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Impact of Heat Stress on Germination and Growth in Higher Plants: Physiological, Biochemical and Molecular Repercussions and Mechanisms of Defence

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Abstract: The present study investigated the effect of heat stress on different stages of growth in wheat, such as germination under various temperature regimes and precocious development of wheat seedlings. The behaviors of two wheat cultivars (*Triticum durum*, cultivar Karim and *Triticum aestivum*, cultivar Salambô) were studied, particularly at the physiological, biochemical levels. Temperature stress caused a harmful effect to plant metabolism, by disrupting cellular homeostasis. A direct result of stress-induced cellular changes is the enhanced accumulation of toxic compounds in cells that include reactive oxygen species. In our investigation made on two wheat cultivars, we were found a similarity in the response to heat stress either at the germination stage or the early development (coleoptiles and first leaf). According to literatures, our results have shown that 10 or 15°C above the optimum of temperature doesn't allow the establishment of wheat seedlings. Furthermore, reserve mobilization, seems to be damaged following heat stress by affecting the enzymes implicated in the starch breakdown and abolish giving nutrients to the wheat embryos. This was demonstrated in our work, in which we were shown that both 5 and 45°C affected the reserve mobilization but this effect is less pronounced after heat treatment of 35°C. At the photosynthetic level, heat stress generated ROS, which could damage both PS I and PS II. The detrimental effect of ROS might be alleviated by antioxidant enzymes and the sequestration of other components in plant cells. Furthermore, adaptation to temperatures changes, at the molecular level, was accompanied by the degradation of the normal proteins and the synthesis of Heat Shock Proteins (HSPs) involved in the mechanism of defence in plants.

Key words: Early growth, germination, heat stress, ionic content, oxidative stress, peroxidasic activity, proteins, wheat

INTRODUCTION

Heat is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall, 2001). In nature, plants are often subjected to environment fluctuations among those salt stress, drought and heat stress. The latter takes priority and capital importance in our research because a big part of Tunsisa, preciously, in the south of country cited in the arid and semi-arid zones. Therefore, Tunisia government's grants to the study of the abiotic stress a crucial role to resolve problems related to irrigation and desertification and to improve the capacity of plants, especially those sensitive to the abiotic constraints mentioned earlier, to cope with, adapt and grow under these severe conditions.

Cereals play a crucial role in the human food and the alimentary security. However, the cereal cultivation occupies an important place in the world agriculture. Several factors of the surrounding environment, such as drought, temperature and salinity (Naheed *et al.*, 2007), hinder the production of cereal in arid and semi-arid regions. Naheed *et al.* (2007) have shown a decline in the productivity of Rice (*Oryza sativa* L.) under salt stress conditions. The screening of tolerant varieties to environmental stresses, based on physiological parameters and biochemical markers, constitutes a powerful tools contributing to resolve this problem.

In response to both biotic and abiotic stresses, plants synthesize a wide set of proteins including chitinases and peroxidases and up-regulate phenylalamine ammonialyase, PAL (Dejaegher *et al.*, 1985). In fact, plants submitted to environment aggressions develop defensive reactions involving an activation of many genes (Collinge and Salusarenko, 1987; Sturm and Chrispeels, 1990) as a

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response to various stimulations. Germination may constitute a precocious investigation for known the threshold tolerance of plants subjected to heat constraints. The study of Dell'Aquila and Spada (1994) has shown that hard wheat seeds have a good aptitude to germinate and give seedlings when seeds were exposed to optimal thermals conditions (25°C). On the other hand, low and high temperatures modify, at the same time, velocity and capacity of germination (Bewley and Black, 1982). In Tunisia, heat stress is a major reason of yield decline in wheat due to delayed germination and plant establishment. Similarly, heat stress is a major challenge to wheat productivity in India (Joshi *et al.*, 2007). Enzyme activity particularly that of peroxidases considered as biochemical markers of stress (Castillo, 1992; Jbir *et al.*, 2001, 2002) varies according to temperature during germination of wheat seeds. In natural environment, plants are usually exposed to the combined effect of temperature and light. But excess of light could be harmful to the photosynthetic apparatus. However, exposure of plants to temperature above the range of optimal temperature caused disturbance to the overall life cycle of the plant. High temperature provoked damage at the physiological, biochemical and molecular levels. Either light or heat stress or both together can generate oxidative stress by accumulating the Reactive Oxygen Species (ROS). In survival cells, many processes are sensitive to temperature stress, such as photosynthesis, respiration, physiological mechanisms (Suzuki and Mittler, 2006). Within the plant cells, when different pathways are uncoupled, electrons that have a high-energy state are transferred to molecular oxygen (O_2) to form reactive O_2 species (ROS) (Asada and Takahashi, 1987; Mittler, 2002). ROS, such as $^1O_2$, H_2O_2, O_2^- and $HO^.$, are toxic molecules capable of causing oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). Under optimal growth conditions, they are mainly produced at a low level in organelles such as chloroplasts, mitochondria and peroxisomes. Therefore, during stress, their rate of production is dramatically elevated.

The present review accentuates on higher plants responses and adaptations to heat stress, particularly during germination and early seedlings development. Further, we sought the threshold temperature consistent with wheat growth and investigated the tolerance mechanisms and strategies established to cope with heat effects.

The environmental and physiological nature of heat stress: Heat stress often is defined as where temperatures are hot enough for sufficient time that they cause irreversible damage to plant function or development. In addition, high temperatures can increase the rate of reproductive development, which shortens the time for photosynthesis to contribute to fruit or seed production. The previous finding showed that the heat stress effect, even though it may not cause permanent damage to development because the acceleration does substantially reduce total fruit or grain yield.

The extent to which heat stress occurs in specific climatic zones is a complex issue. Sensitivity of photosynthesis to heat mainly may be due to damage to components of photosystem II located in the thylakoid membranes of the chloroplast and membrane properties (Al-Khatib and Paulsen, 1999). Studies comparing responses to heat of contrasting species indicated that photosystem II of cool season species, wheat, is more sensitive to heat than photosystem II of rice and Pearl millet, which are warm season species adapted to much higher temperatures (Al-Khatib and Paulsen, 1999).

Physiological responses to heat stress
During germination and early development: In our previous study, we have shown that germination constitutes a precocious test for researching doorstep plant tolerance to heat constraint. Studying wheat seeds behaviour, both Karim and Salambô cultivars, during germination at different temperatures (ranging from 5 to 45°C) in comparison with control temperature (25°C) reveals that low temperature reduced germination capacity and delayed germination (Essemine *et al.*, 2006, 2007). This is in agreement with previous investigations made by Murphy and Thomas (1982), in which they showed that the majority of seeds acquired an optimal temperature of germination comprise between 15 and 30°C. On the other hand, high temperature affected germination capacity and increased slightly the delay time. This cultivar has an aptitude to germinate at 45°C, even the percentage of germination is less (solely 12%). The prolonged delay time, as found at low temperature (5°C), prevent radicle protrusion and defended the establishment of wheat seedlings enable to grow properly. Present results are in agreement with that obtained by Dell'Aquila and Spada (1994). These authors showed that low temperature (5°C) delays radicle protrusion in hard wheat, variety Norba and that imbibition phase extension was related to a process, slow and gradual, of proteins synthesis in wheat embryo. That's why, cold seems to have an advantageous (beneficial) effect on the germination and promote germination of the dormancy embryo. The nature of adaptation process and the intensity of the response vary among plants species and among the different cultivars of wheat (Chen *et al.*, 1982). Consigned data in results

section are in line with that of Petruzzelli and Taranto (1989). These researchers showed a decrease in the rate of reserve mobilization and metabolites of hard wheat seeds, cultivar Appulo, to the embryo after 4 days of heat stress application. This drop was associated with a loss of the seed viability (Petruzzelli and Taranto, 1989; Essemine *et al.*, 2006). This is in agreement with observation of Soltani *et al.* (2002) in chickpea. In this context, researchers found that seedling dry weight reduction was a result of reduction in seed reserve mobilization, not conversion efficiency of mobilized reserve to seedling tissue. We recorded a pronounced decrease in reserve mobilization, mainly, at severe temperature stress (5 and 45°C). Present results are in line with those obtained by Soltani *et al.* (2002, 2006) in chickpea (*Cicer arietinum* L.) where they shown that seed reserve utilization efficiency is decreased only at severe salinities. In addition to that, Blum and Sinmena (1994) showed that seed reserve utilization efficiency of wheat decreased with increasing the temperature.

At very high temperatures, in our previous studies made with wheat 45°C don't allow good rate of germination and caused cell death and embryos damages. Tested during early stage of development (six first days of growth), this temperature (45°C) is not favourable to wheat growth and don't permit the establishment of new wheat seedlings. Present findings on wheat are in line with previous study made by Schoffl *et al.* (1999). Researchers demonstrated that the very high temperatures might provoke severe cellular injury and even death may occur within few minutes and eventually could be attributed to a catastrophic collapse of cellular organization (Schoffl *et al.*, 1999). At moderately high temperatures, injuries or death may occur only after long-term exposure. Direct injuries due to high temperatures include protein denaturation and aggregation and increased fluidity of membrane lipids. Indirect or slower heat injuries include inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein degradation and loss of membrane integrity (Howarth, 2005).

As shown in literature, plants and organisms respond to high temperature stress with the induction of heat shock proteins, HSPs, (Lindquist and Craig, 1988; Vierling, 1991). The latter are postulated to protect organisms from the damaging effects of heat and other forms of stress (Mariamma *et al.*, 1997). An obvious response to heat stress is a decrease in the synthesis of normal proteins. This decline is very pronounced, particularly, at 45°C. Frequently, the drop in normal proteins synthesis is accompanied by an accelerated transcription and translation of a new set of proteins known as heat shock proteins (Perras and Sarhan, 1989). A earlier study performed by Monjardino *et al.* (2005) on maize (*Zea mays* L.) kernels, in which they demonstrated that heat stress decreases protein accumulation and alters composition of the kernels.

Calcium as a signal transduction factor under heat stress conditions: As shown n a ream of literature, calcium was known to play a pivotal role in the both either germination or growth stage. From a studied performed by Lenormand and co-workers (Lenormand *et al.*, 1993) on sunflower seeds we have understanding the involvement of this divalent cation in the seed germination. Thus, authors have shown in their more or less recent study that the triggering reaction controlling cell expansion and germination of sunflower seeds is under the dependence of Ca^{2+}. Calcium has been suggested to play a major role in the initiation of several processes in higher plant cells. During growth and differentiation, it has been demonstrated that its release into cytosol from apoplast or from storage organelles, triggers reactions through calcium binding proteins-dependents processes (Schroeder and Thuleau, 1991). Ca^{2+} is also implicated in the regulation of K^+ current in corn (Ketchum and Poole, 1991), or in senescence of rice leaves (Huang *et al.*, 1990). Thus, large array of reactions are dependent on Ca^{2+}, while very few are not its control (Zhao and Ross, 1989). Ca^{2+} is not only a macronutrient but also a major intracellular messenger involved in the mediation of many diverse physiological processes in plants and calmodulin plays a pivotal role in the calcium messenger system (Roberts and Harmon, 1992; Poovaiah and Reddly, 1993; Gilroy *et al.*, 1993; Bush, 1995). In a study made by Gong *et al.* (1997), it appeared that external Ca^{2+} treatment obviously enhanced heat-shock induced thermotolerance in maize seedlings and this enhancement of heat-shock induced thermtolerance was specified for Ca^{2+} and could not be substituted by Mg^{2+}. In contrast, in the same study (Gong *et al.*, 1997), treatment with the Ca^{2+} chelator EGTA significantly lowered the heat-shock induced thermotolerance. In addition, in other investigations made in around twenty years ago (Graziana *et al.*, 1988; Hosey and Lazdunski, 1988) plasma membrane Ca^{2+} channel blockers La^{3+} and verapamil, which inhibit influx of extracellular Ca^{2+} into cells across the plasma membrane, also weakened the heat-shock induced and Ca^{2+}-enhanced thermotolerance. All of the above mentioned findings indicated that Ca^{2+} was involved in the acquisition of heat-shock thermotolerance and this involvement required the influx of extracellular Ca^{2+} into the cells across the plasma membrane.

Hormonal modulations: Plants owing the capacity to modulate and adapt to adverse environmental conditions, thought the degree of adaptability and tolerance to specific stresses varies among plant species. Hormones play a crucial role in this field. Hormones homeostasis, stability, content and compartmentalization are altered under heat stress (Maestri *et al.*, 2002).

Abscissic acid (ABA) and ethylene (C_2H_4), are the most known stress hormones, are implicated in the regulation of the many physiological properties by acting as signal molecules. Various environmental stresses, such as high temperature, result in increased levels of ABA. The action of ABA in response to stress involves modification of gene expression. Analysis of ABA-responsive promoters revealed several potential cis- and trans-acting regulatory elements (Swamy and Smith, 1999). ABA mediates acclimation/adaptation of plants to desiccation by modulating the up- or down-regulation of numerous genes (Xiong *et al.*, 2002). ABA induction is an important component of thermotolerance, suggesting its implication in biochemical pathways essential for survival under heat-induced desiccation stress (Maestri *et al.*, 2002). Other studies also suggest that induction of several HSPs (e.g., HSP70) by ABA may be one mechanism whereby it confers thermotolerance (Pareek *et al.*, 1998) and can cooperate with other HSPs to reactivate heat-denatured normal proteins (Lee and Vierling, 2000).

Ethylene (C_2H_4), a gaseous hormone, regulates almost all growth and development processes in plants, ranging from seed germination to flowering and fruiting as well as tolerance to environmental stresses. Heat stress changes ethylene production differently in different plant species (Arshad and Frankenberger, 2002). For example, while ethylene production in wheat leaves was inhibited slightly at 35°C and severely at 40°C, in soybean ethylene production in hypocotyls increased by increasing temperature up to 40°C and it showed inhibition at 45°C.

Sequestration of compatible osmolytes: A very important adaptive mechanism in almost plants, including higher plants, grown under abiotic stresses conditions, such as salt stress, drought and /or extreme temperatures, is the accumulation of certain organic compounds of low molecular mass, generally referred to as compatible osmolytes (Hare *et al.*, 1998; Sakamoto and Murata, 2002). Among these components we mentioned: (1) Glycinebetaine (GB), which plays a crucial role as a compatible solute in plants under various stresses, such as salinity or high temperature (Sakamoto and Murata, 2002), (2) Proline is also sequestrated extensively in high plants and usually accumulates in great quantities in

responses to environmental stresses (Kavi *et al.*, 2005). Among other osmolytes, γ-4-aminobutyric acid (GABA), non-protein amino acid, is widely distributed throughout the biological world to act as an osmoticom organic. GABA is derived from the glutamic acid by a single step reaction catalyzed by glutamate decarboxylase (GAD). Several studies have shown that various environmental stresses increase GABA accumulation through metabolic or mechanical disruptions.

Because of their important role to cope with environmental stresses in higher plants, the enhanced tolerance to stresses might be ascribed to the increased accumulation of the compatible solutes.

Biochemical responses to heat stress

Pivotal role played by peroxidase under heat stress: In present studies, we were signalled that the total soluble peroxidases activity, even decreases as function of time at 5 and 45°C, plays a crucial role in adaptation to stress. Thereby, preoxidases were implicated in growth and development processes (Requelme and Cardemil, 1993). Their activities often varied with prevailing conditions (Castillo, 1992). In fact, these enzymes were considered as biochemical markers of stress. Earlier studies provided crucial role of peroxidases in the mechanisms of protection of plants against constrained conditions (Andreeva, 1991; Bellani *et al.*, 2002).

Present results have shown, as in salt stress case that peroxidases activity depends with conditions where germination has taken place (Jbir *et al.*, 2002). Resistance to high temperature and stimulation of peroxidasic enzyme in wheat is probably mediated by synthesis of HSPs during application heat stress, though this might not be the only reason and other mechanisms might exist as well. The HSPs (heat shock proteins) were recognized to protect normal proteins from the direct effects of denaturation during heat stress treatment. Jinn *et al.* (1989) reported a similar correlation between the thermo-stabilization of normal proteins and the amount of HSP synthesized. Understanding the function of HSPs in seeds requires more additional work. More complete characterization of HSP molecular weight by SDS-polyaclrylamide gel electrophoresis was recommended. Furthermore, the changes in cell wall peroxidase activity against ferulic acid (FPOD) and lignin level in roots of NaCl-stressed rice seedlings and their correlation with root growth were investigated by Lin and Kao (2001). Authors demonstrated in their study an increase in FPOD activity in roots preceded inhibition of root growth caused either by NaCl, NH_4Cl, or proline. Egley *et al.* (1983) have shown that peroxidase is involved in the polymerization of soluble phenolics to

insoluble lignin polymers during development of prickly sida (*Sida spinosa* L.) coats, causing the formation of a water-impermeable barrier prior to seed dehydration. In this aim, authors were isolated coats and embryos from seeds harvested at several stages of development and they found that highest peroxidase activity of coats extracts correlated well with the developmental stages of maximum conversion of soluble phenolics to insoluble lignin polymers. Although seed extracts oxidized dihydoxyphenyl-alanine, this activity was eliminated by catalase, indicating that the oxidation of phenolics in the coats is catalyzed by peroxidase rather than polyphenol oxidase (PO).

Molecular responses to heat stress

Heat stress generates oxidative stress by producing Reactive Oxygen Species (ROS): In addition to tissue dehydration resulting in size reduces of whole cell; heat stress can generate oxidative stress by accumulating devastative and harmful reactive species of oxygen (ROS) within different compartments of the cell. Among these activated oxygen species we cite singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). All the species mentioned here are symptoms of cellular injury due to high temperature (Liu and Huang, 2000; Wahid *et al.*, 2007). Cellular homoeostasis is achieved by delicate balance between multiple pathways that reside in different organelles. This coordination may, however, be disrupted during temperature stress, because different pathways within the cells have a different temperature optimum (Chen *et al.*, 1982).

Involvement of antioxidants to alleviate heat stress effects: The levels of these various ROS can be reduced to tolerable levels by antioxidative systems that include ROS-scavenging enzymes, such as superoxide dismutase and ascorbate peroxidase, as well as antioxidants, such as β-carotene and α-tocopherol, under non stress conditions (Asada, 1999). Among the antioxitants considered essential for ROS detoxification during normal metabolism and particularly during stress, we mentioned ascorbic acid and glutathione and ROS-scavenging enzymes such as superoxide dismutase (SOD), ascorbate peoxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peoxiredoxin (PrxR) (Asada and Takahashi, 1987; Iba, 2002; Mittler *et al.*, 2004). These have been found in almost all cellular compartments, demonstrating the importance of ROS detoxification for cellular survival (Mittler *et al.*, 2004). Other earlier studies demonstrated that temperature stress such as heat, cold or freezing is a main cause for yield reduction in crops (Boyer, 1982) and

ROS generated by these stresses have been shown to injure cell membranes and proteins (Larkindale and Knight, 2002; O'Kane *et al.*, 1996). Earlier studies demonstrated that ROS-scavenging mechanisms have an important role in protecting plants against temperature stresses and combination of high light and temperature stress (Iba, 2002; Larkindale and Knight, 2002; Yabuta *et al.*, 2002; Yoshimura *et al.*, 2004).

Implication of HSPs in protective mechanisms against heat stress: Immediately after exposure to high temperatures and perception of signals, changes occur at the molecular level altering the expression of genes and accumulation of transcripts, thereby leading to the synthesis of stress-related proteins as a stress-tolerance strategy (Iba, 2002). Other authors have shown the activation of oxygen-scavenging enzymes when seedlings of rice (*Oryza sativa* L.) cultivar K-sen4 were exposed, at the germination and leaf stages, to 5°C for 7 days (chilling) (Saruyama and Tanida, 1995). The synthesis of the so-called HSPs allows the plants to cope with and adapt to severe abiotic stress. This molecular mechanism is implicated in the processes of adaptation to by triggering the synthesis of a specific range of protein involved in the tolerance to heat or other abiotic stress and increasing the synthesis of normal structural and functionally proteins (Lee and Vierling, 2000).

CONCLUSIONS AND FUTURE PERSPECTIVES

In corollary, obtained results demonstrate that the germination stage in higher plants and particularly in wheat (*Triticum aestivum*) is sensitive to the prevailing conditions of temperature. In other words, the temperature is a key abiotic factor implicated in the monitoring of seeds germination. At the physiological level, high temperature (45°C) was considered as a lethal temperature and might cause death of seeds cells and tissues. However, lower temperature (5°C) has a beneficial consequence in the remove of embryos dormancy allowing a high germination percentage. On the biochemical level, both high (45°C) and low (5°C) temperatures had affected the reserve mobilization to the embryo. This might be ascribed to the influence of temperature on the enzymes involved in the metabolic reactions. In present study, we followed the activity of the soluble peroxidases and we obtained a fluctuation in this activity as function of the temperatures where the germination was performed. Ultimately, on the molecular level, we observed a decrease in the amount of the total proteins after 22 h germination at higher temperature (45°C), this could be owing to the fact that the

degradation of the normal proteins is much faster and important than the synthesis of the so-called protein of heat stress (HSPs).

Finally, we summarize in the short review that both germination and early development in higher plants seem to be much affected by temperature fluctuations and this cause harmful effect to the yield and productivity of plants by affecting all the physiological, biochemical and molecular processes in the plant cells.

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