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Review

Generating high temperature tolerant transgenic plants: Achievements and challenges

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ABSTRACT

Production of plants tolerant to high temperature stress is of immense significance in the light of global warming and climate change. Plant cells respond to high temperature stress by re-programming their genetic machinery for survival and reproduction. High temperature tolerance in transgenic plants has largely been achieved either by over-expressing heat shock protein genes or by altering levels of heat shock factors that regulate expression of heat shock and non-heat shock genes. Apart from heat shock factors, over-expression of other trans-acting factors like DREB2A, bZIP28 and WRKY proteins has proven useful in imparting high temperature tolerance. Besides these, elevating the genetic levels of proteins involved in osmotic adjustment, reactive oxygen species removal, saturation of membrane-associated lipids, photosynthetic reactions, production of polyamines and protein biosynthesis process have yielded positive results in equipping transgenic plants with high temperature tolerance. Cyclic nucleotide gated calcium channel proteins that regulate calcium influxes across the cell membrane have recently been shown to be the key players in induction of high temperature tolerance. The involvement of calmodulins and kinases in activation of heat shock factors has been implicated as an important event in governing high temperature tolerance. Unfilled gaps limiting the production of high temperature tolerant transgenic plants for field level cultivation are discussed.

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1. Introduction

The average temperature of the Earth is rising owing to climate change. The rise in ambient temperature is inducing changes in the patterns of rainfall, droughts and submergence stress. As a result, the distribution pattern of plant communities is being negatively affected. The apprehensions that the vegetation in different landscapes may be drastically affected over a period of time might turn out to be a reality. Despite the fact that plants have the capacity to evolve and cope up with temperature fluctuations, this capacity might not keep pace with global warming. Estimates indicate that the projected moderate warming in the coming decades will negatively affect the yield of rice [1]. Similar projections have also been made for other crop species including brassicas, wheat, etc. The sustainability in agricultural

Abbreviations: HSP, heat shock protein; HSF, heat shock factor; ROS, reactive oxygen species.

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production is being threatened by high temperature stress across the globe.

Technological solution to grow future crops in glasshouses is not an economically viable option. It is rather a more practical solution that genetic machinery of plants is altered such that crops can grow and reproduce under increased ambient temperature. Increased high temperature tolerance would enable plants to survive stress with minimum damage to their cells. All plants have in-built mechanisms to survive increased high temperature conditions. As the capacity of plants for the basal (i.e., the inherent ability to survive under high temperature stress) and the acquired thermotolerance (i.e., the ability to acquire tolerance to otherwise high temperature stress) is differential among different plant species and genera, various components that constitute these two thermotolerance types are important to be identified. It should be feasible to introduce the requisite genes from the more efficient thermotolerance type species into the less efficient type species. If the donor and the recipient species types are sexually compatible, classical breeding methods can work. However, if the donor and recipient species are sexually diverse, it should be possible to transfer the identified genes using transgenic methods. Conventional plant breeding efforts to generate tolerance against abiotic stresses including high temperature stress have not been very successful. This is due to various reasons such as (1) tolerance against abiotic stresses is governed by multiple genes and applying conventional breeding methods for complex traits is relatively difficult, (2) the genes crucial for tolerance are neither identified nor available in sexually compatible species and (3) the 'master' regulator genes (which down-stream would affect the whole cascade of gene expression changes leading to stress tolerance) have mostly not been identified. Thanks to developments in agricultural biotechnology, several breakthrough methods have been developed for genetically improving crops against abiotic stresses (including for high temperature stress tolerance) in the past three decades of research [2,3]. However, most of these efforts have remained limited to laboratory-based experiments. The aim to obtain tolerant transgenic plants that can withstand field-level high temperature conditions remains unfulfilled.

An array of modulations occurs in plant systems in response to high temperature stress. Different plant growth stages respond differentially to high temperatures. High sensitivity of the reproductive stage of crops to this stress has immense agronomic consequences [4]. High temperature negatively affects fertilization in rice plants, as pollen production and viability is highly sensitive to increased temperature stress. Rise of as low as 1 °C ambient temperature for just 1 h at anthesis in rice results in high spikelet sterility [5]. As in rice, the fertilization process is noted to be sensitive to high temperature stress in diverse plant species. The level of cellular injury caused by high temperature stress is compounded by other prevailing abiotic stresses: high temperature stress in combination with drought, salinity, excess UV, ozone, high light intensity, weed competition and pathogens results in highly deleterious effects on crop productivity [6].

Past studies show that a plethora of alterations at morphological, anatomical, physiological, genetic, biochemical, cellular and molecular levels occur as a consequence of high temperature stress [2]. High temperature affects the whole gamut of metabolic reactions and processes including photosynthesis, respiration, nitrogen metabolism, protein turnover, nucleic acid metabolism, lipid metabolism, etc. The term 'high temperature response' encompasses various metabolic changes, which enable the cells to adapt to high temperature stress. In relation to photosynthesis process, high temperature stress affects PSII electron transport activity by affecting the oxygen-evolving complex and results in reduction of the activity of ribulose-1, 5-bisphosphate carboxylase oxygenase (RUBISCO). Thermal denaturation of RUBISCO activase is a key factor responsible for loss of RUBISCO activation during high temperature stress [7]. Arguably, molecular changes represent the foundation of all down-stream adaptations. To understand high temperature-related adaptations, one relevant way thus is to understand what molecular alterations occur during this stress. The genomic and proteomic work reveals that high temperature response is characterized by rapid reprogramming of gene expression machinery leading to up- and down-regulation of thousands of genes within minutes of exposure to high temperature stress [8]. The early changes to high temperature stress involve reprogramming in signal transduction components, transcription factors and proteins associated with the metabolism of reactive oxygen species (ROS) generated by the stressful conditions. This is followed by a phase in which metabolic adjustments predominate. Transcript profiling data show that genes encoding for (a) heat shock proteins (HSPs), (b) osmolytes, (c) enzymes that affect the membrane fluidity and (d) enzymes involved in ROS homeostasis constitute major down-stream players in high temperature response [9]. Detailed analysis suggests that high temperature response involves a coalition of pathways that culminate in the activation/synthesis of heat shock factors (HSFs) and accumulation of HSPs as a major event.

The in-depth biology of high temperature stress response in terms of signal perception and transduction, activation/synthesis of transcription factors, genomic and proteomic alterations, ROS metabolism, and various other detailed biochemical, physiological and cellular changes can be referred in several recent reviews [2–4,6,10–15]. We herewith limit ourselves in discussing the strategies currently being employed for the production of high temperature tolerant transgenic plants and highlight the gaps that exist in this endeavor.

2. Production of high temperature tolerant transgenic plants

Detailed studies have suggested that 'gene discovery' is the most important input that limits genetic engineering for high temperature tolerance. The aspect of gene discovery is thus emphasized in the fore-going account.

2.1. Engineering chaperone activity

As a primary event, high temperature stress disturbs the homeostasis of cellular proteins. The proteins potentially lose their optimal structural and functional attributes with rise in ambient temperature. The loss of biological activity of proteins upon high temperature stress may be due to aggregation and/or protein misfolding [16]. The stress-induced accumulation of aggregated and mis-folded proteins is deleterious to the cell functioning. Proteins associated with chaperone and protein degradation machineries of the cell are stimulated on exposure to high temperature stress as a step toward minimizing damages to other proteins [2]. The specific proteases synthesized upon high temperature stress may be involved in degradation of abnormal protein species.

The synthesis of HSPs is an important event associated with high temperature stress [10,17]. High and rapid up-regulation of *HSP* genes is considered as a hallmark of high temperature stress response [17]. Most HSPs reportedly function as molecular chaperones in protecting cells against damage due to high temperature by stabilizing and/or re-folding of denatured proteins [18]. HSPs were discovered nearly 40 years ago and since then, these proteins have remained as the major subject of research on high temperature stress. The expression levels of HSPs are strongly correlated with high temperature tolerance [16]. Major support in correlating HSPs and high temperature tolerance has come from experiments