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Recent advances in potato genomics, transcriptomics, and transgenics under drought and heat stresses: a review

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Abstract: Sustainable potato production practices are crucial for food security and social sustainability in the future since potato is a highly nutritious food and it is considered as one of the most promising crops to reduce human hunger and poverty in the world due to its high yield potential. However, being a temperate crop, potato is exposed to various environmental stresses, including extended periods of drought and heat. The majority of potato genomics, transcriptomics, and transgenics studies concentrate on the characterization of molecular mechanisms governing cold hardiness of tubers and response and tolerance mechanisms against diseases. Likewise, potato breeding studies focus on increasing the yield, extending the postharvest storage, and developing cultivars that withstand biotic stresses. The number of genomics, transcriptomics, and transgenics studies of drought and heat tolerance in potato is limited, although they are necessary state-of-the-art research procedures to characterize and identify the regulatory mechanism underlying any stresses in order to develop new crop varieties that can tolerate harsh environmental conditions. For these reasons, this review focuses on recent advances in genomics, transcriptomics, and transgenics of drought and heat tolerance in potato.

Key words: Potato, miRNA, transcriptomics, genomics, QTLs, drought, heat, stress, transgenics

1. Introduction

Potato (*Solanum tuberosum* L.) was first domesticated in the highlands of South America and nowadays is cultivated worldwide in Europe, Asia, North America, Africa, and Oceania, in decreasing order of production share (FAO, 2009). Following rice and wheat, potato is the third most produced food crop and the first noncereal crop in the world with an annual production of 376×10^6 t on a total of 19.3×10^6 ha at an average yield of 19.5 t/ha in 2013 (FAO, 2009). The majority of the annual world potato production is contributed by developing countries, where it is cultivated in marginal areas prone to environmental anomalies. Potato yields vary among countries between 2.5 and 45.9 t/ha, with developing countries obtaining the lowest potato yields (McGregor, 2007). Although the average annual potato production has been risen in the last two decades, this increase was mainly due to an increase in the cultivated area, whereas the average yield rates remained nearly stable in developing countries (Walker et al., 2011). Potato is used as a vegetable widely in the food industry in various forms, including table potato, French fries, chips, mash, flour, alcohol, and starch. It is also utilized in animal feeding, fuel production, and industrial purposes (Ortiz et al., 2004; Gerbens-Leenes et al., 2009). Potato is a staple food with its potential in fighting against malnutrition in both developed and developing countries since potato tubers are known sources of carbohydrates, proteins, vitamins, and minerals (Çalışkan et al., 2010). Besides being a highly nutritious food, potato is considered as one of the most promising crops to reduce human hunger and poverty in the world due to its high yield potential as reflected by a very high harvesting index value above 75% (Scott et al., 2000; Thiele et al., 2010). For these reasons, sustainable potato production practices are crucial for food security and social sustainability in the future under ever-changing environmental conditions.

Being a temperate-zone crop, potato growth and productivity are limited by its sensitivity against various abiotic stresses such as drought, salinity, and high/low temperatures (Kikuchi et al., 2015). Although potato shows a higher harvesting index than other major crops (FAO, 2009) and exhibits higher energy productivity

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(5600 kcal) than other crops, such as maize (3860 kcal), wheat (2300 kcal), and rice (2000 kcal) under sufficient irrigation (Renault and Wallender, 2000), it is more sensitive to drought stress due to its shallow root system (Yuan et al., 2003). Limited water supplies lead to potato production losses in areas with inconsistent rainfall or poor irrigation (Evers et al., 2010; Thiele et al., 2010). In addition to drought, potato is also affected by heat stress, mainly in tropic and subtropic regions, since it prefers a cool environment (Simmonds, 1971). Indeed, temperature fluctuations are considered to be the most significant overwhelming environmental factors influencing the quality and yield of potato (Levy and Veilleux, 2007). The loss in total potato yield is expected to reach up to 32% by 2050 (Hijmans, 2003). The main reason for this much reduction in the yield is gradual increase in global temperatures causing higher water demands for agricultural production. Optimal tuber yield is achieved when potato plants are grown at average day temperatures of 14–22 °C (Van Dam et al., 1996). Soil temperature higher than 18 °C causes tuber yield losses when combined with high ambient air temperature (Monneveux et al., 2014), whereas tuber growth is inhibited at temperatures above 25 °C and the growth of above-ground parts is limited when temperatures reach above 39 °C (Donnelly et al., 2007). The severity of decline in tuber quality and yield increases when heat stress accompanies drought stress (Ahn et al., 2004). Potato yield is also influenced drastically by low temperatures because it is a temperate crop that cannot acclimate to frost, which is a major concern that causes damage to crops (Chen and Li, 1980; Barrientos et al., 1994; Vega and Bamberg, 1995).

The frequency and severity of drought conditions as a result of increasing global temperatures are expected to worsen in the following decades, leading to reductions in potato production (Hijmans, 2003). High temperatures and drought could also affect the nutritional quality of tubers by reducing dry matter and accumulation of nutrition (Haverkort and Verhagen, 2008; Lipiec et al., 2013). Yield losses combined with low crop quality could drastically impact the economic output and the overall human food supply (Simelton et al., 2012).

In the last decades, potato breeding has concentrated on improving the yield of final product at lower production costs, reducing the use of chemicals in the field, increasing the cold hardiness of tubers, and extending the postharvest storage duration. Breeders have not focused on improving potato characteristics to tolerate drought and heat for a long time (Thiele et al., 2010; Monneveux et al., 2013). Recent developments in molecular genetics and genomics of crop species, and extensive knowledge obtained from studies of model plants, provide great opportunities for understanding the genetic basis of drought and heat tolerance in order to identify the individual component traits and breed potato plants for favorable alleles in the underlying genes (Tuberosa, 2012). Moreover, developments in transgenic potato research and integration of knowledge obtained from wild relatives may also facilitate the improvement of new drought- and heat-tolerant potato lines (Kikuchi et al., 2015). For these reasons, this review focuses on recent advances in genomics, transcriptomics, and transgenics of drought and heat tolerance in potato.

2. Potato genomics in drought and heat stresses

The most cultivated potato varieties in the world belong to tetraploid (2n = 4x = 48) *S. tuberosum* subsp. *tuberosum*; however, various potato species with different ploidy levels are also cultivated, particularly in South America (Watanabe, 2015). The triploid and pentaploid species are grown only in the highlands of the Andes, but diploid species are grown more widely than triploid and pentaploid ones. Tetraploid and polyploid varieties are self-compatible while diploid cultivars are generally self-incompatible (Pal and Nath, 1942; Cipar et al., 1964). Although tetraploid potato clones are self-compatible, they undergo inbreeding depression and are therefore usually heterozygous (Gebhardt et al., 2007). Complex genetic inheritance and heterozygosity of the cultivated potato (*S. tuberosum* subsp. *tuberosum*) complicate the genetic mapping. To limit the complexity of potato genetics, diploid (2n = 2x = 24) individuals are frequently used as parents for molecular mapping studies (Simko et al., 2007). Diploids can be derived from tetraploid genotypes through anther or pollen culture, or through interspecific hybridization with certain genotypes of *Solanum phureja* (2n = 2x = 24). However, the self-incompatibility feature of diploid potatoes prevents development of pure lines. Therefore, a number of common mapping approaches based on homozygous lines cannot be applied in potato unlike in other crops; instead, an F1-hybrid population can be used for genetic mapping in potato (Simko et al., 2007).

Self-incompatibility of diploid wild potato relatives results in an extremely large secondary gene pool full of genetic variation. They are outcrossing and, consequently, heterozygous parents generate segregating F1 progeny. Since potato shows vegetative propagation (tubers), addition of outcrossing results in naturally abundant phenotypic and genotypic variations in the potato gene pool under variable climatic conditions (Cipar et al., 1964). In this way, the potato resembles humans. Therefore, the models and methods developed in human genetics derived from the Human Genome Project can serve as a model for developing original breeding strategies for potato (Gebhardt et al., 2007).

Many quantitative trait loci (QTLs) for yield, agronomic, and quality traits have been identified in potato (Gebhardt et al., 1991; Freyre et al., 1994; Van Eck et al., 1994a, 1994b; Schäfer-Pregl et al., 1998; Werij et al., 2007; Bradshaw et al., 2008; Śliwka et al., 2008). However, quite a small number of QTLs have been identified for traits of abiotic stress tolerance in potato, and all identified QTLs to date are related to only drought tolerance traits. In the first QTL study for drought tolerance in potato, a total of 23 QTLs were identified from control, PEG stress, and recovery treatments under in vitro conditions. Among these, 10 QTLs were located on chromosome 2, and three QTLs involved in trait of root-to-shoot ratio were identified on linkage groups 2, 3, and 8. In another study by the same group, a total of 47 QTLs were identified in a diploid potato mapping population under well-watered, drought, and recovery conditions (Anithakumari et al., 2012). Among them, 28 QTLs were drought-specific, 17 were specific to the recovery treatment, and 2 were unique to the well-watered condition. Thirty-one significant QTLs were located on chromosomes 5 and 4 for different traits in drought, recovery, and well-watered conditions. Four QTLs for $\delta^{13}C$, three for chlorophyll content, and one for chlorophyll fluorescence (Fv/Fm) were detected to colocalize with yield and other growth trait QTLs identified on other chromosomes.

To date, a limited number of QTLs for complex traits of drought tolerance have been detected by traditional QTL mapping approach. Association mapping is considered as an alternative approach to the traditional QTL mapping for the identification of QTLs. The main advantages of association mapping include: 1) no need to make crosses to generate segregating populations; 2) a collection of various cultivars and breeding lines can be utilized for mapping studies; 3) higher mapping resolution may be reached with many more meiotic recombination events. In view of the advantages and applications of association mapping, it can be applied in potato to develop molecular markers for drought tolerance.

3. Potato transcriptomics in drought and heat stresses

Accounting for 26% among all environmental stresses, drought is the most important stress factor that plants face (Blum and Jordan, 1985). Detrimental effects of drought on plants can be observed at the whole-plant level as the death of plants and/or decrease in productivity (Parida and Das, 2005). Drought decreases vegetative potato plant growth (leaves, stems, shoots, and roots) (Ojala et al., 1990; Deblonde and Ledent, 2001), development (Mahajan and Tuteja, 2005; Kar and Kumar, 2007), and production by reducing the number (Eiasu et al., 2007), size (Schafleitner et al., 2007), and quality (Deblonde and Ledent, 2001) of the tubers. Drought stress mainly influences the final potato yield at two stages of potato development, namely tuber initiation and tuber bulking (Martinez et al., 1995).

Considered to be the most important environmental factor influencing the quality and yield of potato (Levy and Veilleux, 2007), high temperature affects various biochemical and physiological processes in potato plants. High temperature negatively affects the tuber initiation and development by inhibiting the tuberization signal, StSP6A, which is an orthologue of the Arabidopsis FLOWERING LOCUS T (FT) protein (Ewing, 1981; Navarro et al., 2011). High temperature also causes nutrient source-sink problems by decreasing the carbon assimilation in tubers (Wolf et al., 1990, 1991) and inhibits tuber filling (Krauss and Marschner, 1984). Hence, high temperature, in turn, leads to reduced tuber quality and yield (Borah and Milthorpe, 1962). Heat stress also causes a decrease in photosynthesis by decreasing the gas exchange and chlorophyll biosynthesis (Reynolds et al., 1990; Midmore and Prange, 1992).

Both drought and heat cause osmotic and oxidative stresses in plants. Plants have evolved different defense mechanisms, such as avoidance and tolerance, activated under osmotic and oxidative stresses (Kalefetoğlu and Ekmekci, 2005). The avoidance of drought stress is one of the most crucial issues in potato production. Extended periods of drought or high temperatures lead to the production of reactive oxygen species (ROS), which are cytotoxic in high concentrations. Because ROS are toxic but also participate in signaling events, plant cells require at least two different mechanisms to regulate their intracellular ROS concentrations by scavenging of ROS: one that will enable the fine modulation of low levels of ROS for signaling purposes, and one that will enable the detoxification of excess ROS, especially during stress (Mittler, 2002). Potato plants have developed a plethora of biochemical and molecular mechanisms to cope with the deleterious effects of ROS in the cells. These systems are called antioxidant protection mechanisms. Biochemical strategies include synthesis of compatible solutes, changes in the photosynthetic pathway, alteration in membrane structures, and induction of antioxidant enzymes and plant hormones (Parida and Das, 2005). Detrimental effects of all environmental stress types on potato plants can be observed at the transcription level. Responses to drought and heat stress may occur within a few seconds (such as a change in the phosphorylation status of a protein) or within minutes and hours (such as a change in gene expression) (Zhu, 2002). Stress responsive genes can be divided into two categories as 'early-response genes' and 'delayed-response genes'. Early-response genes are induced very quickly and often transiently. Their induction does not require new protein synthesis because all signaling components are already in place. Delayed-

response genes are activated by stress more slowly, and their expression is often sustained. They constitute the vast majority of the stress-responsive genes. The early-response genes typically encode transcription factors that activate downstream delayed-response genes, which take a role in stress tolerance (Kalefetoğlu and Ekmekci, 2005; Sreenivasulu et al., 2007). Hence, understanding the transcriptomic changes in potato under drought and high temperature conditions is essential to identify the response and tolerance mechanisms. Identification of drought and heat response/tolerance genes of potato would assist the molecular breeding of new potato lines tolerant to drought stress.

To date, several transcriptomic studies have been completed in potato development and abiotic/biotic stress response (Bachem et al., 2000; Flinn et al., 2005; Rensink et al., 2005a; Kloosterman et al., 2008; Evers et al., 2010; Massa et al., 2011); however, the number of transcriptomic studies carried out to elucidate the changes in the gene expression profiles of potato under drought and high temperature is limited. There are various studies exploring the expression patterns and functions of individual genes in potato under drought (Monneveux et al., 2013). Several studies reported the expression of genes associated with the production of compatible solutes, such as glycine betaine, trehalose, glucosylglycerol, and proline, increase in potato under drought (reviewed by Byun et al. (2007) and Kikuchi et al. (2015)). In one of the earliest transcriptomic studies of potato in response to abiotic stresses, 20,756 expressed sequence tags (ESTs) from a complementary DNA (cDNA) library were constructed by pooling messenger ribonucleic acid (mRNA) from heat-, cold-, salt-, and drought-stressed potato leaves and roots (Rensink et al., 2005a). In this EST library, 1476 unique sequences were identified in potato leaves and roots exposed to abiotic stresses. Previously the majority of potato transcriptomic studies used a spotted cDNA array produced by The Institute for Genomic Research (TIGR) containing around 10,000 cDNA clones (Rensink et al., 2005b), whereas some researchers designed their own cDNA arrays (Kloosterman et al., 2005). In these early studies, stress-induced genes such as late-embryogenesis abundant proteins, heat shock proteins, and transcription factors were commonly detected as they have functions similar to their orthologues in other plant species.

In 2008, the Potato Oligo Consortium (POCI) array with 44,000 probes representing 42,034 potato unigenes was developed to intensify the transcriptomic research of potato (Kloosterman et al., 2008). The array was incorporated into the functional genomics program of a Canadian consortium to improve disease resistance and tuber quality traits of potato (Regan et al., 2006). Recent developments in high-throughput sequencing technologies of the whole transcriptome, known as RNA-Seq, permit the analysis of all transcripts in a sample for mRNA and miRNA abundance, and detection of aberrant transcripts and transcript splice variants (Wang et al., 2009b) without a prior knowledge of the transcriptome of a studied organism (Morin et al., 2008). Large sets of RNA-Seq data have been generated from two potato genotypes, the doubled monoploid *S. tuberosum* Group Phureja DM1-3 516 R44 and the heterozygous diploid breeding line *S. tuberosum* Group Tuberosum RH89-039-16, by the Potato Genome Sequencing Consortium (PGSC) to facilitate the functional identification of genes in different plant tissues under various environmental conditions (Potato Genome Sequencing Consortium, 2011). In order to complement the efforts of the PGSC, a reference for the potato transcriptome was prepared from different tissues and biotic/abiotic stresses by using the Group Phureja clone DM1-3 516R44, whose whole genome sequence was already completed (Massa et al., 2011). Transcript abundance of more than 22,000 genes was quantified from over 550 million RNA-Seq reads, and genes were classified into 18 gene modules in a weighted gene correlation network analysis (WGCNA), which gives an inclusive identification of highly correlated groups (Zhang and Horvath, 2005). Interestingly, the majority of the modules included genes exclusively overrepresented in a specific tissue type, suggesting the biological variability that accounts for gene expression differences among tissues is highly correlated with the type of genes expressed in different tissues. On the other hand, principal component analysis of biotic and abiotic stresses showed that the abiotic stresses cluster together with hormone treatments and this cluster is different from the cluster of biotic stresses, indicating the involvement of different gene networks in abiotic and biotic stresses. Since 30% of the genes identified in this study did not have a functional annotation, functional characterization of highly expressed genes among gene modules will be among future challenges to understand the biological roles of unknown genes in potato growth, development, and response to environmental conditions. In a recent in silico study, RNA-Seq data generated by the PGSC in response to abiotic and biotic stresses and plant hormone treatments were analyzed comprehensively (Massa et al., 2013). This study showed that the number of genes that overlap between hormone (ABA, BAP, IAA, and GA_3) treatments and abiotic (heat, mannitol, and salt) stresses is much higher than the ones each share with the biotic stresses. Seventeen molecular function ontologies were overrepresented in Gene Ontology enrichment analysis of common genes of abiotic and hormone treatments. One of these ontologies included stress-responsive transcription factors, many of which were induced in

abiotic and hormone treatments. These proteins belong to the MYB, APETALA2 (AP2)/ethylene-responsive element binding factor (ERF), and NAM, ATAF1/2, and CUC2 (NAC) transcription factor families. Almost 53% of these transcription factors were expressed in one or more of the stress conditions analyzed, suggesting that different stress or hormone treatments stimulate common signaling pathways as well as unique gene networks.

In a recent transcriptomic study of potato under drought stress, the transcripts that are differentially expressed under water withholding and rewatering were identified in order to deepen the understanding of the molecular mechanism of potato stolon responding to water stimulus (Gong et al., 2014). For this purpose, *Solanum tuberosum* variety Ningshu 4 was either exposed to drought stress for 3 days (as relative soil moisture was reduced to 35%–40%), or was rewatered and grown for 3 days following the 3 days of drought stress, or was grown at maximum field water-holding capacity (as a control group) at the flowering stage. By analyzing the RNA-Seq data generated from stolon tips of potato plants in each of the three groups, the researchers identified 3189 and 1797 differentially expressed transcripts under only drought treatment and treatment of drought followed by rewatering, respectively. Of these transcripts, 263 genes showed reverse differential expression patterns in plants exposed to only drought and drought followed by rewatering, suggesting that a strict transcriptional regulation of these genes is very important for the survival of potato plants under severe drought conditions. Several of these genes are homologues of known drought-responsive genes in Arabidopsis, including a dehydrin, protein phosphatase, auxin-responsive protein, gibberellic acid (GA)-stimulated gene, calmodulin-like protein, abscisic acid 8'-hydroxylases, and calcium-transporting ATPase. Interestingly, differentially expressed genes were classified into metabolism-related gene ontologies, indicating an obvious impact of water stimulus on general metabolism (especially that of ABA and sugar) in stolon tips at the flowering stage of potato development. Similar to other transcriptomic studies, several transcription factor genes were also differentially expressed in stolon tips after drought stress. Differentially expressed transcription factor genes were classified into 50 different families, with the largest ones belonging to the bHLH, ERF, MYB, C2H2, NAC, WRKY, HD-ZIP, and bZIP families, suggesting that a comprehensive symphony of regulatory pathways is activated under water stress and recovery in potato.

Comparison of transcriptomic changes among different potato varieties or clones was the focus of some transcriptomic studies of potato under drought stress. In a comparative transcriptomic study of two potato clones, the transcriptomic changes of leaves from two moderately drought-tolerant native Andean potato clones, SA2563 and Sullu, grown in the field were analyzed using the TIGR 10K potato cDNA microarray under drought stress (Schafleitner et al., 2007). A total of 1713 genes were found to be differentially expressed, and 186 of these genes were induced in both clones under drought conditions. These genes were grouped into categories related to osmotic regulation, control of carbohydrate metabolism, membrane modifications, consolidation of cuticles, and cell protection mechanisms, such as ROS detoxification and protein stabilization. In another comparative transcriptomic study of two potato clones, the transcriptomic changes in the leaves of drought-tolerant clone Sullu and drought-sensitive clone SS2613 were compared under a continuously increasing drought stress in a field experiment (Evers et al., 2010). Genes involved in photosynthesis and carbohydrate metabolisms were found to be downregulated in Sullu after 28 days of water withholding, whereas downregulation of photosynthesis-related genes was observed only after 49 days of water withholding in SS2613, indicating a slower response of sensitive clone SS2613 to water withholding. Moreover, higher levels of osmolyte accumulation, such as galactose, inositol, galactinol, and proline, were observed in Sullu under drought stress, suggesting the tolerance mechanisms of tolerant clone Sullu became less pronounced in this field water withholding study, while it could still produce three times higher tuber yields compared to the sensitive clone SS2613. In another attempt to relate the transcriptomic differences between potato accessions, accessions Sullu and Ccompis were compared under drought conditions in a field at high altitude (Mane et al., 2008). Sullu was found to tolerate water withdrawal better compared to Ccompis since the expression levels of genes involved in antioxidant, flavonoid, and carbohydrate metabolisms were higher in Sullu, indicating the presence of distinct molecular and biochemical drought responses in the two potato accessions leading to yield maintenance but differential biomass accumulation in vegetative tissues. One explanation for these observations is that the daytime temperatures during the field study were higher than the temperatures potato can tolerate such that the plants were exposed to a simultaneous drought and high temperature stresses during the experiment. Therefore, it is necessary to do these types of drought experiments in temperature-controlled growth chambers in order to reduce any potential additional stresses that may cause unwanted transcriptomic changes in plants.

In another similar study, the transcriptomic changes occurring in tubers of three accessions of *Solanum tuberosum* subsp. *andigena* (VTSA01, 02, and 03) were compared under two cycles of drought stress in a temperature-controlled greenhouse (Watkinson et

al., 2006). VTSA01 adapted to the first cycle of water withholding by recovering the photosynthesis at the end of the first cycle and maintained this drought adaptation in the second cycle of water withholding. VTSA02 showed an intermediate adaptation to water withholding with limited photosynthetic recovery at the end of the first cycle of drought while its photosynthetic recovery was improved at the end of second cycle of drought, suggesting a partial adaptation to the drought stress. VTSA03 could recover the photosynthetic activity only after the second cycle of water withholding, suggesting that it needed an acclimation period before full recovery. Genes involved in antioxidant defense, chromatin remodeling, transcriptional control of gene expression, and flavonoid biosynthesis were highly upregulated in VTSA01 and moderately in VTSA02, whereas their induction was not significant in VTSA03. Interestingly, genes encoding for glutathione-S-transferase and superoxide dismutase did not respond to drought stress. Two zinc-finger transcription factors were induced in tubers of VTSA01 following the first cycle of stress and in tubers of VTSA02 after the second cycle, while they were repressed in tubers of VTSA03 after the first cycle of water withholding. These data suggest two different antioxidant mechanisms activated under drought adaptation and acclimation in potato tubers. Antioxidant defense of metallothioneins is activated at both adaptation and acclimation stages while genes involved in the flavonoid pathway are induced in drought adaptation since they show an induced basal level of expression in drought-adapted accession. Since the tuber development differed between three accessions of potato exposed to drought stress, a follow-up transcriptomic study was carried out to correlate the expression of genes associated with carbon metabolism with the tuber development phenotypes under drought (Watkinson et al., 2008). Adapted accession VTSA01 showed starch deficiency while intermediate accession VTSA02 showed normal starch deposition due to the alterations of expression of genes involved in sucrose and starch metabolism, such as *sucrose synthase*, *starch phosphorylase*, and *glycogen bound starch synthase*, as well as genes associated with trehalose and respiration metabolisms. Taken together, these studies suggest that the drought adaptation of different potato accessions is affected by the metabolic networks associated with starch biosynthesis and deposition in tubers.

In one of the studies, the transcriptome of transgenic potato overexpressing yeast *TREHALOSE-6-PHOSPHATE SYNTHASE1* (*ScTPS1*) was compared to that of nontransformant plants using the POCI microarray in order to identify the molecular basis of improved drought tolerance of transgenic potato lines (Kondrak et al., 2011). Ninety-nine genes were found to be expressed differentially, and 46 of them were assigned into 16 functional groups. Among those groups, seven genes associated with carbohydrate metabolism were all found to be upregulated in transgenic plants. A large proportion of genes were found to be involved in transcriptional and translational regulation of gene expression. Interestingly, it was shown that transgenic potato lines expressing *ScTPS1* under the control of a drought-inducible potato promoter, *StDS2* (Dóczi et al., 2002), grow slower and have a lower CO_2 fixation rate and stomatal density compared to nontransformants under drought conditions, although they could tolerate drought more efficiently than nontransformants (Stiller et al., 2008). Compared to constitutive promoters, stress-inducible promoters usually maintain a low level of expression of the regulated gene, causing negative pleiotropic effects on the plant under conditions where the product of the expressed gene is not necessary (Cominelli and Tonelli, 2010). These pleiotropic effects of *ScTPS1* overexpression in potato may occur due to complex transcriptional, translational, and posttranslational regulatory mechanisms that interact to lead to drought tolerance in potato. In a follow-up study, 379 genes of known function were found to be differentially expressed in drought-treated *StDS2::ScTPS1*-expressing transgenic potato plants (Kondrák et al., 2012). Four transcription factors were found to be uniquely upregulated in transgenic plants, suggesting that they may be used in generation of drought-tolerant transgenic potato plants in the future.

Wild *Solanum* species are adapted to various environments, growing at different altitudes from sea level to above 4000 m, and they do so within a broad range of temperature, photoperiod, and water supplies. Hence, wild potato species represent a great resource for genetic variability that can be used by breeders in identification of traits controlling the drought tolerance responses. In order to identify the molecular mechanisms underlying the drought tolerance in wild potato relatives, transcriptional profiles of two *Solanum* wild species, *S. venturii* and *S. cardiophyllum*, with contrasting behaviors under drought stress was carried out recently (Lucca et al., 2011). Significant differences in transcriptional profiles between the two wild potato relatives were observed with differentially expressed genes varying not only in number but also in their temporal expression profiles. Drought-sensitive wild potato relative showed little differential gene expression (235 genes) associated with cell wall metabolism, transcription, protein metabolism, and signaling while tolerant species displayed at all times a massive differential expression comprising 4133 genes. Species shared 151 differentially expressed genes while there was a set of differentially expressed genes unique to each species. This suggests the activation of a general drought response mechanism in both species while

different unique strategies are used by each species to cope with drought. Several unique genes associated with tolerant species *S. cardiophyllum* can be used as candidate genes to develop new potato cultivars with higher drought tolerance responses in the future.

Improvement of potato heat tolerance has been moderately successful because there are limited numbers of studies on understanding the molecular mechanism of heat tolerance in potato (Broin et al., 2000; Ahn et al., 2004; Levy and Veilleux, 2007). In order to better understand the heat tolerance mechanisms of potato, the key genes and overall network of genes acting in the heat tolerance of potato should be characterized in more detail. To our knowledge, only a few studies are available on the heat response of potato at the molecular level. In one of the studies, 2190 genes were found to be differentially expressed in potato leaves when the plants were exposed to moderately elevated temperatures (30/20 °C, day/night) for up to 5 weeks (Hancock et al., 2014). Heat-responsive genes involved in photosynthesis, lipid metabolism, and amino acid biosynthesis were highly overrepresented at all time points of stress treatment. In tubers, a total of 2886 genes exhibited major changes in their transcript levels associated with the different temperature conditions in the course of stress treatment. Differentially expressed genes in potato tubers were underrepresented in functional categories related to cell wall processes, lipid metabolism, aspects of secondary metabolism, hormone metabolism, biotic stress, DNA metabolism, and development, whereas genes involved in RNA metabolism were overrepresented following moderately high temperature treatment. In k-means clustering of heat-responsive transcripts of potato, genes associated with ABA, ethylene, auxin, and brassinosteroid responses; heat shock proteins and transcription factors; and genes previously associated with abiotic stress responses were identified. These data indicate that the potato plants respond to moderately elevated temperatures differently than other crops such that instead of known symptoms of abiotic stress they exhibit a combination of different biochemical and molecular pathways during tuber development.

In another study, Gangadhar et al. (2014) used a yeast-based functional screening method to identify, characterize, and classify high-temperature stress-responsive genes in potato. A total of 95 potential candidate genes were identified from heat-stressed potato plants after 2 and 48 h at 35 °C. The responses of each of 95 genes to various abiotic stresses were characterized, and 20 genes were found to be responsive to drought, 14 to salt, and 11 to heat/drought/salt stresses. Expression patterns of the majority of the 95 candidate genes varied under heat, drought, and salt stress conditions. Most of these 95 genes were involved in various cellular metabolisms, signal transduction pathways, stress responses, and protein folding mechanisms based on gene ontology analysis, suggesting a possible role of these genes in heat tolerance of potato (Gangadhar et al., 2014).

To identify the genes involved in the periderm response to heat stress, transcriptome profiling of immature tubers was analyzed under high soil temperatures (33 °C) (Ginzberg et al., 2009). Stress-related transcripts were differentially expressed in both the skin and phelloderm while heat-shock proteins (HSPs) were mainly induced in the phelloderm and genes involved in plant/microbe interaction responses were mainly induced in the skin under heat treatment, suggesting that various gene networks are activated in different tissues in response to heat stress.

Rensink et al. (2005b) subjected potato seedlings to cold (4 °C), heat (35 °C), or salt (100 mM NaCl) stresses for up to 27 h in order to identify abiotic stress-responsive genes in potato. A total of 3314 cDNA clones exhibited a significant differential expression in at least one stress condition. While 2584 cDNA clones were differentially expressed under cold stress, 1149 and 998 clones were up- or downregulated under salt and heat stresses, respectively. The functional annotation analysis of differentially expressed clones showed several transcription factors, DNA binding proteins, transporter proteins, phosphatases, and HSPs in response to abiotic stresses. In addition, a suite of genes with unknown functions were also identified (Rensink et al., 2005b).

4. Potato miRNAs in response to drought and heat stresses

MicroRNAs (miRNAs) are endogenous, short (≈21 nt), noncoding RNAs that play important gene-regulatory roles in animals and plants by pairing to the mRNAs of protein-coding genes to direct their posttranscriptional repression (reviewed by Voinnet (2009)). In addition to functions in plant development, such as root initiation and development, leaf development, formation of vascular bundles, transition from vegetative to generative state, and flower initiation and development (reviewed by Flynt and Lai (2008)), miRNAs also have regulatory functions in hormonal control and adaptation to a variety of biotic and abiotic stresses (reviewed by Voinnet (2008)).

As of June 2015, there were 8495 plant mature miRNAs from 72 plant species registered in the miRBase database (Griffiths-Jones et al., 2006). The targets of many miRNAs have been predicted by in silico analyses, and some of them have been experimentally validated in the lab. The first attempt to identify the miRNAs that function in development and their targets in potato was done in 2009 (Zhang et al., 2009). In this study, 48 potential miRNAs were identified in *S. tuberosum* by in silico comparisons of known miRNAs from other plants against potato EST,

GSS, and nucleotide databases. This first study to identify potato miRNAs was followed by several other studies (Kim et al., 2011; Xie et al., 2011; Zhang et al., 2013; Lakhotia et al., 2014; Ou et al., 2014; Zhang et al., 2014), in which the number of predicted miRNAs and their potential targets in potato increased significantly, suggesting that the prediction algorithms employed to identify new miRNAs and their targets have improved in recent years.

To date, all genome-wide identification studies of miRNAs and their targets affected by abiotic stresses in *S. tuberosum* concentrated exclusively on the characterization of miRNAs and their targets under drought conditions. High-throughput DNA sequencing allowed researchers to identify various miRNA families affected under drought stress. In a recent comprehensive deep-sequencing miRNA study, a total of 458 known and 674 new miRNAs in control samples was identified, whereas 471 known and 566 novel miRNAs were predicted in drought samples (Zhang et al., 2014). The researchers proved that 100 of the known miRNAs were repressed whereas 99 of them were induced in entire potato leaves under 20 days of drought stress. Moreover, 151 of the novel miRNAs were repressed while 119 of them were induced in drought-treated potato leaves compared to the controls. In addition, a total of 246 target genes of known miRNAs and 214 target genes of novel miRNAs were identified based on target prediction. Following the transcript abundance analyses of select differentially expressed miRNAs and their target mRNAs, miR811, miR814, miR835, and miR4398 were found to play roles in posttranscriptional regulation of drought-related genes in potato. These miRNAs target a MYB transcription factor, a hydroxyproline-rich glycoprotein, an aquaporin, and a WRKY transcription factor, respectively. These miRNAs and target genes showed negative correlations based on their expression patterns. In one of the studies, the expression of stu-miR396 gradually increased in the first 6 h of drought treatment, while the expression levels of stu-miR156a and stu-miR157a decreased in the first 3 h of drought and then increased at 6th hour 6 of treatment, suggesting that these three miRNAs function as early responders to drought stress and their expressions are under tight regulation (Hwang et al., 2011a). One of the known targets of miR396 is the growth-regulating factor (GRF) family of transcription factors functioning in leaf and cotyledon growth in Arabidopsis (Kim JH et al., 2003). Constitutive overexpression of miRNA396 suppresses the expression of 6 GRF targets and alters leaf growth and stomata development in Arabidopsis (Liu et al., 2009). stu-miR156a and stu-miR157a target Squamosa promoter-binding protein (SBP)-box transcription factors that have regulatory roles in multiple developmental processes including shoot and flower development (Wu and Poethig, 2006; Xie et al., 2006; Schwarz et al., 2008; Wang et al., 2009a; Wu et al., 2009; Jiao et al., 2010).

In a study of miRNAs belonging to the family of miR171, expression levels of stu-miRNA171a, stu-miRNA171b, and stu-miRNA171c were analyzed under air-drying or 15% polyethylene glycol 6000 (PEG 6000) (Hwang et al., 2011c). Transcript levels of all three miRNAs decreased in the first hour of air-drying while they were induced afterwards until the sixth hour of stress treatment. Accumulation of stu-miRNA171a transcript decreased in the first hour of PEG treatment, followed by an increase in the third hour, and this level was kept steady thereafter up to 48 h of drought treatment. During air-drying, the transcript level of stu-miRNA171b decreased in the first hour, and then it recovered back to the control levels in the third hour and exceeded the control levels in the sixth hour of stress treatment. Transcript levels of stu-miRNA171b showed a slight decrease in the first 6 h of PEG treatment while the levels were induced after 12 h of treatment compared to the control conditions. Although the transcript abundance of stu-miRNA171c was similar to that of stu-miRNA171a under air-drying treatment, it decreased in the first hour of PEG treatment and slightly increased during the course of the experiment, reaching control levels in 48 h of stress treatment. Targets of miRNA171 belong to the transcription factors in the GRAS gene family, which is a plant-specific protein family named after the first three members: GIBBERELLIC ACID INSENSITIVE (GAI), REPRESSOR OF GAI (RGA), and SCARECROW (SCR) (Di Laurenzio et al., 1996; Peng et al., 1997; Silverstone et al., 1998; Bolle, 2004). They play roles in plant development and signal transduction, including root development, axillary shoot development, maintenance of the shoot apical meristem, light signaling, gibberellic acid (GA) signaling, nodulation signaling, brassinosteroid (BR) signaling, auxin signaling, and transcriptional regulation in response to abiotic and biotic stresses (Xiaolin et al., 2012). There are 33 *GRAS* genes in *Arabidopsis thaliana*, and transcript levels of some *GRAS* genes are tightly regulated by miRNAs (Lee et al., 2008). Transcript accumulation of *AtSCL6*, *22*, and *27* were negatively regulated by miRNA171/miRNA39 in Arabidopsis. miRNA-dependent cleavage of the mRNAs was shown when *Nicotiana benthamiana* leaves were cotransformed with *miRNA171/miRNA39* and *AtSCL6* or *AtSCL22* (Llave et al., 2002). This mRNA cleavage was developmentally controlled as the truncated RNAs can only be detected in inflorescence tissue. Interestingly, GRAS proteins contain a variable N-terminal domain (N-domain), which causes them to resemble eukaryotic intrinsically disordered proteins (IDPs) that interact with large numbers of diverse proteins, acting as network hubs in various vital processes (Wright and Dyson, 1999; Uversky et al., 2005; Patil and Nakamura, 2006; Radivojac et al., 2007). Hence, miRNA-based posttranscriptional

regulation of GRAS proteins is necessary since these proteins are key regulators of cross-talk between different signaling networks activated under environmental stresses (Sun et al., 2011).

In another similar study, the transcript abundances of stu-miRNA172c, stu-miRNA172d, and stu-miRNA172e were found to be affected by drought treatment (Hwang et al., 2011b). Transcripts of stu-miR172c and stu-miR172d accumulated more in drought-treated potato plants while the transcript level of stu-miRNA172e was not affected by drought. Arabidopsis miRNA172 is a negative regulator of plant-specific transcription factor gene *APETALA2* (*AP2*) (Chen, 2004) and *AP2*-like genes, including *TARGET OF EAT1* (*TOE1*), *TOE2*, *TOE3*, *SCHLAFMUTZE* (*SMZ*), and *SCHNARCHZAPFEN* (*SNZ*) (Aukerman and Sakai, 2003; Schmid et al., 2003). *AP2* is a floral organ identity gene playing key roles in meristem identity during floral development (Drews et al., 1991). *AP2*-like genes, on the other hand, mainly act as flowering repressors by involvement in the determination of time of flowering (Jung et al., 2007; Mathieu et al., 2009). The transcript abundance of miRNA172a, b, and c reach maximum when plants are in the reproductive growth stage, while the transcript levels of miRNA172d and e are always very low and not affected by developmental processes (Jung et al., 2007). Interestingly, miRNA156 regulates the mRNA levels of its target *SPL9*, which in turn controls the expression of miR172 by directly activating its transcription (Wu et al., 2009). Hence, miRNA156 regulates the expression of miRNA172, and the expression patterns of both are inversely correlated to each other (Chuck et al., 2007; Wang et al., 2009a), suggesting that the flowering and floral organ identity is highly regulated by a network of miRNAs. Some *AP2*-like transcription factors such as *RAP2.1* are highly induced under drought stress (Dong and Liu, 2010), indicating a connection between miRNA172 and regulation of drought signaling in plants.

Yang et al. (2014) identified three potato novel miR159 family members, stu-miR159a, stu-miR159b, and stu-miR159c, based on bioinformatics analysis and predicted the *StGAMyb-like1*, *StGAMyb-like2.1*, and *StGAMyb-like2.2* genes as their targets. Their further studies proved an opposite expression pattern between miRNAs and their targets. Three stu-miR159 members exhibited a significant decrease in expression under 25 days of drought stress, while the expression levels of potato GAMyb-like family members were greatly increased. These data suggested that stu-miR159s negatively regulate GAMyb-like genes in response to drought stress, and, in turn, control the drought responses in potato.

Plants accumulate proline as an osmoprotectant against abiotic stresses. Eleven known miRNAs targeting mRNAs of enzymes involved in the proline synthesis pathway were predicted by bioinformatic analyses (Yang et al., 2013). Ten out of 11 predicted miRNAs were successfully validated in potato by qRT-PCR assay, and 9 of 10 miRNAs were downregulated and one was upregulated under drought stress conditions. Moreover, proline contents of potato plants were significantly increased by drought stress. All together these results suggest a negative correlation between miRNA expression and proline accumulation. Therefore, miR172, miR396a, miR396c, and miR4233 were predicted to target the transcript of a key regulator of proline biosynthesis, Δ^1-*PYRROLINE-5-CARBOXYLATE SYNTHETASE* (*P5CS*), whereas *PYRROLINE-5-CARBOXYLATE REDUCTASE* (*P5CR*) and *PROLINE DEHYDROGENASE* (*ProDH*) transcripts were targets of miR2673 and miR6461, respectively. Transcript abundance of miR396 was found to be negatively affected by drought in rice (Zhou et al., 2010) and cowpea (Barrera-Figueroa et al., 2011), but positively affected in drought-stressed Arabidopsis (Liu et al., 2008) and tobacco (Frazier et al., 2011). miR2673 was identified as a regulator of genes involved in signal transduction of auxin and ethylene in *Lotus japonicas* (Hu et al., 2013) and genes involved in polarized cell growth, cellular signaling, and plant defense response in *Picrorhiza kurroa* (Vashisht et al., 2015). It also targets WRKY transcription factor 6 involved in the control of processes related to senescence and pathogen defense and is especially upregulated during tomato and melon late abscission (Xu et al., 2015).

Heat affects the transcript accumulation of various miRNAs in plants (Sunkar and Zhu, 2004). There are a few reports on genome-wide identification of heat-responsive miRNA in *Triticum aestivum* (Xin et al., 2010) and *Populus trichocarpa* (Lu et al., 2008). Accordingly, miR156, miR159, miR160, miR165/166, miR168, miR169, and miR393 were found to be induced whereas miR172 was repressed under heat treatment in *T. aestivum* (Xin et al., 2010). miR170/171 was downregulated in heat-treated *P. trichocarpa* (Lu et al., 2008). Unfortunately, there is no study on the identification of miRNAs that function in heat-response and/or tolerance in potato. Hence, further studies are required to widen our understanding of miRNA-based regulatory mechanisms that govern the posttranscriptional control of heat response and tolerance in potato in order to develop new varieties that can tolerate high temperatures in the near future.

5. Transgenics of drought and heat tolerance in potato

In recent years, much effort was given to the identification of single genes that are necessary for proper drought and/or heat response and tolerance in plants. These genes were used to generate transgenic potatoes that can tolerate drought and/or high temperature conditions. The spinach *BETAINE ALDEHYDE DEHYDROGENASE*

(*BADH*) gene responsible for the biosynthesis of glycine betaine was overexpressed under the control of a stress-inducible *RD29A* promoter in transgenic potato plants to increase the drought tolerance (Zhang et al., 2011). Transgenic potato plants showed abundant BADH activity under stress. Besides, the fresh weight of all transformants increased (17%–29%) under stress conditions compared to nontransgenics (NTs). Furthermore, a negative relationship (r = –0.9495) between BADH activity and the relative electrical conductivity in the leaves of the transformants was observed. The results demonstrated that BADH endowed drought and salinity stress tolerance to potato plants. In a study, the rhizobacterium *codA* gene coding for CHOLINE OXIDASE (COD) producing choline being a precursor of glycine betaine was overexpressed under the control of the stress-inducible *SWPA2* promoter (Kim KY et al., 2003) to confer tolerance to various stresses including drought (Ahmad et al., 2008). Glycine betaine was 3–5 times higher in transgenic lines, and transgenics showed better performance under drought and salinity stress compared to NTs. Transgenics preserved higher water content and had over twice the dry weight of NTs (Ahmad et al., 2008). In another study of the same group, *COD*-transformant potato plants showed higher antioxidant enzyme activity, better photosynthetic efficiency, higher chlorophyll content, and lower MDA content than NTs under drought stress conditions (Cheng et al., 2013). Introduction of the yeast *TREHALOSE-6-PHOSPHATASE SYNTHASE1* (*TPS1*) gene, which functions in the biosynthesis of osmolyte trehalose, into potato leads to drought tolerance by increasing the relative water content in source tissues (leaves) (Kondrák et al., 2012), and by enhancing the accumulation levels of amino acids such as glutamine, glutamate, phenylalanine, proline, and asparagine in sink tissues (tubers), indicating a partial difference in tolerance mechanisms against drought stress in source and sink organs (Juhász et al., 2014). Unfortunately, transgenic plants expressing yeast *TPS1* under the control of the *Cauliflower Mosaic Virus* (*CaMV*) *35S* promoter showed growth retardation, producing small leaves and numerous branches (Yeo et al., 2000). Constitutive expression of the *GG-PHOSPHATE PHOSPHATASE/SYNTHASE* (*ggpPS*) gene from a rhizobacterium under the control of the *RD29A* promoter in potato increased the accumulation of glucosylglycerol in tubers, and transgenic plants tolerated drought more efficiently than untransformed ones (Klähn et al., 2009). There were attempts to develop transgenic potato plants tolerant to drought by overexpression of specialized forms of carbohydrates. For instance, overexpression of *SUCROSE:SUCROSE 1-FRUCTOSYLTRANSFERASE* (*1-SST*) and *FRUCTAN:FRUCTAN 1-FRUCTOSYLTRANSFERASE* (*1-FFT*) genes from the globe artichoke in potato enhanced the accumulation of carbohydrate inulin (Hellwege et al., 2000), which conferred drought tolerance by contributing to the osmotic control via stabilization of membranes and proteins (Pilon-Smits et al., 1995; Vereyken et al., 2003). As an important osmoprotectant, free proline is accumulated more in natural potato plants (Schafleitner et al., 2007), or in transgenic potato plants expressing globe artichoke fructan synthetic pathway genes under drought conditions (Knipp and Honermeier, 2006). Overexpression of *P5CS* is associated with root development and tolerance against stresses (Taylor, 1996). However, there are no reports of transgenic potato plants overexpressing *P5CS* under drought. Overexpression of the *P5CS* gene in potato leads to higher accumulation of proline, lower MDA content, and better root development under 100 mM and 200 mM NaCl stresses in transgenic plants compared to nontransformants (Khan et al., 2015). The other study reporting the generation of transgenic potato plants overexpressing *P5CS* showed an improved tolerance to salinity through higher production of proline (Hmida-Sayari et al., 2005). Interestingly, repression of the gene responsible for the production of a soluble mitochondrial enzyme, FORMATE DEHYDROGENASE (FDA), in potato increases the steady-state levels of formate and accelerates the accumulation of proline in response to drought stress (Ambard-Bretteville et al., 2003), suggesting that the response and tolerance mechanisms against osmotic stresses blend together in plants.

In two studies on identification of new genetic resources for traits that could contribute to the development of more robust potato varieties tolerant to drought, expressions of genes associated with the production of antioxidants, flavonoids, heat shock proteins (HSPs), and late embryogenesis abundant (LEA) proteins were found to be induced under water stress (Watkinson et al., 2006; Schafleitner et al., 2007). These genes were used to generate drought-tolerant transgenic potato plants. In order to improve the drought tolerance of potato plants using low-molecular-weight antioxidants the *Arabidopsis* gene encoding for DEHYDROASCORBATE REDUCTASE1 (DHAR1) was overexpressed in potato, and therefore ascorbate levels increased in transgenic plants, causing better growth in drought conditions (Eltayeb et al., 2011). Transformation of potato with the *Arabidopsis GLUTATHIONE REDUCTASE1* (*GR1*) gene led to the accumulation of glutathione and ascorbate, in turn enhancing the tolerance of transgenic potato plants under drought stress (Eltayeb et al., 2010). Another way to increase the ascorbate levels is enhancing its biosynthesis. For this purpose, strawberry *D-GALACTURONIC ACID REDUCTASE* (*GalUR*) gene (Hemavathi et al., 2009) or *L-GULONO-γ-LACTONE OXIDASE* gene were overexpressed in potato (Hemavathi et al., 2010).

Ascorbate and proline levels as well as antioxidant enzyme activities increased in transgenic potato plants, resulting in drought tolerance. Interestingly, suppression of the gene coding for the enzyme important for the oxygen-evolving complex in photosystem II, namely manganese-stabilizing protein (MSP), in transgenic potato resulted in higher tuber yield, increased photosynthetic activity, and reduced oxygen generation (Gururani et al., 2012). Hence, this led to lower production of ROS while higher accumulation of proline and ascorbate coupled to higher antioxidant enzyme activity was observed in transgenic potato plants, causing drought tolerance (Gururani et al., 2013). In some other studies, genes encoding for antioxidant enzymes were used to develop drought-tolerant transgenic potato plants. In one study, overexpression of *Potentilla atrosanguinea* (Himalayan cinquefoil) *CYTOSOLIC COPPER-ZINC SUPEROXIDE DISMUTASE* (*Cu/ZnSOD*) in potato enhanced the SOD activity as well as the photosynthetic rate and stomatal conductance, resulting in drought tolerance (Pal et al., 2013). The attempt of developing drought-tolerant transgenic potato plants by simultaneous overexpression of genes encoding for two antioxidant enzymes and choline oxidase was proven to be successful (Ahmad et al., 2010), indicating the potential of transgenics to enhance the stress tolerance of crop plants in the future. In this study, *Manihot esculenta* (cassava) *Cu/ZnSOD*, *Pisum sativum ASCORBATE PEROXIDASE* (*APX*) and *Arthrobacter globiformis* (rhizobacterium) *codA* genes were overexpressed in potato plants simultaneously, resulting in higher accumulation of GB and higher activities of SOD, APX, and catalase, and in the end better tolerance to drought (Ahmad et al., 2010). Transgenic potato lines over-expressing the *AtYUC6* gene survived after water withholding for 18 days and exhibited higher water content in leaves and lower ROS content with increased expression of *Cu/ZnSOD* compared to NTs (Kim et al., 2013). *Cu/ZnSOD* transformed to potato lines driven by the *35S* promoter had higher net photosynthetic rate and stomatal conductance under control and salinity conditions compared to NTs (Pal et al., 2013). When *Cu/ZnSOD*- and *APX*-expressing transgenic potato lines driven by the *SWPA2* promoter (SSA plants) were exposed to 42 °C for 20 h, photosynthetic activity decreased by 6% in SSA plants, whereas that of NT plants decreased by 29% (Tang et al., 2006). *NUCLEOSIDE DIPHOSPHATE KINASE2* (*NDPK2*) regulates the expression of antioxidant genes. Arabidopsis *NDPK2*-expressing transgenic potato lines under the control of the *SWPA2* promotor (SN plants) or *CaMV 35S* promoter (EN plants) showed less visible damage at high temperatures compared to NT plants, respectively. Besides, the photosynthetic activity of SN plants was decreased by about 10% after treatment at 42 °C for 20 h compared to the SN plants grown at 25 °C, whereas that of NT plants declined by 30% (Tang et al., 2008). These results proved that the manipulation of the antioxidant system of potato may improve potato tolerance to various abiotic stresses.

Various transcription factors that function in the regulation of stress response and tolerance as well as other proteins controlling cellular homeostasis were also used to generate drought-tolerant potato plants. Belonging to the APETALA2 (AP2)/ETHYLENE RESPONSE FACTOR (ERF) family of transcription factors, DEHYDRATION RESPONSE ELEMENT B1A (DREB1A)/C-REPEAT-BINDING FACTOR3 (CBF3) is highly induced under drought stress (reviewed by Seki et al. (2007)) and therefore was used to confer drought tolerance in various plants (reviewed by Umezawa et al. (2006)). Overexpression of *Arabidopsis thaliana AtDREB1A* in potato plants was evaluated under salt and freezing stresses in in planta experiments (Celebi-Toprak et al., 2005; Behnam et al., 2006, 2007). Recently, drought tolerance of *AtDREB1A*-expressing potato plants was carried out in an in vitro study (Huynh et al., 2014), suggesting that overexpression of *AtDREB1A* enhances tolerance of potato against various environmental stresses. Belonging to the same AP2/ERF family of transcription factors, overexpression of *AtDREB1B* in potato plants increased the accumulation of proline and preserved the relative water content, therefore leading to better overall plant growth under drought conditions (Movahedi et al., 2012). *Arabidopsis DREB* orthologues in potato also enhanced drought tolerance when overexpressed in transgenic potato plants. Transgenic potato plants constitutively overexpressing potato *StDREB1* or *StDREB2* genes accumulated higher levels of proline and showed reduced water loss under drought and salinity (Bouaziz et al., 2012, 2013). Moreover, overexpression of the *ScCBF1* gene from wild potato relative *Solanum commersonii* enhanced drought stress tolerance in transgenic *Solanum tuberosum* and *S. commersonii* plantlets grown in vitro (Pino et al., 2013). Transgenic plantlets demonstrated better root development and overall growth and accumulated higher levels of proline in the stems and leaves in drought. Stress-response genes such as *DEHYDRIN10* (*DNH10*) were highly induced in transgenic potato plantlets under drought stress. Overexpression of the *Solanum tuberosum StMYB1R-1* gene, an R1-type MYB-like transcription factor, in potato plants improved the drought tolerance of transgenic plants by decreasing the relative water loss while increasing the stomatal closure rate (Shin et al., 2011). Several drought-responsive genes including *AtHB-7*, *RD28*, *ALDH22a1*, and *ERD1-like* were induced in transgenic plants under drought conditions, suggesting that *StMYB1R-1* functions as a transcription factor involved in the activation of drought-related genes. In a similar study, the *Ipomoea*

batatas (sweet potato) *IbMYB1* gene, an R2R3-type MYB transcription factor involved in secondary metabolite production, was overexpressed in potato (Cheng et al., 2013). Transgenic potato plants could tolerate the drought since they accumulated higher levels of secondary metabolites such as phenols, flavonoids, and anthocyanin. Interestingly, constitutive overexpression of a gene encoding for *Capsicum annuum* (pepper) *PATHOGEN AND FREEZING TOLERANCE-RELATED PROTEIN1* (*CaPF1*) in potato plants showed improved tolerance to various environmental stresses including drought (Youm et al., 2008). Overexpression of another transcription factor from potato also increased the tolerance of transgenic plants under various environmental stresses (Lee et al., 2007). In this case, constitutive overexpression of the potato *ETHYLENE RESPONSIVE ELEMENT BINDING PROTEIN1* (*StEREBP1*) gene conferred tolerance against salinity and cold temperatures; however, drought response of transgenic potato plants was not investigated in this study. NAC proteins are plant-specific transcription factors and they play important roles in abiotic and biotic stress responses (Puranik et al., 2012). In a recent study, overexpression of the potato *StNAC2* gene led to salt tolerance in vitro and drought tolerance in pot-growing conditions (Xu et al., 2014). Interestingly, expression of *StNAC2* was induced by *Phytophthora infestans*, the causal agent pathogen of potato late blight; however, the tolerance phenotypes of *StNAC2*-overexpressing transgenic potato plants were not characterized in this study.

Drought application at the beginning of the tuberization stage decreases tuber number, growth, and yield (Cavagnaro et al., 1971). In order to investigate the potential effects of transgenic potato plants overexpressing stress-response genes on tuber number, growth, and yield under drought conditions, transgenic potato plants overexpressing *Triticum aestivum* (wheat) mitochondrial *Mn SUPEROXIDE DISMUTASE* (*TaSOD3:1*), *Hordeum vulgare* (barley) *DEHYDRIN*4 (*HvDHN4*), *Brassica napus* (canola) *BnDREB/CBF1*, or *Bromus inermis* (bromegrass) *BiROB5*, a LEA group 3-like protein (similar to dehydrin) (Robertson et al., 1994), under the control of a constitutive promoter or a stress-induced *Arabidopsis COR78* promoter were generated (Waterer et al., 2010). Combining the *BiROB5*, *HvDHN4*, or *TaSOD3.1* transgenes with the *COR78* promoter showed significant potential to enhance the tuber yields under drought stress, proving the strength of genetic engineering studies to enhance the drought tolerance of potato plants. Moreover, transgenic potato expressing *COR78::DHN4* and *COR78::ROB5* could also tolerate high temperatures during tuber development, suggesting some specific proteins function in different stresses, and the overexpression of genes that produce these specific proteins leads to tolerance against various environmental stresses in potato.

The number of transgenic studies to improve the high temperature tolerance in potato is limited. In one of these studies, pyramiding of antioxidant enzymes was proven to be successful to generate heat-tolerant plants. In this study, the chimeric gene construct of *M. esculenta Cu/ZnSOD* (*MeCu/ZnSOD*) and *P. sativum APX* (*PsAPX*) under the control of stress-inducible *SWPA2* promoter from sweet potato (Kim KY et al., 2003; Tang et al., 2004) with a chloroplast-targeting transit peptide was overexpressed in high temperature-sensitive potato cultivar Atlantic (Tang et al., 2006). Transformants were healthier with a minor decrease in photosynthetic activity compared to nontransformants after 10 h of heat shock at 42 °C, suggesting a positive correlation between responses to oxidative and high temperature stresses. In a similar study, transgenic potato (cultivar Atlantic) lines were generated by overexpression of the Arabidopsis *NUCLEOSIDE DIPHOSPHATE KINASE2* (*AtNDPK2*) gene under the control of the *SWPA2* promoter (Tang et al., 2008). NDPK2 is known to maintain the intracellular levels of (d)NTPs and regulate the expression of antioxidant genes in plants (Otero, 2000). Transformants showed less wilting with a minor decrease in photosynthetic activity compared to nontransformants after 10 h of heat shock at 42 °C, indicating the importance of intracellular ROS homeostasis in heat tolerance. In a follow-up study, the *SWPA2::AtNDPK2* transgene was transformed into transgenic potato lines overexpressing *MeCu/ZnSOD and PsAPX* under the control of the *SWPA2* promoter in order to analyze the effects of gene pyramiding on high temperature tolerance (Kim et al., 2010). Transgenic potato plants were found to tolerate high temperature stress at 42 °C, reflected by a lower reduction in photosynthetic activity than nontransformants and transformants of *SWPA2::AtNDPK2* and *SWPA2::MeCu/ZnSOD* and *SWPA2::PsAPX*, indicating that the simultaneous overexpression of *CuZnSOD*, *APX*, and *NDPK2* is more effective than single or double transgene expression for developing plants with enhanced tolerance to various environmental stresses.

In another study, the Arabidopsis gene encoding for an antioxidative enzyme, 2-CYSTEINE PEROXIREDOXIN (At2-CYS PRX), that protects the photosynthetic machinery from ROS damage by removing peroxides (Dietz, 2003) was expressed in the Atlantic cultivar of potato under the control of the *SWPA2* promotor (Kim et al., 2011). Photosynthetic activity of transgenic potato plants did not reduce as much as that of nontransgenic plants when exposed to high temperature stress at 42 °C, indicating that overexpression of *At2-CYS PRX* under control of the stress-inducible *SWPA2* promoter in potato enhanced tolerance to high heat by eliminating the negative effects of oxidative stress.

Transcription factors were also used to improve the high temperature tolerance of transgenic potato plants. For instance, overexpression of a pepper transcription factor gene, *CaPF1*, increased the thermotolerance of transgenic potato plants (Youm et al., 2008). In another study, *Arabidopsis CBF3* (*AtCBF3*) was ectopically expressed in potato plants (Dou et al., 2015). Transgenic potato plants showed higher photosynthetic activity but lower ROS accumulation compared to nontransformants. Moreover, expressions of genes encoding for photosystem and antioxidant proteins were higher in transgenic plants than in nontransformants, indicating that overexpression of *AtCBF3* in transgenic potato plants enhanced high temperature tolerance.

HSPs are synthesized in plants under high temperature stress (Waters et al., 1996; Sun et al., 2002). They function as molecular chaperons to guide and stabilize protein folding and tertiary structure, prevent protein aggregation, regulate protein refolding, and target improperly folded proteins to degradation pathways (Mayer and Bukau, 2005; Kalmar and Greensmith, 2009; Haslbeck and Vierling, 2015). Transgenic approaches utilizing HSPs in order to develop thermotolerance were demonstrated in several plant species, including Arabidopsis (Lee et al., 1995), carrot (Malik et al., 1999), and rice (Katiyar-Agarwal et al., 2003). Similar studies with HSPs should be carried out in potato as well since expression of *HSP70* was found to be induced in potato plants exposed to high temperature (Dou et al., 2015).

6. Future challenges and prospects in drought and heat tolerance of potato

In a world where population growth exceeds food supply, agricultural and plant biotechnologies aimed at overcoming severe environmental stresses need to be fully implemented. Potato forms an important part of the human diet since it is a carbohydrate-rich, energy-providing crop with little fat, and its regular consumption would certainly have a significant impact on human health, especially with nutritional improvements of potato in the near future (Camire et al., 2009). Although potato is a crop of choice of many farmers in more than 160 countries because of its high yield and low cost of cultivation, the average yield of potatoes around the world is far below its physiological potential of 120 t/ha due to various environmental stresses, such as drought and high temperatures.

In order to understand the molecular mechanisms involved in drought and high temperature responses of potato, wild *Solanum* species could be used since they are adapted to grow in various environments. Wild crop relatives have been used by plant breeders for their undeniable benefits in providing a wide pool of potential genetic resources to improve the undesirable traits of modern crops (Hajjar and Hodgkin, 2007). Likewise, wild potato species represent a great resource of genetic variability that can be used by breeders in identification of traits controlling the drought and heat stress responses, and in improving the abiotic stress tolerance in breeding programs. Various wild *Solanum* species have been used as genetic donors for introgression of biotic stress resistance and some abiotic stress tolerance to *S. tuberosum* (Kikuchi et al., 2015). Although the genome of *S. commersonii* was sequenced and it was used in various transgenic studies to generate cold-tolerant plants (Pino et al., 2013), the traits that are responsible for abiotic stress responses have not been transferred into *S. tuberosum*.

Recent developments in molecular genetics and genomics of crop species, and extensive knowledge obtained from studies of model plants, provide great opportunities for understanding the genetic basis of drought and heat tolerance in order to identify the individual component traits and breed potato plants for favorable alleles in the underlying genes (Tuberosa, 2012). Identification of genotypic and phenotypic variations in natural accessions of *Arabidopsis thaliana* in The 1001 Genomes Project will generate massive amounts of data that can be incorporated into studies of potato genomics, transcriptomics, and transgenics to facilitate the identification of new traits under drought and heat stresses and shows the massive potential of utilization of wild relatives to incorporate the molecular mechanisms underlying natural variations (Weigel and Mott, 2009).

Characterizing and understanding various plant phenotypes accurately in a short period of time has been rising to prominence in recent years. Although molecular breeding approaches require genotypic data for selection of breeding lines, development of molecular markers used in molecular breeding is still in need of a strong correlation with phenotypic data (McMullen et al., 2009). Recent developments in high-throughput phenotyping will provide an opportunity for generating a fast, inexpensive, and massive collection of data that will together provide a reliable assessment of trait phenotypes for many of the underlying genotypes in a typical plant breeding population. High-throughput phenotyping for drought tolerance traits would facilitate the discovery of new QTLs in potato.

Plants are exposed to a combination of abiotic stresses, such as drought and heat, in nature. The plant responses to these various stresses involve many shared molecular components. However, plants can respond differently to a combination of abiotic stresses than they do to individual stresses. Hence, detailed evaluation of multiple stress response mechanisms of crops may be required for improving abiotic stress tolerance. Even though the response of potato to drought stress has been

studied in details, there is still a need for further studies to understand the molecular mechanisms underlying the potato response to heat stress and a combination of heat and drought stresses.

There has been elaborative research on the generation of transgenic potato plants that can tolerate individual environmental stresses, yet potato research may also take advantage of recent advances in the transgenics of other plant species to develop multiple stress tolerance in potato. Precise genome engineering using CRISPR/Cas systems has been proven to be especially successful for the development of transgenic plants tolerant to various environmental stresses (reviewed by Belhaj et al. (2013)). Moreover, gene pyramiding and targeted expression of transgenes have been utilized to generate transgenic plants resistant to various biotic and abiotic stresses (Sree and Rajam, 2015; Surekha et al., 2015). Therefore, research in potato transgenics can utilize the strategies that have been proven to be successful in other plant species.

Overall, integration of genotypic, phenotypic, proteomic, physiological, transcriptomic, and epigenetic data is required to understand the responses of potato against drought and heat stresses, and research strategies utilizing the power of the combination of traditional breeding and transgenics will provide great potential to improve the resilience of potato crops under multiple environmental stresses.

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