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Seeds and Stress: Integration of Endogenous and Environmental Signals in Multiple Adaptive Response Pathways

Emilio Cervantes[1*] • Juana G. de Diego[2]**

[1] IRNASA-CSIC, Salamanca, Spain
[2] Department of Biochemistry, University of Salamanca, Spain
Corresponding author: * ecervant@usal.es, ** dediego@usal.es

ABSTRACT

The term "stress" is widely used in relationship with plants, but there is no evidence at all of plants suffering any stress. The application of the term to the plant sciences is metaphorical, but nevertheless it helps to reveal new aspects of plant physiology, especially concerning environmental adaptation and responses during seed germination. New DNA sequences, not previously reported for Arabidopsis, or in plants, and even not similar to any sequences reported in databases, were identified as being expressed during germination of the model plant *Arabidopsis thaliana*, suggesting that complex genome dynamics operate in this process. The mRNA encoding prohibitin was identified as up-regulated during germination. It contains features suggesting that it may be involved in translational control. Seeds are fascinating systems to study genome dynamics and translational control.

Keywords: genome, germination

INTRODUCTION

Giambattista Vico published his book entitled '*Principi di scienza nuova*' in Naples, in 1744. At the beginning of the second section, the author writes: "*Man, by the undefined nature of the human mind, when this submerges into ignorance, makes of himself the rule of the Universe*". In this sentence, the author recognises the risks of personification, also called anthropomorphism; that is, the attribution of human attributes to non-human beings. Interestingly, more than two hundred and fifty years later, the scientific literature offers many examples of personification. In the long term, personification may result in a poor and restricted view of the world around us, by which we will think of nature in general, or some of its elements, as having characteristics of human beings that only we humans, have. But, on the other hand, many of the words and concepts that exemplify personification give very graphical representations of the behaviour of organisms which contribute to advances in their understanding. Thus, language in science is a two-edged sword that we need to use with precaution. Metaphors have a very precise objective: their use is recommended while they contribute to the advance in our analysis of living processes. Once the analysis is complete, to maintain an interpretation of life based on metaphors becomes artifact and results in a poor and restricted view of reality. This may be particularly true in the case of studies with plants. Plants have physiological properties and attributes that make them substantially different to humans. In their analysis, it may be convenient to use terms that have their origins in human physiology, but to keep using these terms exceeding the purpose of an initial analysis may be, in the long term, misleading.

THE MEANING OF STRESS

The term 'stress' was first used in human physiology meaning a status of acute tension of the organism, that becomes forced to mobilise its defences to confront a menacing situation. It was introduced by the Hungarian physiologist Hans Selye (1907–1982), first in a book and later in a scientific article (Selye 1956, 1964). Since then, it has been used widely in the biological sciences as well as introduced in the colloquial language of most countries throughout the world. Initially, the term 'stress' was intended to be applied to human physiology, and, in this sense, it was associated with increased levels of suprarenal hormones in the blood. Later on, and accordingly with the increased interest in psychology, "stress" was applied to human behaviour. The term "stress" is often used in this sense, meaning the situation of an individual that does not invest enough energy directly to confront the real problems in its interaction with the environment, being in the obligation to keep a reserve of energy to keep safe his integrity and psychological equilibrium (Thines and L'Empereur 1975). More recently, the term has expanded to become widely used, in particular in the plant sciences. Even if we do not know whether or not plants really 'suffer', or go through any status similar to 'acute tension' or if an event as frequent for humans as a 'threatening situation' has, for plants, a meaning outside that of metaphorical personification, nevertheless the use of the term and the investigation of the 'stress' situations have contributed considerably to our understanding of biological processes in plants. The application of the term to plants is thus "metaphorical", in the sense that there is no evidence of real "stress" in them. Nevertheless, with this "metaphorical" use, we obtain a very accurate description of many processes. Paradoxically, the definition of stress that applies to psychology is particularly useful to gain understanding of the process of seed germination.

One of the main characteristics of living beings is their capacity to interact with others and with the environment. For this, they have developed biochemical mechanisms adapted for sensing variations in the environment that constitute the stimulus, and integrating diverse stimuli in the elaboration of a response (**Fig. 1**).

In Biology, stress was first described in terms of hormonal or endocrine modifications resulting in the activation of defence-response mechanisms in humans. This is of par-

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Research Note

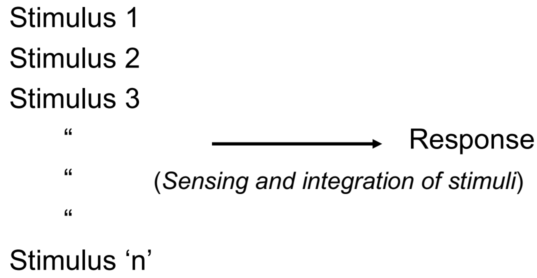

Fig. 1 Mechanism of response to the environment.

ticular interest because the analysis of the defence–response mechanisms activated under stress reveals essential aspects in the general mechanisms of sensing and integration of stimuli and elaboration of responses. Defence not only consists in the activation of a series of functions directed to confront the changing environmental conditions but, on the other side, it involves as well multiple mechanisms whose function is to check the internal conditions in the organism, or, as stated long ago by Claude Bernard, to maintain the homeostasis of its internal medium ("*milieu interieur*"). Such mechanisms include DNA repair, control of the integrity of the elements required for transcriptional and translational regulation, of protein integrity and degradation, coordinated organelle biosynthesis and stability, etc… Therefore, whether or not the term is appropriate, it opens the door for the analysis of signal-transduction mechanisms that are essential components of biological processes. The meaning of the term in psychology introduces, thus, a new and interesting component. The individual that is victim of a stress situation becomes partly unable to confront the real problems in its interaction with the environment, being in the obligation to keep a reserve of energy to keep safe his integrity and psychological equilibrium. This remarks the complexity that exists in the integration of stimuli, that not only consists in providing rapid responses in terms of activating a set of functions, but also means organic adaptation, i.e., the complex set of reactions, that even if they do not result in immediate visible effects, are essential for setting up metabolic adjustments. Seeds are excellent models to study the adjustment of the internal milieu with the environmental conditions in complex organisms.

Dormant and germinating seeds sense the conditions of the environment and integrate them together in signal transduction and metabolic pathways responsible for cell integrity and growth.

NEW ENVIRONMENTAL RESPONSE MECHANISMS

Seeds are very interesting structures. They are specialised in two activities that they are able to do better than humans. First, they are specialised in resisting harsh environmental conditions and changes during prolonged periods of time. A human being may resist without drinking water for a few hours, or a few days at the maximum, while a seed may resist years, often without any detectable modification. Changes in the environmental variables that can put humans or animals under severe difficulties are tolerated easily by seeds without showing any major alteration. But even more surprising than their capacity to resist strain, is the capacity of seeds to sense variations in the environment, to integrate these sensations together with reactions of its own metabolism in order to give a growth response such as germination. From this point of view, seeds may be considered as structures specialized in survival from stress and germination, a stress response.

In the plant's life cycle, germination fixes the time and place for further development. It marks the transit between a dynamic structure, the seed, and a static organism, the plant. Being of crucial importance, germination must be regulated tightly. The seed possess sensing mechanisms that allow it to germinate only under certain conditions.

Many important advances in plant physiology are the result of the study of the responses of seeds to environmental factors, thus the phytochrome photoreceptor was discovered by the study of light effects on germination in lettuce (Borthwick *et al.* 1952); but now with genomics, understanding the process of seed germination has, as an obligatory first step, the analysis of seed germination in the model plant, *Arabidopsis thaliana*. The comparison of the process in the model with other plant species (such as legumes and cereals) is the second stage of the investigation. It is becoming clear today, that even in the simplest and better known model plant, Arabidopsis, germination is not a simple process. It reveals new and unexpected features, among which there may be new mechanisms for sensing environmental variations and their integration in multiple biochemical pathways that constitute a developmental process. In other plants, such as legumes with larger genomes, germination will be a fascinating process full of surprises and unexpected results awaiting future research, but we are still far from understanding it completely in Arabidopsis.

Light, temperature, water, nutrients, salts, metals and other physical factors of the environment may affect seed viability and germination in Arabidopsis. The transcriptome of dry seeds includes many mRNA molecules synthesized during embryogenesis and regulated by Abscisic Acid (ABA; Nabakayashi *et al.* 2005). Light, via the phytochrome photoreceptor activates the synthesis of the enzymes of the pathway leading to gibberellic acid (GA; Yamaguchi *et al.* 1998), a hormone involved in the transcriptional regulation (Ogawa *et al.* 2003). Accordingly, GA is required in the synthesis of proteins induced during germination (Gallardo *et al.* 2001, 2002). GA exerts its effect by interacting with DELLA proteins repressors of growth (Tyler *et al.* 2004). Germination is the result of cell elongation, cell division occurs mainly after radicle protrusion (Barroco *et al.* 2005). Germination is associated with reorganisation of cell components and DNA repair (Hunt *et al.* 2007) and transcription is not strictly required for germination (Rajjou *et al.* 2004).

UNKNOWN GENOME SEQUENCES AND TRANSLATIONAL CONTROL

In a recent experiment designed to identify and clone nucleotide sequences expressed during seed germination in Arabidopsis, a total of 50 different sequences were cloned (de Diego *et al.* 2006). Among these sequences some corresponded to known genes of Arabidopsis involved in transcription, antioxidant defences, hormone responses, mitochondrial biogenesis and cell cycle. Others corresponded to Arabidopsis genomic regions in BAC clones that were not previously annotated as expressed units, thus representing DNA fragments transcribed, at least, during germination. A third group contained an important proportion of the sequences, that were not similar to any available regions from the Arabidopsis genome, but resembled more DNA sequences in databases from other organisms, such as fish species, and some of them may encode transposons. Finally, in a fourth group, a number of the sequences isolated showed no homology with any sequences in the databases, suggesting that previously unknown genome sequences are mobilised during the process of seed germination. This work (de Diego *et al.* 2006) was presented at several meetings where it raised questions repeatedly. First, do the new sequences really correspond to the Arabidopsis genome? Secondly, why are there so many new sequences, and why are those new sequences expressed during germination?

Many of the new sequences found have been re-amplified from Arabidopsis DNA or used as probes in northern and southern blots. Therefore, to answer the first question, they do indeed correspond to the Arabidopsis genome. The answers to the other questions require more speculation. One possibility is that the constant light condition used for seed germination in the initial cDNA-AFLP experiment could be the source of an additional stress superimposed on the natural stressful situation of germination and resulting in an exceptional genomic response. Since the pioneering work of Barbara McClintock (1984), transposons are known to be involved in dynamic responses of the genome when submitted to external changes (stress). A large population of retrotransposons have been described recently in oocytes and their role in the regulation of gene expression during the early stages of vertebrate development has been suggested (Peaston *et al.* 2004). Also recently, it has been shown that many non-coding RNAs may be expressed in a variety of tissues and developmental conditions. Among them, some may be precursors of microRNAs, whose roles in differentiation processes have been demonstrated recently both in plant and in animal systems. Interestingly, translational control by micro-RNA has been already demonstrated during Arabidopsis seed germination (Reyes and Chua 2007). Results of a cDNA-AFLP experiment by Bove *et al*. (2006) indicated that an important proportion of mRNA molecules related with translational apparatus and protein translation were up-regulated during dormancy breaking in seeds of *Nicotiana plumbaginifolia*. Translational control may be important during germination to regulate the synthesis of proteins according to their co-ordinate participation in multi-protein complexes or organelle assembly. Interestingly one of the sequences identified in the cDNA-AFLP approach reported above (de Diego *et al*. 2006), corresponds to prohibitin, a mitochondrial protein, whose mRNA was involved in the control of cell cycle in animals (Manjeshwar *et al*. 2003). DNA sequences in the 3′ UTR region of prohibitin share similarity with micro-RNA precursors and may be involved in translational control (de Diego *et al*. 2007).

PROTEIN DEGRADATION AND THE UNFOLDED PROTEIN RESPONSE

Many trends in biology originate from work done on mammalians and then expand to studies on plants. Recently the Unfolded Protein Response (UPR; Schroder and Kaufman 2005) is acquiring importance as a mechanism controlling many cellular processes in mammals. Basically, UPR departs from the existence in the cell of a series of mechanisms that recognise the signals from unfolded proteins and use them as a trigger to initiate developmental responses including changes in the organelles and apoptosis. During long storage periods, seeds accumulate unfolded and degraded proteins, and immediately upon imbibition important changes in the organelles accompany the cellular elongation that results in germination. In a short time upon germination, vascular bundles need to be formed and for this developmental process, apoptosis is necessary. Evidence has accumulated in recent years that plant hormones interact with each other and with central pathways of cellular metabolism. Reactive oxygen intermediates have multiple cross-interactions with ABA and ethylene (Meinhard *et al*. 2002; Cervantes 2006). Selective protein degradation is an important aspect in hormone action, and hormones interact with the machinery of protein degradation in multiple ways (Tyler *et al*. 2004; Dharmasiri *et al*. 2005; Kepinski and Leyser 2005). Free radicals are produced during active metabolism and respiration and are mediators of the stress response. The relationships between free radicals and hormone action are multiple and constitute a promising line of investigation.

Seeds are an excellent system to investigate these interactions. Nature has more richness than we think and seeds are a good example of how rich and varied processes may still remain undiscovered. As Heraclitus said: "*Nature loves to hide*".

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