

BIOMARKERS OF PLANTS WITH DIFFERENT TYPES OF ECOLOGICAL STRATEGIES

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Summary. Adaptive changes driven by ecological stresses can be investigated at various hierarchical levels – from molecular up to coenotic. Of particular interest is adaptation directed at altering key plant physiological processes in response to negative and unusual ecological factors. Patterns of physiological adaptation can be classified within the framework of ecological strategy. One of the most widely used classifications has been proposed by Ramensky and Grime (1971, 1988). Adaptation to stress in plants is regulated by a complex of molecular and genetic mechanisms that activate stress responses to prevent protein degradation and cellular component destruction and restore homeostasis. Since protein biosynthesis is a key link in the metabolic chain and because phytohormones regulate and coordinate such fundamental processes as growth, development, photo- and biosynthesis and respiration, their expression profiles are likely to vary between plants that adopt different stress response strategies. In this study, we investigated the utility of proteins, especially members of stress protein families, and of phytohormones as biomarkers for different ecological adaptation strategies.

Key words: adaptation, ecological strategy, ecophysiology, phytohormones, stress proteins.

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Abbreviations: ABA – abscisic acid; HSPs – heat shock proteins; IAA – indole-3-acetic acid; PGFs – plant growth factors.

INTRODUCTION

The question of how environmental stresses affect and influence plants has been one of the focal areas of modern biological research. This is especially relevant because of the potential for the radical ecological changes and reduction in biodiversity brought about by large-scale anthropogenic phenomena such as global warming.

Adaptive changes driven by ecological stresses can be investigated at various hierarchical levels – from molecular up to coenotic. Of particular interest is adaptation directed at altering key plant physiological processes in response to negative and unusual ecological factors. Our understanding of these types of changes has been greatly advanced in the recent decades due to the active development of ecological plant physiology.

Professor N.A. Maximov pioneered an ecological component in physiological research, starting in his classical works on frost- and drought resistance (Maximov, 1927, 1952). The term “ecological physiology” was introduced in 1952 by professor V.Billing. Ecological physiology studies how environmental effects influence and shape key physiological processes in plants. This has clear practical importance for agriculture, forestry and related fields. For instance, physiological plasticity is a major determinant of survival and productivity of plants. Ecological and physiological phenotypes and associated genetic loci are important for the selection of donors with agriculturally valuable characteristics, tolerance to extreme environmental conditions, in identifying the species for initial introduction to a new environment and for system engineering in adaptive agriculture.

The analysis of activation and synthesis of stress proteins and phytohormones can potentially generate a set of biomarkers to discriminate between different ecological adaptation strategies in plants. Very little is known about the role that phytohormones, their localization and distribution play in plant adaptation strategies. A number of open questions also remain about the correlation of activation and expression patterns of stress proteins

with ecological adaptation strategies. Further, our understanding of the respective roles of stress proteins and phytohormones in shaping stress responses can reveal the fundamental mechanisms of plant stress adaptation and elucidate new approaches for maintaining global biodiversity and mitigating the effect of global environmental changes.

TYPES OF ECOLOGICAL STRATEGIES

Patterns of physiological adaptation can be classified within the framework of ecological strategy. Modern strategy classifications take into account both negative and positive environmental factors and make also use of biological productivity. One of the most widely used classifications has been proposed by Ramensky (Ramenskiy, 1971) and Grime (Grime et al., 1988) and is based on the relative importance of competition, stress and disturbance – the three major determinants of vegetation. According to this classification plants are allocated into one of three categories: competitors, stress – tolerants and ruderals. Such division is based on the ratio of growth potential to realized growth.

Ruderal species are typified by weeds – primarily annual, with rapid growth and development cycles. Stress-tolerants are usually slowly growing perennial plants that are characterized by small to moderate size and are capable of maintaining vegetation and reproduction under stress conditions. Grime (1974) and Rabotnov (Rabotnov, 1985) described the basic features upon which to base the classification of plants into ecological strategies.

Competitors (or violents, C – strategists) are the plants that have a high growth potential and high realized growth. Competitors are equally susceptible to stresses and disturbances. They lack adaptation necessary for survival in unfavourable or resource-limited conditions. These plants are characterized by highly developed and extensive root system and canopy and are capable of efficient utilization of nutrients, water and space.

Stress-tolerants (or patients, S-strategists) are a morphologically and ecologically diverse group, including plants found in low-resource and continuous disturbance setting. These plants have a low growth potential, nearly fully realized by means of highly specialized physiological stress-

induced adaptation, including metabolic pathways.

Ruderals (or ex erlevers, R-strategists) similarly to competitors grow in low-competition setting. These are “antisocial” plants, because they thrive when disturbances prevent the growth of competitors or when there is a temporary relief from stress or competition. Ruderals are equally sensitive to abiotic and biotic stresses. Their survival is ensured by allocating a greater proportion of available resources to reproduction at the expense of growth and vegetation. Adaptations in ruderals result in rapid growth under favourable conditions but focus on maintaining seed production under stress conditions.

STRESS PROTEINS IN PLANT ADAPTATION

Stress proteins are critical for maintaining homeostasis under stress. The mechanism of stress responses in plants (for instance due to heat shock) is analogous to that in animals, insects and yeast and is characterized by the following key steps (Fig. 1). A temperature increase of 8-10°C downregulates the synthesis of normal proteins and significantly upregulates the expression of heat shock proteins (HSPs). The synthesis of HSPs is preceded by the transcription of HSP genes. A number of these genes have been sequenced. When normal temperature conditions are restored, HSP expression levels decrease, while typical rates of synthesis are restored for other proteins. This is accompanied by a rapid degradation of HSP mRNA (half-life of up to 2 hours). The period of elevated protein synthesis rates (6-10 h from the onset of the heat shock) is followed by their rapid reduction to nominal levels. Kinetic parameters of HSP synthesis and accumulation depend on the specific family. Early HSPs (70 and 90 kDa) are expressed first, followed by HSPs with lower molecular weights. Different plant species synthesize identical early HSPs (specifically HSP70) in response to a variety of exogenous stresses (Lee and Vierling, 2000). This observation suggests that the early HSP response is nonspecific and plays a role in erecting a generic defensive barrier against primary cellular damage. The more specific synthesis of later proteins is likely due to their roles in restorative processes (Voinikov and Borovskiy, 1994; Kotak et al., 2007).

Molecular chaperones control protein transport, assembly, translocation

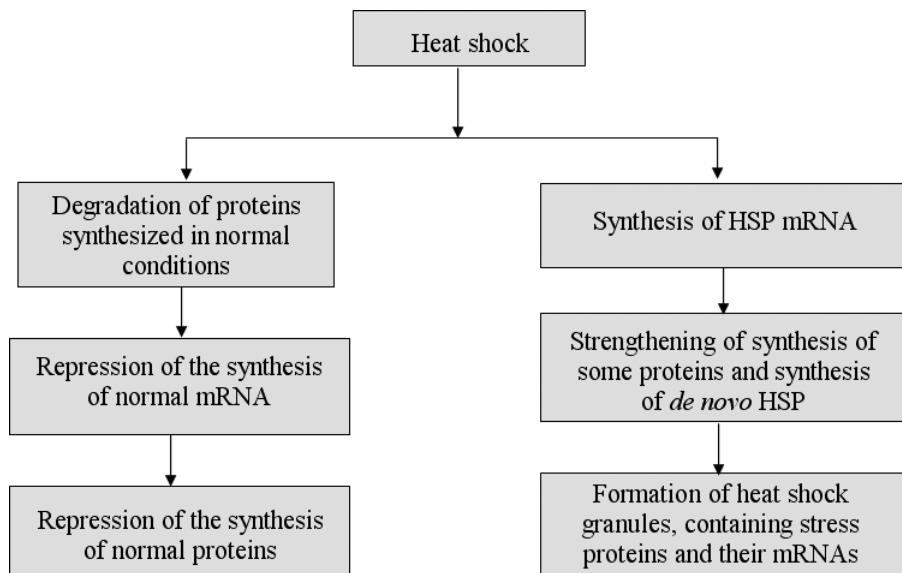


Fig. 1. The sequence of responses in plant biosynthesis induced by heat shock.

and degradation, and predominantly belong to one of the stress protein families. They are localized in the cytosole and organelles, particularly in the nucleus, mitochondria, chloroplasts, and the endoplasmic reticulum (Wang et al., 2004). There are five basic families of stress proteins, including heat shock proteins HSP70 and HSP60 which have been studied extensively. They are localized in the cytosole and organelles, particularly in the nucleus, mitochondria, chloroplast, and endoplasmic reticulum (Wang et al., 2004; Sangster and Queitsch, 2005) (Table 1). The upregulation of stress proteins, which occurs against a background of depressive changes in polypeptide formation, relative to normal environmental conditions, is one of the main components of the adaptive response. As is known, the stress proteins of HSP70 kD family locate mainly in cytoplasm, where they represent stress-induced and constitutive polypeptides (Derocher and Vierling, 1995; Perdue et al., 1998). Proteins of this family are found also in the nucleus and mitochondria (Brodsy et al., 1998). The support and maintenance of proteins translocation through out the mitochondrion, chloroplast and endoplasmic reticulum membranes concerns the chaperone function of

HSP70 kD family (Rassow et al., 1997) (Table 2). As was shown in our researches the reaction of proteins of this family to temperature stresses is more expressed at patients, whose ecological strategy is directed to fast realization of the life cycle.

The stress proteins of HSP 60 kD family are found in mitochondria and chloroplasts both under stress-induced and constitutive conditions (Cloney et al., 1994). The basic function of the proteins of this family is the maintenance of conformational reorganizations of mitochondrion polypeptides (Panasenko et al., 2003). Proteins of HSP 60 kD family in significant amounts are found in control and stressful conditions in explorers, whereas the patient contains small amounts of these proteins in control.

The stress proteins of HSP 110 kD family are found in cytoplasm,

Table 1. Localization of the basic HSP families.

HSP family	Cell compartment
HSP 70 kD stress – induced constitutive mitochondrion's 70 kD nuclear	cytoplasm cytoplasm mitochondrion nucleus
HSP 90 kD Synthesis of 90 kD polypeptides 86 kD Localization 90 kD polypeptides	cytoplasm mitochondrion mitochondrion chloroplasts endoplasmic reticulum
HSP 110 kD	cytoplasm chloroplasts mitochondrion
HSP 60 kD stress – dependent stress – independent	mitochondrion chloroplasts
Low molecular weight HSP	cytoplasm chloroplasts mitochondrion endoplasmic reticulum

chloroplasts and mitochondria (Schirmer et al., 1994). They prevent the aggregation of proteins in the cytosole and mitochondria (Queitsch et al., 2000). Stress proteins from this family were detected in patients under temperature stresses.

The synthesis of low molecular weight stress proteins as is known, is characteristic for plants and occurs in chloroplasts under the control of chloroplast DNA (Lee et al., 2005). In our investigations was shown, that the plants with different types of ecological strategy differed in their spectrum of low molecular weight HSP both in the control and in experiment. The patients contained an insignificant amount of these proteins in the control. At temperature stresses the intensity of synthesis of these proteins essentially grew.

Between low molecular weight HSP isolated from plants – explorers certain distinctions in the control and in temperature stress conditions have been revealed. Hence, the revealed differences in biosynthesis of proteins in response to temperature stress in plants with different types of ecological strategy specify that such plants differ from each other at a molecular

Table 2. The basic HSP families and their possible functions.

Family	Function
Chaperons	Support and assembly of proteins
HSP 70 kD	Support of proteins Mediators of proteins translocation through out mitochondrion's, chloroplasts and endoplasmic reticulum membranes
HSP 90 kD	Support of proteins
HSP 110 kD	Prevention of proteins aggregations in citozole and mitochondrion Interaction with proteases
HSP 60 kD	Maintenance of conformational reorganizations of mitochondrion's proteins
Low molecular weight HSP	Prevention of proteins aggregations, participation in support of proteins

level. The character of genome expression and activation of stress protein biosynthesis depends on the type of ecological strategy. The synthesis of stress proteins in patients occurs more actively. Under temperature stress contents of mitochondrial and chloroplast HSP are increased. The fast adaptive reactions of patients promote a survival of these plants in unfavourable conditions.

Thus, the essential distinctions in the reaction of protein system of plants to temperature stress revealed in our researches, allow considering the proteins, including stress, as biomarkers of plants with different types of ecological strategy.

ECOPHYSIOLOGICAL CHARACTERIZATION OF PHYTOHORMONES

Considering the role of phytohormones (or plant growth factors, PGFs) in regulation and coordination of key physiological processes, including growth, development, photosynthesis, liquid exchange, respiration and assimilators transport, one may surmise that plants following different survival strategies will exhibit different PGF compositions.

Specifically, the type of ecological strategy is determined by the PGF composition as a whole, as well as the distribution of specific factors in individual plant organs. There are very few previous studies that compared phytohormonal composition in plants with different types of ecological strategies. For instance, the comparison of abscisic acid (ABA) and indole-3-acetic acid (IAA) content in two ruderal and three stress-tolerant species revealed that independent of the level of mineral nutrients, ABA concentration per 1 g of wet mass was higher in ruderal species (Usmanov et al., 1990). ABA is accumulated more rapidly in leaves and reproductive organs of ruderal species. Ruderals also tend to maintain higher concentrations of ABA in roots compared to stress-tolerants (Borzenkova et al., 2001). Higher concentrations of ABA in leaves of ruderal plants is likely conducive to a more rapid completion of vegetative phase and transition to the reproductive phase. Intensive accumulation of ABA is characteristic of ruderal species. In contrast, stress-tolerant species are characterized by lower concentrations of cytokinins in roots, whereas

their reproductive organs contain considerably lower amounts of ABA relative to cytokinins (Borzenkova et al., 2001). ABA is a first-response mediator, sensing environmental changes and causing certain adaptive metabolic adjustments. By regulating the closure of stomata, ABA partially ameliorates the effect of stress; by further upregulating gene expression and new polypeptide synthesis it is also instrumental in enabling defensive reactions and increasing the hardiness of the plant (Allagulova et al., 2007; Rodgriguez et al., 2005). Abiotic stresses are accompanied by the accumulation of endogenous ABA, and sometimes also an increase in IAA and cytokinins. The changes in PGF composition depend on the nature of the stress and plant species. They can be putatively associated with the ecological adaptation strategy, help to determine the type of strategy and serve as a biomarker. The composition of phytohormones under stress is thus one of the factors that form the adaptive response (Kosakivska 2003, 2008).

MODERN VIEW-POINT ON PHYSIOLOGICAL ADAPTATION

The formation of new survival strategies via ecologically driven physiological adaptation is one of the primary evolutionary goals of plants. Adaptation to stress in plants is regulated by a complex of molecular and genetic mechanisms that activate stress responses to prevent protein degradation and cellular component destruction and restore homeostasis. While responses to biotic stresses are frequently determined by a single genetic locus, abiotic stress responses tend to have complex multiple genetic bases. This makes the control or genetic engineering of resistance difficult to achieve and requires expression of genes involved in signaling and regulatory pathways, synthesis of stress proteins and functional and structural metabolites.

Primary stresses, such as drought, salt, cold, heat, chemical pollutants and others, frequently act together to cause cellular damage and precipitate secondary stresses, including osmotic and oxidative stresses (Fig. 2). The primary stress signal (for example, changes in temperature, membrane permeability etc.) triggers adaptive and protective mechanisms that in turn shield proteins and membranes from damage and destruction and restore the

parameters of homeostasis. Inadequate signalling or gene expression can irreversibly destabilize cellular homeostasis, lead to protein and membrane degradation and eventually to cell death (Vinocur and Altman, 2005).

The multifaceted nature of the evolutionary process necessitates the use of multiple investigative approaches. Hence, a broad integrative

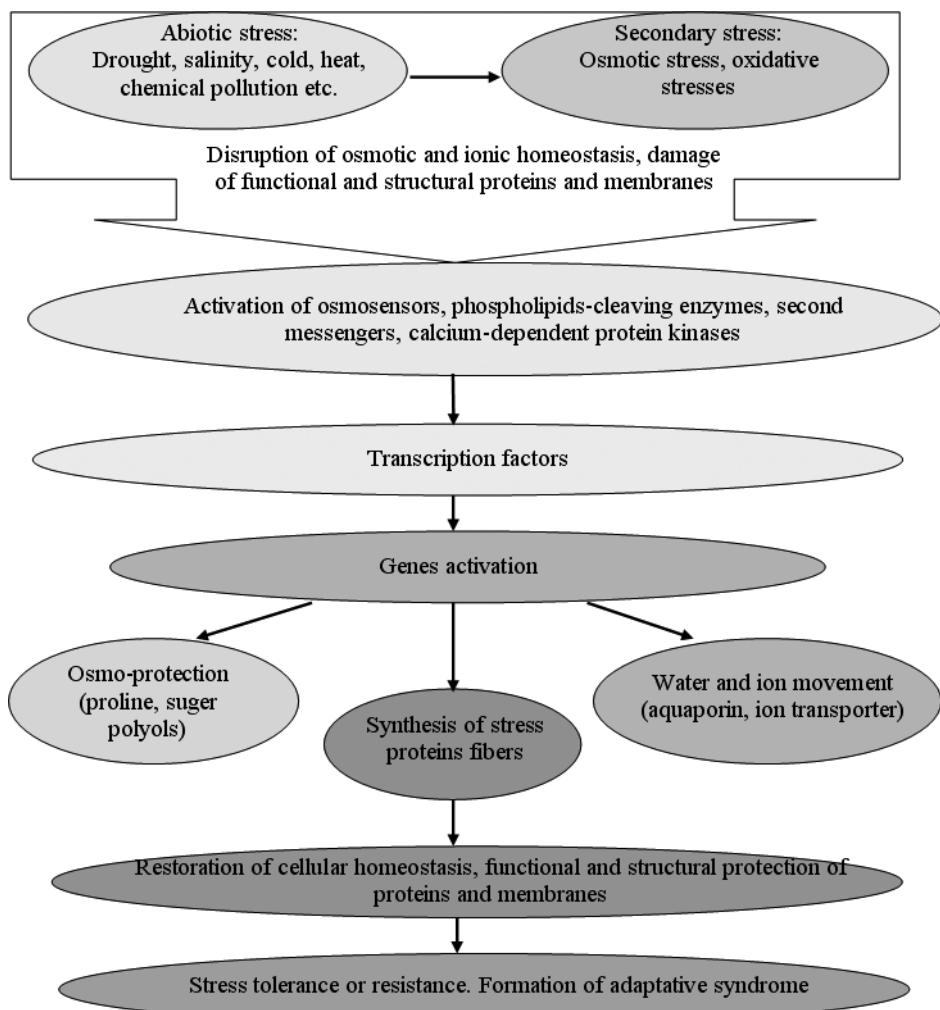


Fig. 2. Schematic presentation of plant response to abiotic stresses.

approach to understanding ecophysiological evolutionary aspects is particularly relevant given current global ecological challenges that affect population dynamics and competition among plant species. A promising ecophysiological research direction is characterization and analysis of interdependence between physiological transformations and the adoption of a specific ecological strategy. A comprehensive description of proteins and phytohormones and analysis of metabolic processes involving these factors under a variety of conditions, will permit their use as biomarkers, identify genotypic and phenotypic changes and determine evolutionary pathways of adaptation.

References

- Borzenkova, R.A., M.Yu. Yashkov, V.I. Pyankov, 2001. Soderzanie abscisovoi kisloti i citokininov u dikorastuschih vidov s rasnimi tipami ekologicheskikh strategiy, *Phyziol. Rast.* 48 (2), 229-237.
- Brodsky, J., M.Bauerle, M.Horst, A.McClellan, 1998. Mitochondrial Hsp 70 cannot replace BiP in driving protein translocation into the yeast endoplasmic reticulum, *FEBS Letters*, 435 (2), 183-186.
- Cloney, L.P., D.R.Bekkaqui, G.L.Feist et al., 1994. Brassica napus plastid and mitochondrial chaperonin 60 proteins contain multiple distinct polypeptides, *Plant Physiol.*, 105 (2), 233-241.
- Derfling, K., 1985. Gormony rasteniy. Sistemny podhod. M.: Mir, 304c.
- Derocher, A., E.Vierling, 1995. Cytoplasmic Hsp70 homolog of pea differential expression in vegetative and embryonic organs, *Plant Mol. Biol.*, 3 (27), 441-456.
- Grime, J.P. Vegetation classification by reference to strategies, 1974. *Nature*, 250, 26-31.
- Grime, J.P., J.G. Hongson, R. Hunt, 1988. Comparative plant ecology, L.: Univin Human, 739 p.
- Kotak, S., J. Larkindale, U. Lee, P. von Koskoll-Doring, E. Veirling, K.D. Scharf, 2007. Complexity of heat stress response in plants, *Current Opinion in Plant Biology*, 10, 310-316.
- Kosakivska, I.V., 2003. Physiological and biochemical aspects of plant adaptation to stresses. Kyiv, Steel, 191 p.

- Kosakivska, I.V., 2008. Stress proteins of plants. Kyiv, Ukr. Phytosociol. Cent., 151 p.
- Lee, G.J., E. Vierling, 2000. A small heat shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein, *Plant Physiol.*, 122 (1), 189-198.
- Lee, U., M. Escobar, B. Williams, S.-Wh. Hong, E. Vierling, 2005. Genetic analysis reveals domain interactions of *Arabidopsis* Hsp100/ClpB and cooperation with the small heat shock protein chaperone system, *The Plant Cell*, 17, 559-571.
- Maximov, N.A., 1927. Kratkiy kurs physiologii rasteniy dlya agronomov. M.; L.: Gosizd-vo, 378c. (In Russian).
- Maximov, N.A., 1952. Izbrannye raboty po zasuchoustoichivosti I zimostoikosti rasteniy, M.: Izd-vo AN SSSR, 294c. (In Russian).
- Panasenko, O.O., M.V. Kim, N.B. Gusev, 2003. Structura i svoistva malych belkov teplovogo shocka, Uspechy boil. chimii, 43 (1), 59-98. (In Russian).
- Perdue, D., E. Mitcham, L. Neven, 1998. Transient expression of the HCS 70 protein in cherry fruit subjected to heat stress, *J. Agr. Food Chem.*, 46 (10), 4447-4450.
- Queitsch, C., S.-W. Hong, E. Vierling, Lindquist, 2000. Heat shock protein 110 plays a crucial role in thermotolerance in *Arabidopsis*, *Plant Cell*, 12, 479-492.
- Rabotnov, T.A., 1985. O typach strategiy rasteniy, *Ecologia*, 3, 3-11. (In Russian).
- Ramenskiy, L.G., 1971. Izbrannyye trudy. L.: Nauka, 334c. (In Russian).
- Rassow, J., O. von Ashen, U. Bomer, N. Pfanner, 1997. Molecular chaperones: towards a characterization of the heat shock protein 70 family, *Trends in Cell Biol.*, 7 (3), 129-133.
- Rodriguez, E.M., J.T. Svensson, N. Malatarasi, 2005. Barley Dh13 encodes a KS-type dehydrin with constitutive and stress responsive expression, *Theor. Appl. Genet.*, 110, 852-858.
- Sangster, T., Ch. Queitsch, 2005. The HSP90 chaperone complex, an emerging force in plant development and phenotypic plasticity, *Current Opinion in Plant Biology*, 8, 86-92.
- Schirmer, E.C., S.L. Lindquist, E. Vierling, 1994. *Arabidopsis* heat shock

complements in thermotolerance defect in yeast, *Plant Cell*, 6, 1899-1909.

Usmnov, I.Yu., G.R. Kudoyerova, A.V. Martyniva, V.Z. Guli-Zade, A.S. Mustafina, 1990. Sootnoshenie indoliluksusnoi I abscizovoi kislot u rasteniy s raznymi tipami adaptivnykh strategiy, *Physiol. i biochim. kult. rast.*, 22 (1), 65-68. (In Russian).

Vinocur, B., A. Altman, 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitation, *Current Opinion in Biotechnology*, 16, 123-132.

Voinikov, V.K., G.B. Borovskiy, 1994. Role stressovykh belkov v kletkach pri hypertermii, *Uspechy sovr. Biol.*, 114(1), 85-95. (In Russian).

Wang, W., B. Vinicur, O. Shoseyov, A. Altman, 2004. Role of plant heat shock proteins and molecular chaperones in the abiotic stress response, *Trends in Plant Sciense*, 9 (5), 244-252.