

The response to vernalization in wheat is controlled by at least five genes (Stelmah et al. 2000), of which three main, *VrnA1a*, *VrnB1a* and *VrnD1a*, are respectively localized on chromosomes 5A, 5B and 5D (Kocsy et al. 2010). Winter type of plant is sown only if three key genes are recessive alleles. In this case the presence of only one dominant gene *VrnA1a* provides complete insensitivity of plants to vernalization and dominant alleles *VrnB1a* and *VrnD1a* only partially reduce the need for it. *Vrn* genes have been cloned and few of their allelic variants have been described in recent years for wheat and barley (Distelfeld et al. 2009; Kane et al. 2005; Loukoianov et al. 2005; Ndjido et al. 2005; Sandra et al. 2009).

The response of wheat to photoperiod, i.e., the length of daylight, is controlled by a system of genes *Ppd*, localized on chromosomes 2D, 2B and 2A. Gene *PpdD1a* is seen as a key locus determining photoperiodic sensitivity

of hexaploid wheat. The gene belongs to a family of PRR (Pseudo Response Regulator), known regulators of diurnal rhythms in *Arabidopsis*.

Phenotypic expression of the genes of these systems is well studied in soft wheat. As the studies on this culture show, *Vrn* genes play a more important role in determining the trait—the speed of transition to heading (their contribution is 75%) (Trevaskis 2010; Trevaskis et al. 2006a,b). Surely these genetic systems are interconnected with each other (Dubcovsky et al. 2006; Trevaskis et al. 2006a). It is shown that under the influence of short photoperiod in the absence of vernalization (18–20°C) in winter wheat varieties with a dominant locus *Ppd D1a* slowed the transition to a heading, and in all sorts of recessive loci *Ppd*, on the contrary, accelerated. At the same time all sorts of *Vrn* carried loci in the recessive state, which required vernalization to go to the heading (Zhmurko 1999).

Adaptation of wheat to growing conditions is largely due to the genetic diversity of systems of genes *Ppd* and *Vrn*, controlling the reaction of genotypes to temperature and light environmental factors, including photoperiodism, the response to vernalization and its duration, light intensity and temperature of growth (Worland et al. 1994; Worland and Snape 2001).

Genes of photoperiodic and vernalization control of wheat development probably are involved in resistance to abiotic stress. It is shown that winter wheat varieties that carry all the loci *Ppd* and *Vrn* in the recessive state are more hardy than varieties with a dominant locus *Ppd D1a* and the recessive loci genes *Vrn* (Zhmurko 1999).

The most appropriate models for studying the effects of genes on the manifestation of some physiological and biochemical characteristics can be nearly isogenic lines (NILs) of wheat that differ in the genes *Vrn* and *Ppd*. Isogenic lines, as more fully meeting the rule of the only difference, have minimal differences in all characteristics except the marker once. Therefore, based on the results of research carried out on the isogenic lines, we can make a conclusion on the effect of a gene on the processes occurring in plant organisms.

In today's changing climate conditions, followed by regular droughts and extreme high temperatures (Morgun et al. 2010), it seems urgent to study the effects of these genetic systems that control the type and pace of plants' development in their degree of drought and heat resistance of common wheat. However, this issue has hardly been investigated.

Heat Resistance of Plants

Heat resistance is the ability of the plant organism to withstand higher temperatures without irreversible damage.

Reaction to changes in ambient temperature can be divided into three categories depending on the impact duration of this factor: 1. Direct responses of metabolic systems. The duration of these reactions are measured in minutes or hours. 2. Slower response to temperature changes with duration of several weeks or more. 3. Genotypic responses to constant exposure to the temperature factor is realized over many generations.

Effects of high temperatures lead to a number of negative changes in plant life: severe dehydration and dryness, burning, destruction of chlorophyll, irreversible respiratory disorders, other physiological processes, the cessation of protein synthesis enhancing their degradation, and accumulation of toxic substances, particularly ammonia. At very high temperatures permeability of membranes dramatically increases, followed by a thermal denaturation of proteins, coagulation of the cytoplasm and cell destruction. Adaptive mechanisms providing resistance to unfavorable temperatures can act at all levels of organization—molecular, cellular, organismal and population. Heat resistance of plants consists of two components: endurance—the ability of the cytoplasm to endure extreme temperatures by physical and chemical properties; and avoidance—the complex of existing plant safety devices which reduce the harmful effect of the factor, and slow down or prevent the development of lesions.

Molecular mechanisms of adaptation are realized through:

- change in the catalytic properties of enzymes, i.e., their activation energies—the enzyme conformational changes caused by rupture or formation of weak bonds;
- modification of the primary structure of the enzyme;
- maintaining the relative constancy of the ratio between the Michaelis constant and the concentration of the substrate;
- change in the content of the enzymes in the cells;
- change in viscosity of the bilayer membrane; and
- change in the degree of unsaturation of fatty acids and the length of the acyl chains in lipids is controlled by several enzymes: desaturase, tioesterazy and elongazy.

Physiological and biochemical mechanisms of adaptation are realized through:

- inhibition of growth;
- two-phase reaction of physiological processes—increase in the initial stages and further reduction of photosynthesis and respiration rates; and
- status change of phytohormonal plants, etc.

Heat Resistance of Wheat Isogenic Lines on the Genes *Ppd* and *Vrn*

Analysis of the heat resistance of the seed. Germination of seeds is a crucial stage in the development of plants. At this point plants begin to grow from very few cells of the embryo. Heat shock may disrupt the stability of the enzyme systems of seeds that does not allow to use reserve nutrients of the endosperm on the formation of the germ sufficiently. The impact of temperature can adversely affect the germination of seeds to varying extent: from the embryo not developing at all, to its development and growth being substantially impaired. The seeds of different varieties, respond differently to HS which is probably due to genotype (Amtmann et al. 2005).

We have assessed the ability of the wheat seeds isogenic on the genes *Ppd* and *Vrn* lines to germinate under heat shock. Heat resistance was evaluated by reduction of seed germination (% of control). The obtained results have shown (Table 1) that under the conditions of heat shock the overall level of germination of all lines of *Vrn* was lower than the lines *Ppd*. Since all the investigated lines are created in gene pool of the same grade, Mironovskaya 808, it suggests that the effects of *Ppd* genes on the heat resistance of wheat seeds are expressed more significantly than genes *Vrn*. Heat resistance of tested lines of seeds was different. Line of *Ppd-A1a* has the largest resistance, line of *Ppd-D1a* and grade has less (for a complete recessive on gene loci *Ppd* and *Vrn*), which have equal rates on this basis. The least heat-resistant seed lines showed *Ppd-B1a*. Among the lines of *Vrn* lowest heat resistance of the seeds was found in line *Vrn D1a*, while the highest in line *Vrn A1a*. Heat resistance of seed varieties was lower than that of the line *Ppd A1a*, but higher than all other lines (Table 1).

Table 1. Effect of heat shock on seed germination isogenic lines of wheat cultivar Mironovskaya 808, %.

Isogenic line*	Seed germination, %		Heat resistance, % to control
	Control, 22°C	Experiment, 55°C	
<i>Ppd-D1a</i>	100.00	40.50	40.50
<i>Ppd-B1a</i>	98.00	37.50	38.26
<i>Ppd-A1a</i>	100.00	44.50	44.50
<i>Vrn-A1a</i>	100	39.88	39.88
<i>Vrn-B1a</i>	100	25.87	25.87
<i>Vrn-D1a</i>	100	24.83	24.83
Cultivar**	96	39.50	41.14
LSD _{0.5}	1.83	2.22	2.14

*—Dominant loci; **—All loci in a recessive state.

Thus, already at the stage of germination, genes *Ppd* and *Vrn* show the effects on plant resistance to elevated temperatures. The level of manifestation of the effect depends on the specific loci of these genes (dominant and/or recessive).

Accumulation of seedlings biomass at a temperature shock. It is known that heat shock has a negative impact on the growth processes of plants. In some plants a few degrees temperature rise leads to plants growth retardation which is a protective reaction. Resistant forms quickly adapt to stress and recover growth.

We assessed growth response to heat shock (50°C) on changes in dry matter accumulation. In experiments on plants we visually observed the accumulation of anthocyanins. This can be explained by the fact that anthocyanins are antioxidants. Thus, synthesis of anthocyanins is a protective mechanism against elevated temperatures (Shao et al. 2008).

The results of determining the changes in mass of seedlings have shown (Table 2) that in all the investigated *Ppd* lines the weight of overground parts and roots in the conditions of thermal shock was higher than that of all the lines of *Vrn*, regardless of the specific loci of these genes. This may indicate a stronger effect of genes *Ppd* on the heat resistance of seedlings. In addition, the weight gain of the overground part of seedlings during heat shock inhibited much stronger than the increase in mass of roots. However, the level of heat resistance of seedlings is linked to the state of specific gene loci, *Ppd* and *Vrn*. Thus, the lowest heat resistance showed germ line *Ppd-B1a*, and the highest showed germ line *Ppd-A1a*. Seedling line *Ppd-D1a* showed somewhat lower heat resistance compared with the line of *Ppd-A1a*. Seedlings *Vrn* lines also differed on the accumulation of biomass in thermal shock. The lowest level of accumulation was detected in seedlings of line *Vrn B1a*, seedling lines of *Vrn D1a* had a higher level

Table 2. Effect of temperature shock on changes in seedlings biomass accumulation of isogenic lines of wheat cultivar Mironovskaya 808, % of control.

Isogenic line*	Change in biomass accumulation, % of control		Heat resistance, %
	Aboveground part of seedlings	The root system	
<i>Ppd-D1a</i>	22.76	40.65	31.71
<i>Ppd-B1a</i>	16.55	28.74	22.65
<i>Ppd-A1a</i>	23.74	46.67	35.21
<i>Vrn-A1a</i>	19.02	33.25	26.13
<i>Vrn-B1a</i>	15.46	18.01	16.74
<i>Vrn-D1a</i>	19.31	29.22	24.27
Cultivar**	21.40	29.51	25.45
LSD _{0.5}	2.32	1.82	1.04

*—Dominant loci; **—All loci in a recessive state.

and the maximum was observed in line *Vrn A1a*. Consequently, the most heat-resistant seedlings are lines *Ppd A1a* and *Vrn A1a*. The level of heat resistance of Mironovskaya 808 seedling varieties was lower than that of the lines *Ppd D1a*, *Ppd A1a*, *Vrn A1a* and *Vrn D1a*, but higher than the other lines (Table 2).

The temperature threshold for proteins coagulation. Coagulation is adhesion of the protein particles and their precipitation in the sediment. Coagulation of proteins that are part of the protoplasm takes place at temperatures above 50°C. Due to the irreversibility of the process, coagulated protein loses its functions. It occurs in different plants at different temperatures. The temperature threshold of proteins coagulation (TTCP) is judged on the extent of heat resistance of cells protoplasm colloids (Kosakovskaya 2008). Accordingly, the higher protoplasm TTCP, the more heat-resistant plant is.

Our data have shown (Table 3) that TTCP depends on the plants, age of the studied lines, e.g., a five-week plant had lower index than four-week plants, regardless of the status of a particular *Ppd* and *Vrn* gene locus. This is probably due to ontogenetic differences in metabolism. However, the lines differed in terms of TTCP. *Ppd-A1a* is characterized by the greatest value of its line, the lower line of *Ppd-D1a*, and the lowest, i.e., the line of *Ppd-B1a*. Among the four-week plant lines with *Vrn* TTCP line *VrnB1a* and *Vrn D1a* was similar and lower than the line *Vrn A1a*. At five-week seedlings this figure was the lowest in the line *Vrn B1a* (Table 3). TTCP varieties of plants of both ages were lower than that of all the *Ppd* lines, but higher than in all lines of *Vrn* (Table 3). Thus, the threshold temperature of proteins coagulation is associated with the condition (dominant and/or recessive) gene loci, *Ppd* and *Vrn*.

Assessment of heat resistance level of isogenic on genes *Ppd* and *Vrn* lines of wheat using three different methodological approaches has shown dependence of plants properties on individual loci status of these genes.

Table 3. The temperature threshold for coagulation proteins isogenic lines of wheat cultivar Mironovskaya 808 (°C).

Isogenic line*	TTCP four-week plant	TTCP five-week plant
<i>Ppd-D1a</i>	61.00 ± 0.25	59.00 ± 0.15
<i>Ppd-B1a</i>	60.00 ± 0.35	58.00 ± 0.25
<i>Ppd-A1a</i>	62.00 ± 0.22	61.00 ± 0.35
<i>Vrn-A1a</i>	59.01 ± 0.32	53.05 ± 0.21
<i>Vrn-B1a</i>	58.00 ± 0.45	51.08 ± 0.33
<i>Vrn-D1a</i>	58.52 ± 0.34	52.32 ± 0.26
Cultivar**	60.05 ± 0.50	53.25 ± 0.37

*—Dominant loci; **—All loci in a recessive state.

Drought Tolerance of Plants

Drought resistance is the property of plants to withstand drought during the ontogeny and to grow and develop in these circumstances thanks to a number of adaptive properties resulting ultimately in the ability to generate offsprings.

Physiological and biochemical mechanisms of adaptation to drought. At the level of the whole body all the plants respond to water deficit in the same way. Their shoots growth is inhibited, there occurs stimulation of root growth, accumulation of ABA and decreased stomatal conductance. Shortly after the start of the drought, the program of accelerated aging of the lower leaves and their death switches on.

The main features enhancing the ability of plants to withstand dehydration are high elasticity of the cytoplasm, a large water-holding capacity and increase in the intensity of metabolism (photosynthesis, respiration, enzyme activity) leading to the formation of metabolic water, and conservation of synthetic reactions in times of drought.

At the cellular and molecular levels at least three mechanisms responsible for the formation of the overall systems resilience to water scarcity operate (Fleury et al. 2010; Ingram and Bartels 1996; Kordum et al. 2008):

1. Expression induction of different genes groups that determine the growth of a number of functional macromolecules, such as key enzymes of synthesis and degradation of osmolytes, analogues of heat shock proteins which function as molecular chaperones. Ubiquitin system synthesizes, and protease inhibitors are formed, as well as ions sequestration proteins, dehydrins and LEA proteins (Dure 1993). There is a new formation of water channels proteins (aquaporins). All these newly synthesized macromolecules protect "normal" cellular proteins under stress, provide the correct assembly of oligomeric structures, remove denatured polypeptides and regulate water status of the body.
2. An active change in the microenvironment of macromolecules due to rapid accumulation of compatible osmolytes such as proline and other amino acids (Samuel et al. 1992), betaine, sugar-alcohols, etc. These low molecular organic compounds have protector, osmo-regulatory and antioxidant effects.
3. Activation of antioxidant enzymes involved in the inactivation of hydrogen peroxide, superoxide radical and hydroxyl radical (Shao et al. 2008). In these reactions superoxide dismutase, peroxidase, catalase and ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase are involved. Active oxygen forms are formed by the action of all, without exception, stress factors on the plant.

Drought in Isogenic *Vrn* Genes *Ppd* and Wheat Lines

Drought tolerance of seeds. Germination of seeds is a process of transition from seed dormancy to intensive activity, resulting in the growth of the embryo starts to move and form seedling from which the young plant develops. Seed germination occurs only under sufficient provision of moisture. When water gets to the seeds they swell, they are activated by enzymes that amplify the processes of storage substances conversion (starch, fats, proteins) with primary disintegration into sugars, fatty acids, and amino acids. This provides the seeds with energy and plastic nutrients necessary for the synthesis of materials going to build a new seedling's tissues. Thus, a sufficient amount of water is one of the key conditions for seed germination.

To simulate the conditions of water deficit in determining the drought resistance of seeds, we used an increased osmotic pressure created by high concentration of mannitol solution (20%), in which seeds germinated. Drought resistance was evaluated by germination of seeds under these conditions. Among the lines *Ppd* ability to germinate under artificial drought was highest in seeds of line *Ppd-A1a*, significantly lower at the *Ppd-B1a* line and the lowest at lines of *Ppd-D1a* (Table 4). Seed germination of *Vrn* lines under drought conditions also depended on the condition of the individual loci of these genes. It was highest in the seeds of line *Vrn A1a*, slightly lower than in line of *Vrn D1a*, minimum in line of *Vrn B1a* (Table 4). Thus, drought tolerance of seeds isogenic on genes *Ppd* and *Vrn* of wheat lines is associated with the condition (recessive and/or dominant) of specific loci of these genes. Grade's seed germination in drought conditions was significantly higher than that of all the investigated lines *Ppd*, but lower than that of the lines *Vrn A1a*, and *VrnD1a* (Table 4).

Table 4. Effect of artificial drought on isogenic lines seed germination of wheat cultivar Mironovskaya 808, %.

Isogenic lines*	Seed germination, %		Drought, (% of control)
	Control	20% mannitol	
<i>Ppd-D1a</i>	100.00	33.23	33.23
<i>Ppd-B1a</i>	99.00	28.67	28.95
<i>Ppd-A1a</i>	100.00	39.00	39.00
<i>Vrn-A1a</i>	100.00	45.56	45.56
<i>Vrn-B1a</i>	84.00	25.28	30.09
<i>Vrn-D1a</i>	96.00	41.17	42.89
Cultivar**	96.00	40.50	42.18
LSD _{0.5}	2.22	4.42	4.45

*—Dominant loci; **—All loci in a recessive state.

Biomass accumulation during drought. Of all the physiological processes the growth process is most sensitive to lack of moisture. Growth stops in the initial period when the plant lacks water, although photosynthesis and respiration take place normally. There are several reasons for this phenomenon. Reduction of water content discontinues DNA replication and hence cell division. The second phase of cell growth (elongation phase) occurs due to the strong income of water. In conditions of water shortage this phase is dramatically hindered. The cells formed in drought conditions are distinguished by their small size. Lack of water leads to other anatomical changes—greater development of mechanical tissues. Inhibition of the growth processes observed at water shortage can also be a consequence of hormonal metabolism (Kordum et al. 2003).

Thus, inhibition of growth is an important indicator that the plant is under stress. Resistant forms quickly adapt to stress and reduced growth.

In our experiments, all plants of isogenic lines studied under the influence of 7-day drought (soil moisture content is 40% lower than in the control) reduced the increase in biomass both of aboveground parts, and roots. This is more pronounced in lines *Ppd*, than in lines of *Vrn*. Root growth in all lines was significantly more suppressed than the growth of aboveground parts (Table 5). However, the lines differed in terms of slowing growth. For example, in lines *Ppd* biomass gain of aboveground parts and roots was the lowest in plants of line *Ppd B1a*, higher in line *Ppd A1a*, and the highest in line *Ppd D1a*. Among the lines of *Vrn* the smallest increment of biomass was in line *Vrn B1a*, higher in line *Vrn D1a*, and the maximum in line *Vrn A1a*. Drought resistance of the plants variety was lower than that of all the lines (Table 5). These results, as well as data on seeds drought

Table 5. Effect of drought on plant growth of isogenic lines of wheat cultivar Mironovskaya 808, % of control.

Isogenic line*	Reduction of biomass growth, % of control		Drought plant, %
	Aboveground part of seedlings	The root system	
<i>Ppd-D1a</i>	76.15	65.22	70.69
<i>Ppd-B1a</i>	71.23	60.30	65.78
<i>Ppd-A1a</i>	74.32	70.54	72.43
<i>Vrn-A1a</i>	89.3	87.0	88.15
<i>Vrn-B1a</i>	77.0	68.0	72.50
<i>Vrn-D1a</i>	81.3	75.1	78.20
Cultivar**	73.47	61.47	67.47
LSD _{0.5}	2.02	2.23	2.86

*—Dominant loci; **—All loci in a recessive state.

resistance, suggest that the genes of the photoperiodic sensitivity of wheat and genes of vernalization need can be involved in shaping the properties of soft wheat drought resistance.

Water content of leaves. Maintaining the hydration of plant tissue is a required condition of plants survival and their adaptation to environmental factors. The hydration degree is an important indicator of water treatment plants. Water content depends on the concentration of cell sap, water potential of individual plant organs, and response to stress exposure. Water content of plant organs is an important indicator of how the plant tolerates drought. The higher water content, the more drought-resistant plant is.

According to our data, water content of the leaves of the investigated lines under drought conditions decreased (Table 6). But the reduction degree was different, depending on the state of a locus of genes *Ppd* and *Vrn*. In line *Ppd* lowest water content of leaves was detected in line *Ppd B1a*, higher in line *Ppd D1a*, and the maximum in line *Ppd A1a*. Among the lines of *Vrn*, lowest water content of leaves was at the line of *Vrn B1a*, slightly higher than it is in line *Vrn D1a*, and had the largest line of *Vrn A1a*. Water content in leaves of the grade's plants was higher than that of the lines *Ppd-D1a* and *Ppd-B1a*, but lower or the same as the rest of the lines (Table 6).

The content of free proline in leaves. Among the mechanisms of plant adaptation to abiotic stresses accumulation of compatible osmolytes plays an important role, one of which is proline (Shao et al. 2008). The increase of this amino acid in plant cells contributes to increased resistance to water, salt and temperature stresses. Accumulation of this amino acid is considered as a mechanism of biochemical adaptation to stress. Proline prevents osmotic shock, as it is osmo-regulator. The concentration of free proline in direct proportion depends on the intensity of drought. The more drought-resistant

Table 6. Effect of drought on the water content of leaves of isogenic lines of wheat varieties Mironovskaya 808, %.

Isogenic line*	Water content of leaves, %		Drought plants (% of control)
	Control 70% FFC	Experiment 30% FFC	
<i>Ppd-D1a</i>	85.45	72.17	84.46
<i>Ppd-B1a</i>	84.79	69.62	82.11
<i>Ppd-A1a</i>	86.72	76.03	87.67
<i>Vrn-A1a</i>	87.02	78.34	90.03
<i>Vrn-B1a</i>	85.01	73.26	86.18
<i>Vrn-D1a</i>	86.73	76.96	88.73
Cultivar**	85.29	75.45	88.46
LSD _{0.5}	1.75	2.00	2.73

*—Dominant loci; **—All loci in a recessive state.

species is, the more proline it accumulates, which is probably due to genotype (Samuel et al. 1992).

The studied lines differed in general level of proline content. In leaves and roots *Ppd* lines, it was significantly lower than that of *Vrn* lines in both the control and during drought. In all the investigated lines under the influence of drought proline content increased, indicating the processes of plants adaptation to water deficit (Table 7). At the same time dependence on the increase in the proline content on the condition of specific gene loci, *Ppd* and *Vrn* (dominant and /or recessive) has been found out. The content of proline in leaves and roots increased less in line *Ppd D1a*, more in line *Ppd A1a*, and the maximum content rise was in *Ppd A1a*. In line *Vrn*, proline content in leaves and roots under drought conditions have increased less significantly in *Vrn B1a*, a little more in line *Vrn D1a* and to the greatest extent in line *Vrn A1a*. The content of proline in the leaves of plants of the variety was higher than that of all the lines *Ppd*, but lower than that of all the lines of *Vrn* (Table 7). Consequently, changes in proline content under the influence of drought indicate its dependence on the state of specific gene loci, *Ppd* and *Vrn* (dominant and /or recessive) in isogenic lines of wheat.

Table 7. Effect of soil drought on free proline content in isogenic lines of wheat varieties Mironovskaya 808.

Isogenic line*	Increasing the content of free proline, % of control		Drought plants (% of control)
	In leaves	In roots	
<i>Ppd-D1a</i>	50.43	33.86	42.15
<i>Ppd-B1a</i>	44.54	20.53	32.54
<i>Ppd-A1a</i>	55.23	41.12	48.18
<i>Vrn-A1a</i>	72.80	61.25	67.03
<i>Vrn-B1a</i>	65.90	52.57	59.24
<i>Vrn-D1a</i>	66.40	56.80	61.60
Cultivar**	58.15	45.00	51.58
LSD _{0.5}	1.03	1.15	1.56

*—Dominant loci; **—All loci in a recessive state.

Conclusion

Generalization of the investigation results of heat resistance of isogenic lines of wheat (Table 8), obtained by three different methods, allows us to conclude that in the high-stress germination accumulation of plant, biomass and TTCP were the lowest in line *Ppd-B1a*, significantly higher in line *Ppd-D1a* and had the highest in line of *Ppd-A1a*. Among the lines of *Vrn*, the

Table 8. Heat resistance of isogenic lines of wheat varieties Mironovskaya 808, %.

Isogenic line*	Indicators of heat resistance, % of control		TTCP, °C
	Reduction in seed germination	Reduced the accumulation of plant biomass	
<i>Ppd-D1a</i>	40.50	31.71	61
<i>Ppd-B1a</i>	38.26	22.65	60
<i>Ppd-A1a</i>	44.50	35.21	62
<i>Vrn-A1a</i>	39.88	26.13	59
<i>Vrn-B1a</i>	25.87	16.74	58
<i>Vrn-D1a</i>	24.83	24.27	58.5
Cultivar**	41.14	25.45	60
LSD _{0.5}	2.14	1.04	0.45

*—Dominant loci; **—All loci in a recessive state.

lowest biomass growth and TTCP was in line *Vrn D1a*, and the highest rates in all lines of *Vrn A1a*. The above-mentioned information gives grounds to believe that the most heat-resistant lines in the early stages of ontogeny are the lines of *Ppd-A1a* and *Vrn A1a* and the least heat-resistant are lines of *Ppd-B1a* and *Vrn B1a*.

Analysis of the generalized results of the study of drought resistance (Table 9) indicates that under drought conditions the highest rates among the investigated lines were in line of *Ppd-A1a*, and the lowest performance was in line of *Ppd-B1a*. In line *Ppd-D1a* content of proline in leaves was higher, and seed germination, accumulation of dry plant biomass and water content in leaves was the same as in line of *Ppd-B1a*. Therefore, among the most drought-resistant lines is a line of *Ppd-A1a*. The level of

Table 9. Drought resistance of wheat varieties isogenic lines Mironovskaya 808, %.

Isogenic line*	Indicators of drought, % of control			
	Reduction in seed germination	Reduced the accumulation of plant biomass	Reduced water content of leaves	Increased content proline
<i>Ppd-D1a</i>	33.23	70.69	84.46	42.15
<i>Ppd-B1a</i>	28.95	65.78	82.11	32.54
<i>Ppd-A1a</i>	39.00	72.43	87.67	48.18
<i>Vrn-A1a</i>	45.56	88.15	90.03	67.03
<i>Vrn-B1a</i>	30.09	72.50	86.18	59.24
<i>Vrn-D1a</i>	42.89	78.20	88.73	61.60
Cultivar**	42.18	67.47	88.46	51.58
LSD _{0.5}	4.45	2.86	2.73	1.56

*—Dominant loci; **—All loci in a recessive state.

drought resistance varieties is much higher than that of *Ppd-D1a* lines and *Ppd-B1a* and the same as that of the line *Ppd-A1a*. Among *Vrn* lines *B1a* is characterized by the lowest rates of drought resistance, and the highest in line *Vrn D1a*, which indicates the relation of specific gene loci *Vrn* condition with the drought resistance property.

Since the investigated lines differ in conditions of genes loci of photoperiodic sensitivity and vernalization requirements, it suggests that these genes may be involved in resistance of common wheat *Triticumaestivum* L. to heat and drought. Since heat- and drought resistance are polygenic traits, they are likely to be formed by the interaction of oligogenes which involves genes *Ppd* and *Vrn*. It is logical to assume that among the mechanisms of heat- and drought-resistance may be the interaction of individual genes *Ppd* and *Vrn* with each other. It is possible that it depends on the individual loci of these genes.

Apparently, the level of heat- and drought-resistance of *Ppd* lines to a great extend is associated with the recessive condition of loci *B1b* and *D1b* and the dominant state of locus *A1a*, but to a lesser extent with the recessive loci condition *A1b* and *D1b* and the dominant state of locus *B1a*. Since the grade level of drought is the same as in line *Ppd-A1a*, it is associated with a recessive gene condition *B1b* and *D1b*. This assumption is supported by the fact that the level of drought indicators in line of *Ppd-D1a*, which has recessive genes *A1b* and *B1b*, is lower than in line of *Ppd-A1a*.

At this stage of research it is not possible to explain specific physiological and biochemical mechanisms of the effects realization of photoperiodic sensitivity genes and vernalization requirements on heat- and drought-resistance of wheat. However, the obtained results with a significant probability give reason to suppose that the formation of these important agronomic properties of wheat is indirectly related to the genotype of these genes—dominant and/or a recessive condition of their specific loci.

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2

Plant WRKY Gene Family and its Response to Abiotic Stress

*Siddanagouda S. Biradar, Pingchuan Deng
and Song Weining**

ABSTRACT

Abiotic and biotic stresses greatly influence plant growth and development. It is necessary to understand the molecular basis of plant response to these stresses. In plants, WRKY proteins are a super family of transcription factors and they have well conserved WRKY binding domains which specifically bind the W-box *cis*-acting element present in the promoter region of several downstream genes and regulate their expression. A large number of WRKY genes have been reported in higher plants in recent years. WRKY proteins are involved in regulating response to stress (biotic and abiotic) such as drought, salinity, dehydration, heat and cold stress, nutrient deficiency and UV radiation tolerance. Overexpression of some WRKY transcription factors in plants was shown to enhance tolerance to abiotic stresses. A single WRKY transcription factor could display different responses to different kind of stresses and in turn regulates multiple signalling pathways. WRKY proteins are involved in signalling cross-talk of both biotic and abiotic stress response while some WRKY genes also possess W-box sequences, indicating that there is co-regulation between WRKY genes

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy and Yangling Branch of China Wheat Improvement Center, Northwest A&F University, Yangling 712100, Shaanxi, China.

* Corresponding author: sweining2002@yahoo.com

in a signalling pathway. This chapter summarizes recent advances in understanding the role of WRKY transcription factors in abiotic stress in higher plants, especially crops.

Key words: Abiotic stress; transcription factors; WRKY gene family; WRKY binding domain; zinc-finger-like motif; W-box; co-regulatory network; signalling cross-talk.

Introduction

Abiotic factors such as drought, salinity, low and high temperature, nutrient deficiency, and UV radiation greatly affect the growth and development of plants. It is estimated that > 50% of average annual yield loss of major crops is caused by abiotic stress (Arzani 2008). Plants are sessile and exposed to multiple stresses at different growth stages with series of metabolic, morphological, physiological and molecular changes (Wang et al. 2001). Plants perceive stress and show adaptive response to various abiotic and biotic stresses. Stress tolerance in plants is generally of very complex nature with multiple genes involved in regulatory networks. Such response to tolerate or resist abiotic stress involves many biochemical pathways mediated by genes which are in turn regulated by transcription factors. Transcription factors (TF) are protein complexes that bind to specific *cis*-acting promoter elements thereby activating or repressing the transcriptional rates of their target genes. These TFs are usually multigene families and individual members within a family often respond differently to various stress conditions. Alternatively, different stress responsive genes may share the same TFs and activate similar cellular responses like stomatal closure, accumulation of stress proteins and anti-oxidants (Bohnert et al. 2001). A range of transcription factors like ERF, NAC, DREB and WRKY are involved in abiotic stress response in plants.

WRKY Gene Family: Structure and Classification

Plant specific WRKY transcription factors comprise a large gene family regulating response to biotic and abiotic (drought, salt, dehydration, cold, heat, nutrient deficiency and UV radiation) stresses and developmental processes such as trichome development, leaf senescence, biosynthesis of pigments, seed dormancy and germination (Rizhsky et al. 2004). They are broadly distributed across the genome and highly diversified in different crop plants. They play both as positive and negative regulator

via transcriptional regulation and protein-protein interactions. Based on conserved WRKY amino acid sequence in their DNA binding domain, these transcription factors are named as WRKY (pronounced as worky). The DNA binding domain of WRKY protein is called as WRKY domain and the DNA binding site as W-box (Rushton et al. 1996). Each WRKY protein has at least one WRKY domain defined by a region of approximately 60 amino acids which contains a well conserved heptapeptide WRKYGQK amino acid signature (which shows high affinity to W-box *cis* element) at its N-terminus and zinc-finger-like motif at its C-terminus (Eulgem et al. 2000). The binding site W-box (C/T)TGAC(T/C) is an *cis*-acting element which is usually found in the promoter region of many stress-related plant genes. Specificity of binding also depends on sequences flanking the W-box element (Ciolkowski et al. 2008). Exceptionally, barley WRKY protein SUSIBA2 specifically binds to a SURE element (TAAAGATTACTAATAGGAA) instead of W-box (Sun et al. 2003). WRKY domain binds to W-box *cis*-element where zinc-finger-like motif provides interface to protein-protein interactions. They have potential to differentially regulate the expression of a several target genes. Some WRKY proteins directly conjugate with signal transduction genes like MAP Kinases (Qiu et al. 2008).

WRKY proteins are mainly classified into three groups based on the number of WRKY domains and the structural features of their zinc-finger-like motifs. Proteins with two WRKY domains belong to group I while all other proteins with single WRKY domain are classified into groups IIa, IIb, IIc, IId, IIe and III (Rushton et al. 1996). WRKY proteins belonging to group I and II have the same zinc-finger-like motif (C₂H₂) unlike group III (C₂HC). Five subgroups are made in group II based on additional short conserved structural motif. Many variant WRKY proteins with the WRKY amino acid sequence replaced by WKKY, WKRY, WRRY, WVKY or WSKY have also been reported and are categorized into group III. Group I WRKY proteins are of ancestral type whereas group III is newly evolved. The presence of one intron is a common feature in this gene family. There is no correlation between the classifications of WRKY genes into groups with their stress response or expression patterns. Expansion of this gene family in higher plants might be because of segmental duplication and deletion events in evolution (Bowers et al. 2003).

Since the cloning and characterization of WRKY cDNA in sweet potato (*SPF1*) (Ishiguro and Nakamura 1994), a large number of corresponding WRKY genes have been reported in different crop plants (Table 1), in addition to the model species *Arabidopsis thaliana*. WRKY genes had long been considered plant specific before they were identified in the non-

Table 1. WRKY gene family in different plants.

Sl.No	Name of the crop	Number of genes	References
1	<i>Arabidopsis thaliana</i>	74	(Eulgem and Somssich 2007)
2	<i>Oryza sativa</i> (rice)	102	(Ross et al. 2007)
3	<i>Triticum aestivum</i> (wheat)	306	(Wu et al. 2008; Niu et al. 2012; Biradar and Weining 2012)
4	<i>Hordeum vulgare</i> (barley)	120	(Mangelsen et al. 2008; Biradar and Weining 2012)
5	<i>Glycine max</i> (soybean)	197	(Schmutz et al. 2010)
6	<i>Helianthus annuus</i> (sunflower)	97	(Giacomelli et al. 2010)
7	<i>Sorghum bicolor</i> (sorghum)	68	(Pandey and Somssich 2009)
8	<i>Zea mays</i> (maize)	136	(Wei et al. 2012)
9	<i>Brachypodium distachyon</i>	86	(Tripathi et al. 2012)
10	<i>Cucumis sativus</i> (cucumber)	55	(Ling et al. 2011)
11	<i>Populus trichocarpa</i>	104	(He et al. 2012)
12	<i>Carica papaya</i> (papaya)	66	(Pandey and Somssich 2009)
13	<i>Brassica napus</i> (canola)	46	(Yang et al. 2009)

photosynthetic eukaryotes like *Dictyostelium discoideum* (slime mold) (Ulker and Somssich 2004) and *Giardia lamblia* (protist) (Pan et al. 2009).

Role in Abiotic Stress

WRKY transcription factors are not constitutively expressed. They are induced in response to external stimuli like abiotic and biotic stresses. They act both as activators or repressors, and thus play key roles in plant developmental processes both by repression or de-repression of signalling pathways. Plant receptors sense abiotic stress and generate signals which activate the signal transduction pathways and in turn WRKY gene will be expressed. Binding of these WRKY genes to W-box activates defense related genes and shows quick response to the stress.

WRKY transcription factors are involved in plants responses to various abiotic stresses like drought and high salinity, dehydration, cold and heat stress (Zhou et al. 2008), nutrient deficiency (Chen et al. 2009) and UV radiation (Izaguirre et al. 2003) (Table 2). WRKY TFs are likely to be involved both in ABA-dependent and ABA-independent signalling pathways wherein components may cross-talk with other signalling pathways. Abiotic stress like drought, heat and salt stress increases the biosynthesis and accumulation of ABA, an essential component of drought

Table 2. WRKY genes involved in abiotic stress response.

Plant	Name of the gene	Induced by abiotic stress	References
<i>Arabidopsis</i>	<i>AtWRKY2</i> , <i>AtWRKY18</i> , <i>AtWRKY25</i> , <i>AtWRKY33</i> , <i>AtWRKY17</i> and <i>AtWRKY28</i>	Salt	(Chen et al. 2009; Jiang and Deyholos 2009; Seki et al. 2002; Liu et al. 2011; Li et al. 2011; Devaiah et al. 2007; Ramamoorthy et al. 2008)
	<i>AtWRKY46</i>	Salt, osmotic stress and low Pi	
	<i>AtWRKY6</i> , <i>AtWRKY42</i> and <i>AtWRKY75</i>	Low Pi	
	<i>AtWRKY25</i> , <i>AtWRKY26</i> and <i>AtWRKY39</i>	Heat	
	<i>AtWRKY33</i> and <i>AtWRKY34</i>	Cold	
	<i>AtWRKY4</i>	Cold and salt	
	<i>AtWRKY25</i> and <i>AtWRKY33</i>	Salt, cold and heat	
	<i>AtWRKY25</i> and <i>AtWRKY26</i>	Heat and cold	
	<i>AtWRKY6</i> , <i>AtWRKY75</i> and <i>AtWRKY70</i>	Oxidative stress	
	<i>AtWRKY25</i>	Oxidative, heat and osmotic stress	
	<i>AtWRKY33</i>	Salt, cold, oxidative stress and UV radiation	
	<i>AtWRKY48</i>	Osmotic stress	
	<i>AtWRKY25</i>	Salt, cold and heat	
	<i>AtWRKY33</i>	Salt, cold, oxidative stress and UV radiation	
	<i>OsWRKY02</i> , <i>OsWRKY01</i> , <i>OsWRKY26</i> , <i>OsWRKY50</i> , <i>OsWRKY81</i> and <i>OsWRKY46b</i>	Drought	
Rice	<i>OsWRKY03</i> , <i>OsWRKY55</i> , <i>OsWRKY78</i> , <i>OsWRKY82</i> , <i>OsWRKY87</i> , <i>OsWRKY96</i> , <i>OsWRKY46a</i> , <i>OsWRKY28</i> , <i>OsWRKY04a</i> , <i>OsWRKY23</i> , <i>OsWRKY31a</i> , <i>OsWRKY67</i> , <i>OsWRKY63a</i> , <i>OsWRKY13</i> , <i>OsWRKY08</i> and <i>OsWRKY44</i>	Salt	(Ramamoorthy et al. 2010; Qiu and Yu 2009; Ricachenevsky et al. 2010; Wang et al. 2007)
	<i>OsWRKY08</i> , <i>OsWRKY07</i> , <i>OsWRKY69</i> , <i>OsWRKY53a</i> , <i>OsWRKY83</i> , <i>OsWRKY85</i> , <i>OsWRKY45</i> , <i>OsWRKY61</i> , <i>OsWRKY78a</i> , <i>OsWRKY56</i> , <i>OsWRKY101a</i> , <i>OsWRKY12</i> and <i>OsWRKY23i</i>	Drought and salt	
	<i>OsWRKY45</i> , <i>OsWRKY79</i> and <i>OsWRKY90a</i>	Cold	
	<i>OsWRKY45</i>	Salt, cold and heat	

Table 2. contd....