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When water runs dry and temperature heats up: Understanding the mechanisms in rice tolerance to drought and high temperature stress conditions

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Abstract

Rice production, owing to its high-water requirement for cultivation, is very vulnerable to the threat of changing climate, particularly prolonged drought and high temperature. Such threats heighten the need for abiotic stress-resilient rice varieties with better yield potential. This review examines the physiological and molecular mechanisms of rice varieties to cope with stress conditions of drought (DS), high temperature (HTS) and their combination (DS-HTS). It appraises research studies in rice about its various phenotypic traits, genetic loci and response mechanisms to stress conditions to help craft new breeding strategies for rice varieties with improved resilience to abiotic stresses. This review consolidates available information on promising rice cultivars with desirable traits as well as advocates synergistic and complementary approaches in molecular and systems biology to develop new rice breeds that favorably respond to climate-induced abiotic stresses. The development of new breeding and cultivation strategies for climate-resilient rice varieties is a challenging task. It requires a comprehensive understanding of the various morphological, biochemical, physiological, and molecular components governing yield under drought and high temperature, but possible by implementing cohesive approaches involving molecular and systems biology approaches in genomics and molecular breeding, including genetic engineering.

Keywords: rice, abiotic stress, high temperature, drought, physiological responses, molecular mechanisms, systems biology

1. Introduction

Among the most important cereals globally produced and consumed, rice ranks second to maize (Awika, 2011). World rice production has been forecasted at a record high of 481.3 million tons in 2017 with the bulk of the production coming from Asia (Childs, 2017). In the US, rice production is projected at 201 million hundredweight [cwt] (10.1 million tons) and is mainly centered in the states of Arkansas, California, Louisiana, Mississippi, Missouri, and Texas. With a burgeoning global population expected to reach 9 billion in 2050, food production needs to increase by 70% to meet the growing nutritional demands (Cramer et al., 2011). In rice, global yield levels must rise by 1.0-1.2% annually to feed the continually growing population and to sustain economic development (Normile, 2008).

Owing to climate variation and climate extremes, rice yield fluctuates by up to 32%, which is about 3 million tons of annual yield loss (Ray et al., 2015). This yield vulnerability in rice production stems from irregular rainfall pattern during growing season resulting to widespread drought (Nguyen, 2002) as well as increasing mean surface air temperature (Peng et al., 2004, Tenorio et al., 2013), solar radiation, atmospheric CO2 concentration, and other greenhouse gases (Nara et al., 2014). Additionally, climate change has been predicted to increase the frequency and intensity of extreme weather conditions such as severe drought, heat stress and floods (Nguyen, 2002).

Rainfed-based rice cultivation systems are the most vulnerable to irregular rainfall pattern. Rainfed areas account to 34% of the total rice production, 50% is irrigated areas and the rest is grown in upland and flood-prone areas that are broadly defined ecosystems (Almanac, 2002). Approximately, 45 million hectares comprise rainfed areas (Haefele and Bouman, 2009) and about half is affected by drought (Serraj et al., 2011). For instance at reproductive stage, estimated moderate and severe yield reduction are about 45-60% and 65-91%, respectively as a result of drought stress (Ghimire et al., 2012, Sandhu et al., 2014, Vikram et al., 2011).

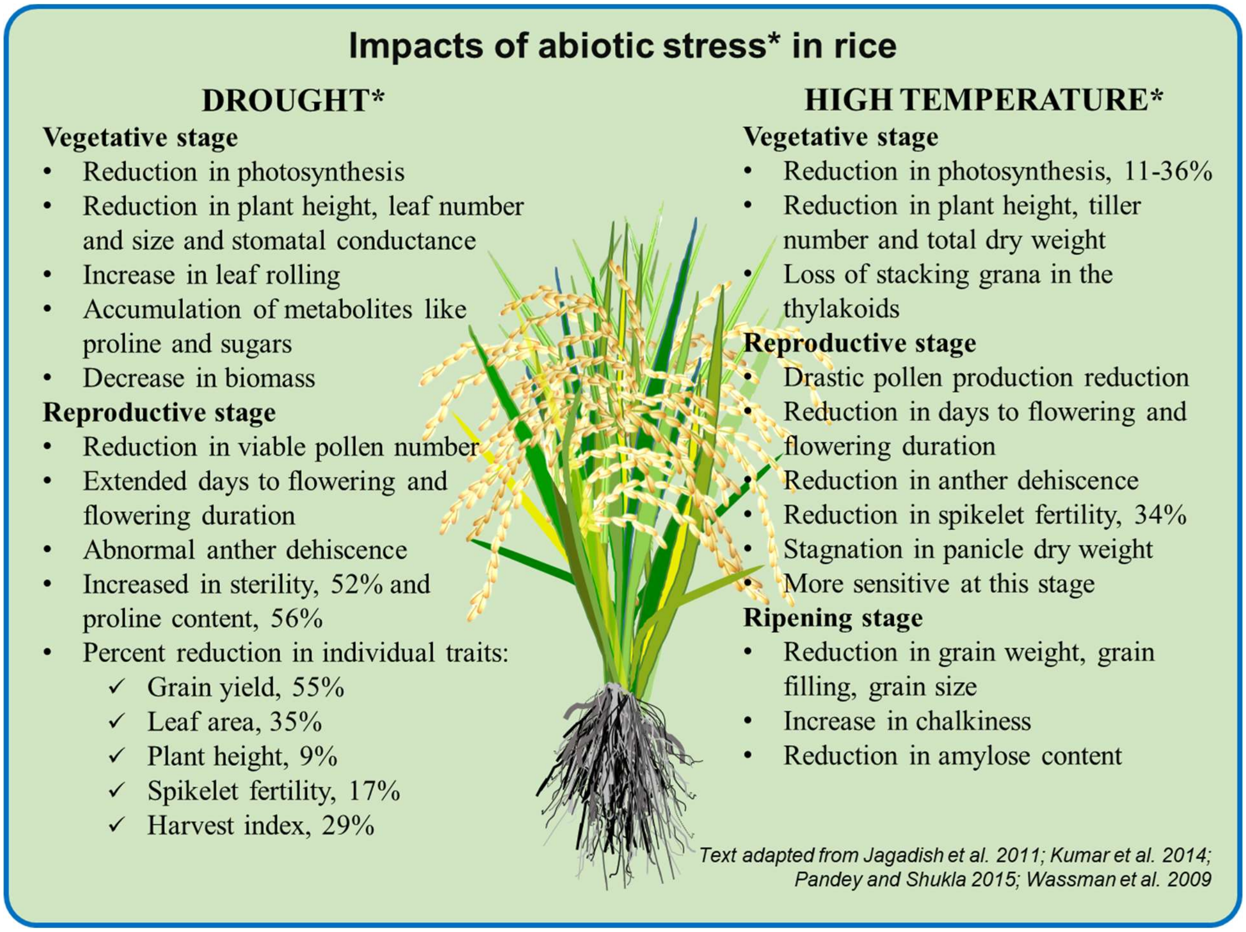
Similarly, high temperature can reduce rice yields by up to 10% for every 1°C increase in temperature depending on cropping season and varietal use (Peng et al., 2004, Tenorio et al., 2013). Reduction in yields results from speeding up the plant development and reducing the period available for yield production (Erda et al., 2005).

The adverse effects of drought stress (DS) and high temperature stress (HTS), including their combined occurrence (DS-HTS) among other abiotic stresses in rice production magnify the need to develop new rice technologies, especially rice varieties that are resilient to environmental stresses and can significantly increase productivity (GRiSP, 2013). Progressing biotechniques in genomics and other OMICs approaches provide alternative tools to identify useful genetic materials for genomic-assisted breeding of rice cultivars that are resilient to abiotic stresses like drought and high temperature. For instance, genomics can decipher genes, alleles or quantitative trait loci (QTL) that make crops adapt to abiotic stresses (Kole et al., 2015). Genomics breeding has the greatest potential for ensuring a sustainable increase of food by adapting available crops to biotic and abiotic stresses and breeding novel crop varieties (Kumar et al., 2014a). Integration of various advanced tools allows molecular breeding of climate-ready crops (Wassmann et al., 2009).

Thus, this review aims to highlight the physiological and molecular coping mechanisms of rice plants under drought and high-temperature conditions, including combined drought-high temperature stress. It sums up research studies in rice on drought and high temperature tolerance conducted thus far and offer strategies involving advanced and emerging approaches to develop rice varieties resilient to stress conditions such as drought, high temperature and their combination. Broadly, this review advocates the synergistic and complementary use of advanced molecular and systems biology approaches in trait or gene discovery and introgression to develop climate-resilient rice varieties for sustainable development in agriculture as well as ensure food supply despite adverse environmental growing conditions.

2. Drought and high temperature stress effects and response mechanisms

Several studies have shown various effects of drought (DS) and high temperature (HTS) stress conditions throughout the growth and development of rice plants as well as corresponding response mechanisms. DS and HTS conditions limit yield potential of rice plants and in worse case, render the plants extremely unproductive. Figure 1 sums up the impacts of drought and high temperature on the various growth and development stages of rice plants (Boonjung and Fukai, 1996a, Jagadish et al., 2011, Kumar et al., 2014a, Pandey and Shukla, 2015, Serraj et al., 2011). For example, at reproductive stage DS and HTS conditions influence spikelet fertility by 17% and 34%, respectively. At vegetative stage, both stress conditions inhibit plant height and tiller development but have opposite effects on flowering. DS prolongs flowering days and flowering period while HTS shortens flowering days and flowering period. Overall, both stresses – high temperature and drought affect rice in its various growth and development stages (Krishnan et al., 2011, Wassmann et al., 2009).



Impacts of abiotic stress* in rice

DROUGHT*

Vegetative stage

- Reduction in photosynthesis
- Reduction in plant height, leaf number and size and stomatal conductance
- Increase in leaf rolling
- Accumulation of metabolites like proline and sugars
- Decrease in biomass

Reproductive stage

- Reduction in viable pollen number
- Extended days to flowering and flowering duration
- Abnormal anther dehiscence
- Increased in sterility, 52% and proline content, 56%
- Percent reduction in individual traits:
  - ✓ Grain yield, 55%
  - ✓ Leaf area, 35%
  - ✓ Plant height, 9%
  - ✓ Spikelet fertility, 17%
  - ✓ Harvest index, 29%

HIGH TEMPERATURE*

Vegetative stage

- Reduction in photosynthesis, 11-36%
- Reduction in plant height, tiller number and total dry weight
- Loss of stacking grana in the thylakoids

Reproductive stage

- Drastic pollen production reduction
- Reduction in days to flowering and flowering duration
- Reduction in anther dehiscence
- Reduction in spikelet fertility, 34%
- Stagnation in panicle dry weight
- More sensitive at this stage

Ripening stage

- Reduction in grain weight, grain filling, grain size
- Increase in chalkiness
- Reduction in amylose content

Text adapted from Jagadish et al. 2011; Kumar et al. 2014; Pandey and Shukla 2015; Wassman et al. 2009

Figure 1. Impacts of abiotic stresses – drought and high temperature on the growth and development of rice plants (Jagadish et al., 2011, Kumar et al., 2014b, Pandey and Shukla, 2015, Wassmann et al., 2009).

High temperature stress and response mechanisms. In a temperature increase of 28°C to 34°C, yield decreased to 7-8% (Korres et al., 2017). As high temperature speeds up plant development and reduces the period available for yield production, potential yields decline (Erda et al., 2005)

(Peng et al., 2004, Matthews et al., 1997). In dry season cropping, yield declines to as much as 10% for every 1oC increase in growing-season minimum temperature (Peng et al., 2004). High temperature causes early physiological maturity, shortens growth period (Wassmann et al., 2009, Krishnan et al., 2011), reduces accumulation of assimilates demonstrated by spikelet sterility (Korres et al., 2017) that leads to irreversible losses (Tenorio et al., 2013). Additionally, high temperature inhibits swelling of pollen grains resulting in poor thecae dehiscence (Zhang et al., 2013) and eventually causes panicle dry weight reduction (Wassmann et al., 2009).

Rice grows optimally at a temperature ranging from 22°C to 30°C, and the current rise in temperature averages would certainly affect global rice production (Korres et al., 2017). The critical temperature for rice production varies depending on the genotypes and physiological status of the plant but extreme temperature is detrimental to its growth and development (Krishnan et al., 2011). Contingent on the geographical location, temperature during rice cropping season could vary e.g. 33°C in Pakistan and 17°C in Japan (GRiSP, 2013) and elevated temperature affects rice at various growth and development stages.

At germination, 97% germination can be achieved at 27-37°C but no growth at 45°C (Ambavaram et al., 2014). During seedling development, the growth rate is linear between 22 and 31°C but stops above 40°C (GRiSP, 2013, Krishnan et al., 2011). Rice can tolerate relatively high temperature (35/25°C day/night temperature) but beyond this, temperature can reduce plant height, tiller number and total dry weight (Wassmann et al., 2009).

At reproductive stage, effects of high temperature are very significant. Under HTS conditions, tolerant rice varieties were able to dehisce at the beginning of flower opening stage, while heat-intolerant rice varieties failed to dehisce (Kobayashi et al., 2011). The unsuccessful dehiscence could be due to the failure of the septum to rupture under high temperature (Kobayashi et al., 2011). Based on multiple regression model analysis of data from 227 intensively managed and irrigated rice farms in rice-producing countries, a study found that higher minimum temperature reduced grain yield, while higher maximum temperature improved it (Welch et al., 2010).

The immediate plant responses upon exposure to HTS include protein denaturation, increased membrane permeability and disruption of the lipid bilayer, decrease in photosynthetic efficiency, more specifically photosystem II (PSII)-mediated electron transport chain, and reduced activities of chloroplastic and mitochondrial enzymes. This eventually leads to abscission thereby affecting plant growth and productivity (Guo et al., 2016b, Gururani et al., 2015). Heat stress also triggers several physiological responses in rice such as senescence of leaf, reduction in loss of water and finally remobilization of nutrients thus enabling plant survival (Zhang and Zhou, 2013). As developing parts of the plant compete for inputs, florets degenerate, peduncle growth stagnates and spikelet becomes sterile (Jagadish et al., 2010, Shi et al., 2013). The hormonal signals generated from hormonal crosstalk in response to HTS is translated into physiological and morphological adaptations including changes in the above and below biomass of the plant, stomatal movement, transport of water and grain filling rate (Skalák et al., 2016, Dobrá et al., 2015).

Nighttime HTS or high night temperature (HNT) stress also affects the growth and development of rice plants. During its vegetative growth, leaf weight and specific leaf area increased at 27°C

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night temperature, while grain yield increased under cooler night temperature (Zhang et al., 2013a). HNT also affects rice productivity through altered pollen germination and spikelet fertility (Mohammed and Tarpley, 2010). In short, high temperature occurring either day or night or much worse at nearly constant high of 30°C is detrimental to the growth and development of rice plants.

A comparative study between low night temperature (LNT) and HNT under field chamber conditions showed a reduction in yield traits in 36 varieties (Zhang et al., 2013a) (Figure 2). This study showed that 4°C difference between LNT and HNT led to 11% reduction in grain yield. When exposed to 29°C night temperature, a similar effect was observed in six tropical hybrid rices across all cropping season with higher yield reduction (19%) during the wet season (Shi et al., 2016). As much as 90% yield reduction was observed in rice plants grown at 32°C night temperature (Mohammed and Tarpley, 2010). This high yield reduction was attributed to high spikelet sterility (61%) and low pollen germination (28%) under these conditions.

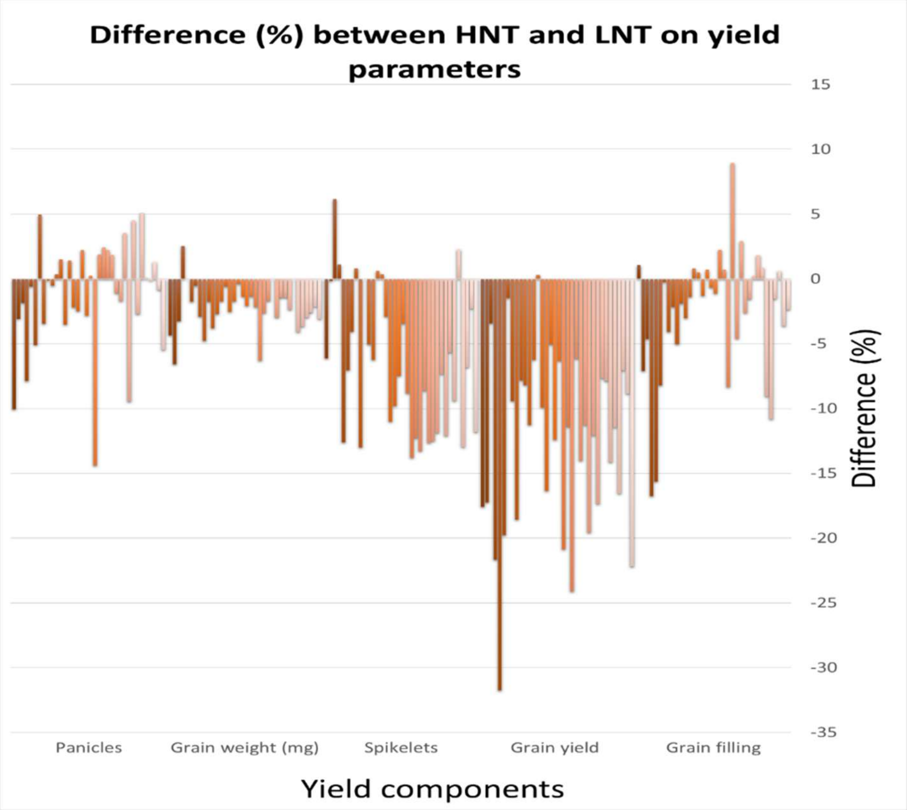

Figure 2. Difference (%) in various yield parameters among 36 rice varieties grown at LNT and HNT. Data adapted from Zhang et al. (Zhang et al., 2013).

In terms of metabolomics, a study has shown the major effects of HNT on the TCA cycle and amino acid and polyamine biosynthetic pathways in HNT-sensitive varieties. Amino acids synthesized from oxaloacetate and α-ketoglutarate were among the metabolites that increased significantly in all sensitive varieties Glaubitz et al., 2015). Also, HNT sensitivity was correlated to accumulation of putrescine, which is the first compound in the synthesis of spermidine (Glaubitz et al., 2015).

Drought stress and response mechanisms. DS can occur at any developmental stage and can make rice production highly vulnerable because of the plant's high-water requirements for growth and development. A study has shown that variable rainfall amount and distribution limited the yield of rainfed rice, moisture stress severely damaged rice planted in freely drained upland, and even killed plants that received 200 mm of precipitation in a day and no rainfall in the next 20 days (Nguyen, 2002). In the rainfed production ecosystem, drought effects on rice vary greatly in terms of phenomorphological and physiological characteristics, still all results in yield reductions (Serraj et al., 2009, Wassmann et al., 2009).

Rice is highly sensitive to drought stress during its reproductive stage resulting in yield loss (Kumar et al., 2008). For example, as drought causes a decrease in the number and weight of rice grains, yield loss can reach yields up to 55% (Wassmann et al., 2009). Drought also impedes the development of the rice plant's reproductive organs such as its ovary and pollen at meiosis along with process inhibition, like anther dehiscence, pollen shedding, pollen germination, fertilization and panicle exsertion (Glaubitz et al., 2015, Li et al., 2015a, Wassmann et al., 2009). Panicle exsertion is affected when the rice plant's peduncles hold back their elongation process due to water stress, causing about 70-75% spikelet sterility (Serraj et al., 2009). Drought also reduces grain number per panicle and single grain weight at panicle initiation (Pantuwan et al., 2002) as well as dry weight accumulation in all plant organs resulting in shortened life cycle (Kamoshita et al., 2008).

At the vegetative stage, drought minimizes the growth of photosynthetic and storage organs effecting lower yields (Wassmann et al., 2009, Pandey and Shukla, 2015). During flowering, the onset of drought limits the rice plant's pollen viability, its stigma receptivity, and seedset, and inhibits grain formation (Boonjung and Fukai, 1996a, Boonjung and Fukai, 1996b). Hence, flowering pattern has been proposed as a selection criterion for drought-tolerant varieties in rainfed lowland rice (Monkham et al., 2015).

At the physiological level, drought causes stomatal closure, which reduces the leaf CO_2/O_2 ratio, resulting in photosynthesis inhibition and subsequent reductions in biomass production and the life cycle of the plant (Korres et al., 2017). Depending on rice genotypes, photosynthesis is inhibited by drought and is apparent in genotypes with low stomatal (gs), and mesophyll (gm) conductances (Serraj et al., 2009). Studies have reported of stomata closure and reduction of leaf CO2/O2 (Serraj et al., 2009) resulting in inhibition of photosynthesis and of substantial decreases of chlorophyll concentrations and total soluble proteins, with parallel reductions in photosynthetic primary products and sucrose-phosphate synthase activity (Serraj et al., 2009, Wassmann et al., 2009).
During water stress, leaf rolling also occurs induced by loss of turgor and poor osmotic adjustments, while delayed leaf rolling is an indication of turgor maintenance and dehydration avoidance (Kumar et al., 2014b). Osmotic adjustment, through the accumulation of compatible solutes such as proline, sucrose, polyamines and glycine betaine, is the most common response in plants under water stress (Pandey and Shukla, 2015). Sucrose accumulated in roots and leaves of some dicotyledonous plants (e.g. tobacco) under drought stress conditions (Rabara et al., 2015b).

Physical manifestations of drought in leaf morphogenesis are reductions in leaf growth, leaf area, and shoot biomass (Boonjung and Fukai, 1996a). Leaf shape and three-dimensional architecture play important roles in influencing photosynthesis, respiration, and transpiration rates (Liu et al., 2016). Phenotyping of IR64-derived near-isogenic lines (NILs) with varying leaf size showed that plants with broad leaves performed better than those with short and narrow leaves (Farooq et al., 2010). Change in leaf size, together with stomatal opening and closure can be an adaptive mechanism for plants to minimize water loss (Farooq et al., 2010, Hu et al., 2017). Leaf size is an important agronomic trait in rice and was shown to moderately correlate to panicle numbers (Rabara et al., 2014a). The same study also noted that the width of the flag leaf positively correlated with grain width and caryopsis width.

Concurrent stresses of drought and high temperature (DS-HTS) and response mechanisms. Response to simultaneous drought and heat stresses is dependent on the plant species as well as the severity and duration of the stresses. Although DS and HTS conditions have been observed previously, knowledge on their combined effects remains limited (Jagadish et al., 2011, Rang et al., 2011).

In a study using five rice genotypes at flowering exposed to HTS, DS and concurrent DS-HTS, stress effects were quantified through spikelet sterility response. A strong relationship between number of germinated pollen on stigmas and spikelet sterility was established though microscopic analyses of anther dehiscence (Rang et al., 2011). The authors also observed that better anther dehiscence was assessed in two N22 accessions exhibiting tolerance to three stress treatments compared to anther dehiscence in Apo, Moroberekan and IR64.

Similarly, N22 outperformed several rice cultivars studied extensively with known drought and heat tolerance. N22 had a significantly lower yield decline than IR64, Moroberekan and Azucena suggesting better heat and drought tolerance in both dry and wet seasons (Jagadish et al., 2011). Candidate cultivars having drought tolerance should be simultaneously screened for high temperature tolerance in both dry and wet seasons as well as in cool temperature to determine true drought tolerance as well as those exhibiting plasticity (Jagadish et al., 2011). Additionally, to further understand the impact of combined heat and drought stress in rice growth and productivity, physiological and molecular analysis must be conducted in well-planned controlled environment and field set-up (Jagadish et al., 2011).

Both DS-HTS affect the metabolic profile of rice plants (Liu et al., 2015). The difference in biochemical profiles was observed in the flowers of sensitive (var. ‘Moroberekan’) and tolerant (var. ‘N22’) rice varieties even under control, non-stress conditions (Li et al., 2015a). The authors added that the metabolites distinguishing the two varieties include proteinogenic amino acids, sugars, and sugar phosphates. Moreover, accumulation of sucrose in the anthers of N22 rice variety was attributed to its tolerance to DS-HTS.

4. Advances in developing climate-resilient rice

Drastic yield decline in cereals production by 2020 due to climate change has been predicted to reach as much as 11% in rice, 14% in wheat and 9% in corn (Hisas, 2011). In the context of global climate change, it is then crucial to improve crop tolerance to abiotic stresses particularly, DS and HTS including tolerance to their combinatorial occurrence (DS-HTS).

The choice of cultivar is one of the possible and immediate adaptation strategies to cope with climate changes and water availability (Korres et al., 2017). In the Philippines, drought-tolerant rice varieties for farms under rainfed (varieties: 'Rio Grande', 'Sacobia', and 12 varieties of 'Sahod Ulan') and upland conditions (var. 'Pasig', 'Apo', and 'Katihan 1') (Santos-Peralta, 2016) were recommended jointly by the International Rice Research Institute (IRRI) and the Philippine Rice Research Institute of the Department of Agriculture (DA-PhilRice).

Another optional strategy is harnessing technological advances in sciences that could further develop the resilience of crops to abiotic stresses to ensure food and nutrient security despite adverse environmental growing conditions. Among these sciences are genomics-assisted breeding, other OMICs approaches or systems biology tools for improving crop resiliency for adverse growing environments owing to climate change (Kole et al., 2015, Rabara et al., 2014b, Tripathi et al., 2012). These tools increase the exploitability of rice genetic resources like wild rice species of which desirable traits have been incorporated in modern rice varieties through molecular approaches and tissue culture (Sanchez et al., 2013). To date, advanced techniques can detect the presence of genes or traits expressing tolerance to DS and HTS conditions and measure the efficiency of their use in breeding populations, thus serving as alternative tools for precise breeding of crops that are resilient to adverse growing environments.

Figure 3 shows the concept of using a wide-base of breeding populations from various rice genetic resources for new breeds of rice cultivars with tolerance to DS, HTS and DS-HTS conditions through the synergistic and complementary use of molecular, genomics and other OMICs approaches for marker-assisted breeding as well as genomics breeding and genetic engineering. Rice varieties with genes/traits of interest conferring tolerance to HTS and DS can be developed through synergistic and complementary use of advanced and emerging approaches in molecular and systems biology.

For example, genome-wide association studies (GWAS) allow identification of genomic regions governing traits of interest and a better understanding of the genetic basis of complex traits in rice such as, major associations for flowering time, grain size and weight, abiotic stress tolerance and disease resistance to name a few (McCouch et al., 2016, Famoso et al., 2011, Huang et al., 2010, Kole et al., 2015, Huang et al., 2011, Zhao et al., 2011). Genetically, these desirable traits expressing drought and high temperature tolerance called genetic loci or QTL are inherited by offsprings or progenies from breeding populations. OMICs approaches such as genomics, metabolomics, transcriptomics, proteomics and interactomics among other recent advances in systems biology can characterize genes, metabolites, transcripts, proteins and interactions of protein-protein and protein-DNA (Tripathi et al., 2012, Yang et al., 2014a) expressing the desirable phenotypic traits for precise crop productivity improvement. For example, integrated analyses of transcript and metabolite profiles in rice under HNT stress reveal that GABA-signaling provides a connection to the inositol phosphate and TCA cycle associating jasmonate to HNT response (Glaubitz et al., 2016) .

Generally, these advances in molecular and systems biology approaches can potentially increase the chances of exploiting the desired traits in under-utilized rice genetic resources as breeding populations. Ensuing discussions show some research studies conducted on these approaches as

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well as their importance and potential as integral parts of synergistic and complementary breeding strategies for new rice varieties with tolerance to DS and HTS.

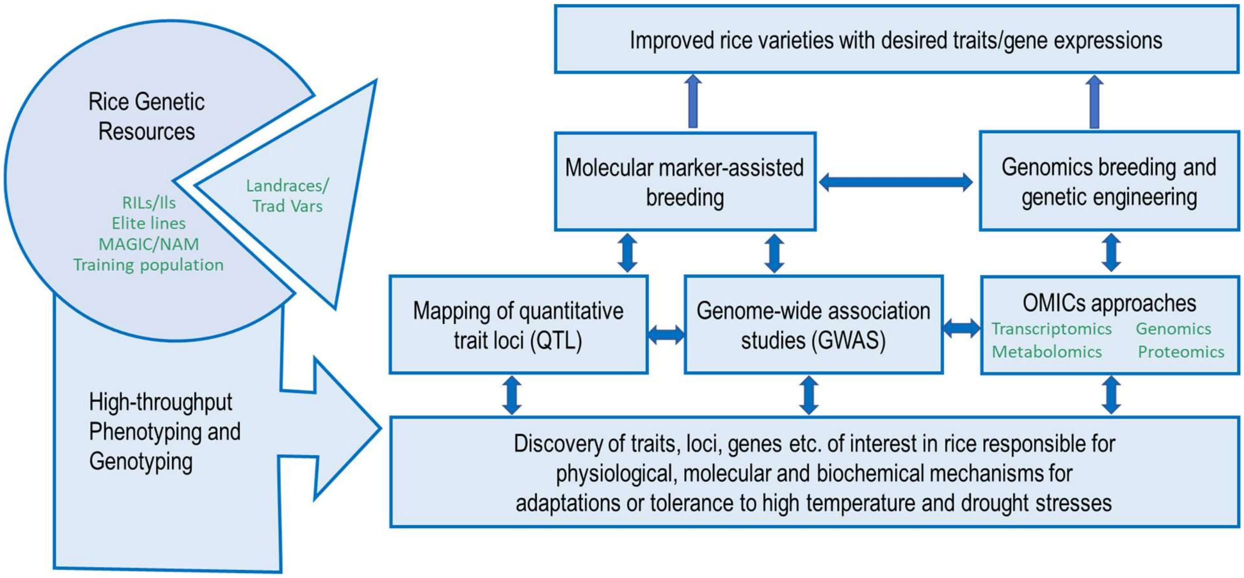

Figure 3. A molecular- and systems-level framework of advanced rice breeding through use of markers, genomics and genetic engineering and employing molecular, genomics and OMICs approaches on a wide-base pool of rice genetic resources as breeding populations.

5. Phenotyping and genotyping of rice genetic resources

To open up new opportunities for environmental resilience and future gains in quality and yield, useful genetic traits are moved across the breeding barrier and expand plant genetic diversity as well (McCouch et al., 2013). Rice diversity possesses a vast wealth of genetic resources that are available for breeding climate resilient varieties. Rice germplasms including landraces and traditional varieties serve as sources of genetic materials essential to develop new rice cultivars through advanced rice breeding techniques (Rabara et al., 2014a). In particular, wild rice species have many useful genes for resistance to major biotic and biotic stresses (Sanchez et al., 2013). Their existence endures owing to farmers who continually cultivated and nurtured thousands of rice varieties for several years thereby contributing to rice diversity (Bellon et al., 2000).

The development of IR8, dubbed as miracle rice is classic example of rice germplasm use. IR8 was the first high-yielding rice variety averaging 9.4 tons/ha and has initiated the “Green Revolution” in agriculture (Hargrove and Coffman, 2006). It was the product of a cross between two landraces: a semi-dwarf rice ‘Dee-geo-woo-gen’ and tall, vigorous rice ‘Peta’ (Ronald, 2012).

Prior studies have shown that landraces and traditional cultivated rice varieties collected from farmers have considerable phenotypic variabilities (Figure 4A) and tolerance to drought and high temperature (Ferrer, 2015, Rabara et al., 2015a, Rabara et al., 2014a). Assessment of phenotypic diversity of 307 Philippine farmer-cultivated rice cultivars using a Shannon-Weaver diversity index (H’) (Rabara et al., 2014a) showed low (average H’=0.45) and high (average H’= 0.73) diversity in qualitative and quantitative traits, respectively (Figure 4B).

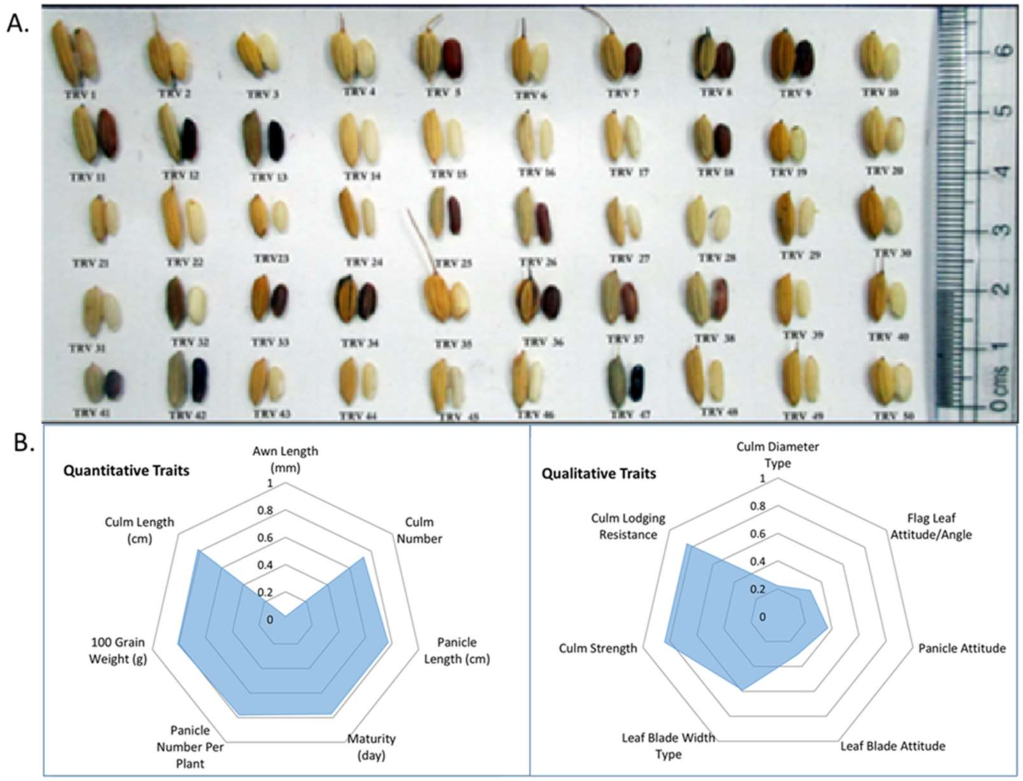

Figure 4. Diversity in (A) grain traits (Ferrer, 2015) and (B) phenotypic characteristics (Rabara et al., 2014a) of rice varieties in the Philippines. (A) Grains of farmers-cultivated traditional rice cultivars demonstrate diversity in size and color of both hull and grain among other traits. (B) Using a Shannon-Weaver diversity index, 307 traditional rice varieties collected in the Philippines showed variant phenotypic diversity: high quantitative (average H=0.73) and low qualitative (average H’= 0.45) traits.

Phenotypic screening of 511 varieties comprised of genebank accessions, breeding lines and varieties from hot regions that grow rice were grown in fields and validated in controlled growth chamber conditions resulted in the identification of 23 rice germplasms with potentially useful applications in breeding for heat tolerance (Tenorio et al., 2013). These 23 accessions underwent further screening in growth chambers at high-temperature treatment of 39°C and 38°C at booting and flowering stages, respectively. Notable accessions exhibiting heat tolerance were Giza 178 at both stages, Milyang 23 and IR2006-P12-12-2-2 at flowering stage and Dular and Todorokiwase at booting stage (Tenorio et al., 2013). In a germplasm screening, two rice cultivars: ‘Bocao’ and ‘Bolinao’ were identified exhibiting up to 22% increase in yield under drought conditions (Ferrer, 2015).

Root morphology is also an important trait in rice tolerance to drought. Figure 5 shows a comparison of root architecture and harvested panicles of two rice varieties: IR64 and PSB Rc14 under well-watered and drought conditions (Ferrer, 2015). Under drought, a 56% reduction in root

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length was noticed in drought-sensitive variety (IR64) compared to 23% in tolerant PSB Rc14 variety (Ferrer, 2015). Therefore, the plasticity of root system development is a rice plant's key trait for water stress adaptation and QTLs responsible for various root traits such rooting depth, length, and thickness were identified (Serraj et al., 2009). The use of deep-rooting rice cultivars in the tropical or subtropical growing regions has been suggested to avoid immediate to high severity of drought stress, as well as the practice of a water-saving technique to avoid high generative stress (Korres et al., 2017).

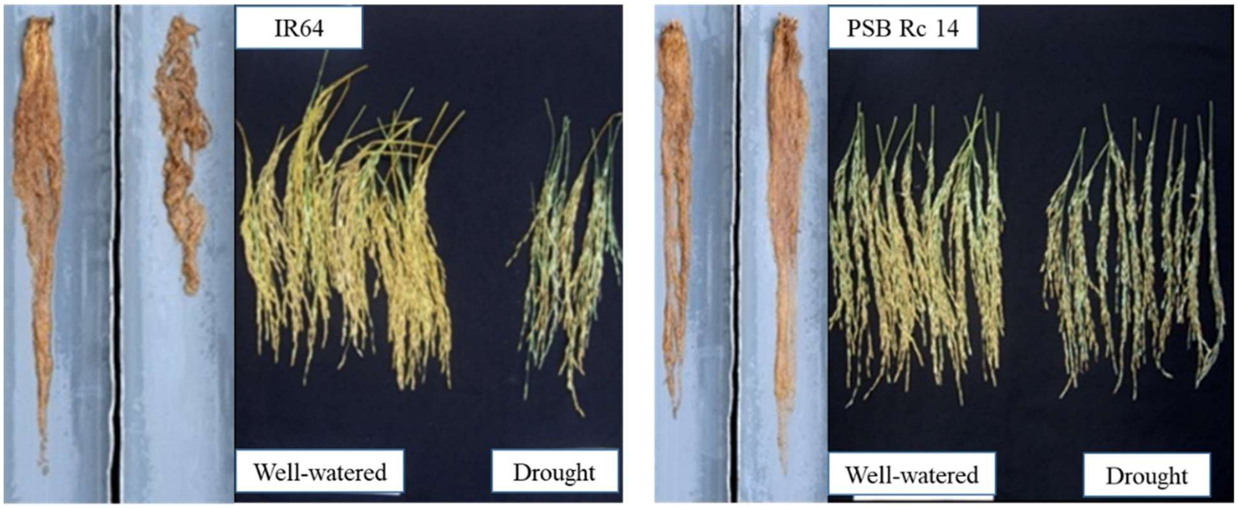

Figure 5. Effects of drought on the panicles and root systems of 'IR64' (drought-sensitive) and 'PSBRc 14' (drought-tolerant) varieties. Photo adapted from (Ferrer, 2015).

6. **Mapping of genetic loci (QTL) and genome-wide association studies (GWAS)**

Advances in genomics, molecular marker technology, fluorescence in situ hybridization and tissue culture opened avenues in utilizing the genetic diversity of rice through interspecific hybridization (Sanchez et al., 2013). For example, wild hybridization aided in the development of two Arizona rice varieties with heat tolerance (Sanchez et al., 2013).

QTL for drought and high temperature tolerance. In molecular marker technology, the identification and introgression of QTLs have become widely used and now a preferred breeding strategy for improving grain yield in rice under various abiotic and biotic stress conditions. QTL mapping is a tool to split up phenotypic traits and their components to help understand the genetic bases of traits during diversification selection throughout crop evolution (Bo et al., 2014).

Various QTLs have been categorized as responsible for tolerance in DS and HTS (Figure 6), some through QTL fine mapping. Using a 5K SNP genotyping chip on a N22/IR64 RIL mapping population, two QTLs: *qSTIPSS9.1* and *qSTIY5.1/qSSIY5.1* for heat tolerance having 65 and 54 genes, respectively were confirmed in less than 400kbp genomic regions (Ps et al., 2017). A novel QTL, *qSTIPSS9.1* was found in chromosome 9 at 394 kbp region and showed heat tolerance at reproductive stage. The second QTL *qSTIY5.1/qSSIY5.1*, which has been previously reported was established in chromosome 5 at a narrower 331 kbp region compared to 23Mb in the original study.

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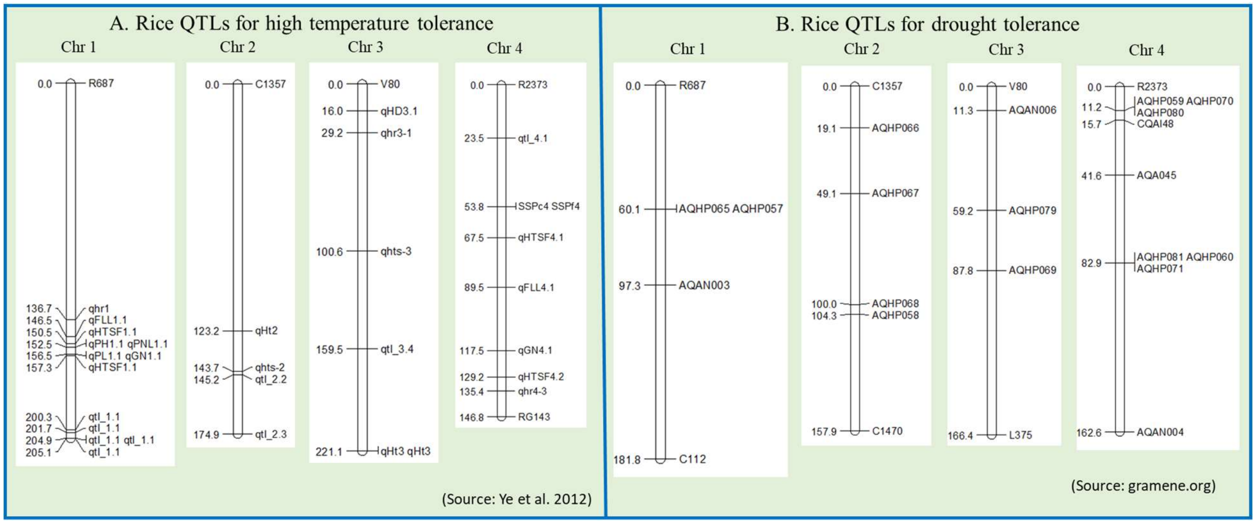
A. Rice QTLs for high temperature tolerance

B. Rice QTLs for drought tolerance

(Source: Ye et al. 2012)

(Source: gramene.org)

Figure 6. QTLs identified in various rice genotypes associated with tolerance to high temperature (A) and drought (B) in chromosomes 1 to 4. QTL/marker data [accession, locus ID and genetic distance (cM)] were adapted from Ye and associates (2012) and Gramene.org and visualized into select rice chromosomes.

Although several QTLs control heat tolerance at flowering stage, QTL *qHTSF4.1* can be an importance source for HTS tolerance since it was found consistently across genetic backgrounds of RILs involving crosses of IR64, N22, Giza178 and Milyang23 (Ye et al., 2015). Figure 6A shows the various published QTLs associated with heat tolerance at flowering stage in different rice genotypes including Azucena, Bala, Toyonishiki, T226, T219 and IR64 (Ye et al., 2012). Four QTLs related to spikelet fertility in rice tolerance to heat stress were identified in F2 ($qHTSF_{1.1}$, $qHTSF_{4.1}$, $qHTSF_{9.1}$ and $qHTSF1_{1.1}$) and BC$_1$F$_1$ ($qHTSF_{1.1}$, $qHTSF_{4.2}$, $qHTSF_{5.1}$ and $qHTSF_{7.1}$) populations of IR64 x N22. The two major QTLs in chromosomes 1 ($qHTSF_{1.1}$) and 4 ($qHTSF_{4.1}$) accounted for 12.6% and 17.6% of spikelet fertility variation under high temperature (38/24ºC) (Ye et al., 2012).

At anthesis stage, two QTLs on spikelet fertility (SF) were detected under heat stress but not under normal conditions (Cheng et al., 2012). These QTL *qSF4* and *qSF6*, apparently stress-induced were found in Xiushui 09 and IR2061 alleles, respectively and possible to use in breeding rice tolerant to HTS (Cheng et al., 2012). Also, a QTL encoding the α2 subunit of the 26S proteasome was identified in the heat-tolerant wild African rice (*Oryza glaberrima*) that is involved in the degradation of ubiquitinated proteins and OgTT1 when overexpressed in rice, conferring tolerance (Li et al., 2015b).

Selection of rice varieties that flower early in the morning (EMF) is one criterion to avoid daytime HTS and reduce spikelet sterility (GRiSP, 2013). NIL lines of 'IR64' introgressed with EMF QTL showed 71% reduction in spikelet sterility during the dry season by achieving peak of anthesis around 8:30 am, ~2 h ahead of 'IR64' (Bheemanahalli et al., 2017). A potential donor for EMF trait is *O. glaberrima* (CG14) which flowers immediately after dawn, potentially escaping high temperatures during the late hours of the day (Wassmann et al., 2009). The "STAY-GREEN" trait in leaf or delayed leaf senescence should be considered for its capability to regulate photosynthesis in HTS (Abdelrahman et al., 2017).

Under DS at reproductive stage, *qDTY1.1* in chromosome 1 showed consistent grain yield increase of about 29%, 24% and 16% in multiple elite genetic backgrounds comprised of N22/Swarna, N22/IRG4 and N22/MTU1010, respectively (Vikram et al., 2011). In the N22/MTU1010 populations but non-stress situation, *qDTY1.1* also showed a positive grain yield effect, suggesting consistency as a QTL for grain yield (Vikram et al., 2011). Similar additive effect on grain yield was observed in *qDTY1.1* in Dhagaddheshi progenies from crosses with Swarna and IR64 varieties with 24.9% and 8.6% mean yields, respectively (Ghimire et al., 2012). Dhagaddheshi is a traditional drought-tolerance rice cultivar in India, while IR64 and Swarna are two popular high-yielding varieties in Southeast and South Asia. Findings of both studies (Ghimire et al., 2012, Vikram et al., 2011) add merit for QTL *qDTY1.1* as a suitable candidate for marker-assisted breeding use owing to its consistent performance in multiple elite rice genetic backgrounds. Figure 6B shows QTLs for drought tolerance found in chromosomes 1 to 4 that are publicly accessible at the Gramene website (http://www.gramene.org).

A simultaneous study of DS and HTS effects at anthesis in Baja/Azucena RILs has identified three QTLs *qtl_1.1*, *qtl_8.2* and *qtl_10.1* influencing multiple abiotic stress tolerance (Jagadish et al., 2010). Found close to marker B1065E10, *qtl_1.1* was recommended as an ideal candidate region for future breeding programs in multiple abiotic stress resistance. QTLs *qtl_8.2* and *qtl_10.1* were localized in chromosome 8 and chromosome 10, respectively.

In summary, several QTLs or genetic loci that control tolerance to DS and HTS in rice plants are quantitatively inherited traits, thereby can be expressed in progenies or offsprings in breeding new varieties through marker-assisted selection.

Genome-wide association studies (GWAS) in rice for drought and high temperature tolerance. By utilizing evolutionary and historical recombination events at the population level, GWAS serve as an instrument to define complex trait differences at the sequence level (Lafarge et al., 2017). Likewise, GWAS can construe the evolution trend and/or the manmade selection of an essential trait (Wang and Qin, 2017). Methodologies for more advanced models have evolved from its simplest form called marker-by marker analysis (Lafarge et al., 2017). To date, GWAS approach have been used to explore traits in rice conferring tolerance to DS and HTS as well as in combination with other analytic tools.

For example, a GWAS of 517 rice landraces involving 14 agronomic traits including drought tolerance, four loci that account to 36% phenotypic variation in the population were identified in chromosomes 1, 5, 6 and 11 (Huang et al., 2010). In a three-method GWAS study on a panel of 167 rice indica landraces, 14 loci associated with spikelet sterility under 38^{o}C growth condition were located (Lafarge et al., 2017). Yet, only a few accessions assembled the favorable alleles at all loci in effective donors such as N22 and some Indian and Taiwanese varieties (Lafarge et al., 2017). The three methods were based on single marker regression, haplotype regression and simultaneous fitting of all markers.

Moreover, a recent review on GWAS and QTL mapping concluded that natural variations responsible for drought resistance occur in crops and comprehensive approaches such as systems biological studies can be utilized to further elucidate the molecular mechanisms of identified loci

(Wang and Qin, 2017). Combined analyses of GWAS and high-throughput phenotyping in rice showed meaningful insights into the genetic architecture of 15 essential traits related to morphology, biomass and yield (Yang et al., 2014b). The combined approach was construed as an innovative instrument to replace traditional phenotyping and for studies of plant breeding, genomics, genetics and gene characterization (Yang et al., 2014b). Likewise, an integrated approach of sequence-based GWAS and functional genome annotation has identified 18 associated loci illustrating the method's potential to link complex traits in rice to their causal polymorphisms (Huang et al., 2011).

Despite of the numerous promising loci identified thus far, elucidation of their molecular mechanisms responsible for improved stress tolerance remains to be explored through systems biological studies and comprehensive approaches (Wang and Qin, 2017).

7. Gene discovery and regulatory mechanisms

Aside from understanding phenotypic trait changes in plant responses, it is doubly important to understand the molecular and biochemical mechanisms involved in plant responses under stress conditions (Shivani et al., 2017). Developing rice varieties with tolerance to DS and HTS is imperative because both DS and HTS are serious limiting factors to productivity and yield. Breeding climate-resilient varieties also poses great challenges to scientists owing to the complexities of screening techniques, component traits, developmental stage or age of plants, environmental interactions including a profound understanding of the various morphological, physiological and biochemical mechanisms specific to DS and HTS. Although complex traits such as genetic loci have been discovered, these should be dissected functionally through comprehensive approaches like systems biology (Wang and Qin, 2017).

For instance, a significant increase in yields was credited to SPIKE (SPIKELET NUMBER) gene, a NAL1 (NARROW LEAF) allele from the tropical japonica rice landrace 'Daringan' (Fujita et al., 2013). SPIKE increased yield by 18% in newly-released variety 'IRRI146' and increased spikelet number in 'PSB Rc18', 'TDK1', 'Ciherang', 'Swarna', and 'BR11' (Fujita et al., 2013). Near-isogenic lines of SPIKE had higher total spikelet number, flag leaf width, root dry weight, and rate of filled grain which resulted in a consistent higher grain yield per m2 compared to 'IR64' variety over four cropping seasons (Fujita et al., 2013).

Several studies on comprehensive transcriptomics and metabolic analyses on the effect of drought and heat stresses on rice had been reported (González-Schain et al., 2016, Glaubitz et al., 2016, Li et al., 2015a). Figure 7 summarizes the chromosomal location of various genes in rice that have been identified for drought and heat tolerance.

Recent molecular studies have shown that heat shock proteins (HSP) and heat shock transcription factors (HSF) are major factors for HTS response in rice. HSPs have tissue-specific expressions that play central roles in plant survival (Chang et al., 2007) such as protecting the proteins from denaturation and acting as molecular chaperones (Baniwal et al., 2004). For example, expression analysis of HSP87 in rice showed that this group is induced early upon exposure to heat stress and return to normal levels once the stress conditions seized, suggesting possible involvement in thermo-tolerance in rice. Further analyses showed that heat acclimation in rice plants and thermotolerance of rice seeds were regulated post-transcriptionally due to a positive feedback loop between HSP101, and a heat stress-associated 32-KD protein (HSA32) (Lin et al., 2014). Heat stress at reproductive stage during anthesis in particular showed altered expression of the small heat shock proteins (sHSPs) in rice plants, which help maintain the electrochemical potential, as well as proteins controlling cellular metabolism (Lee et al., 2007).

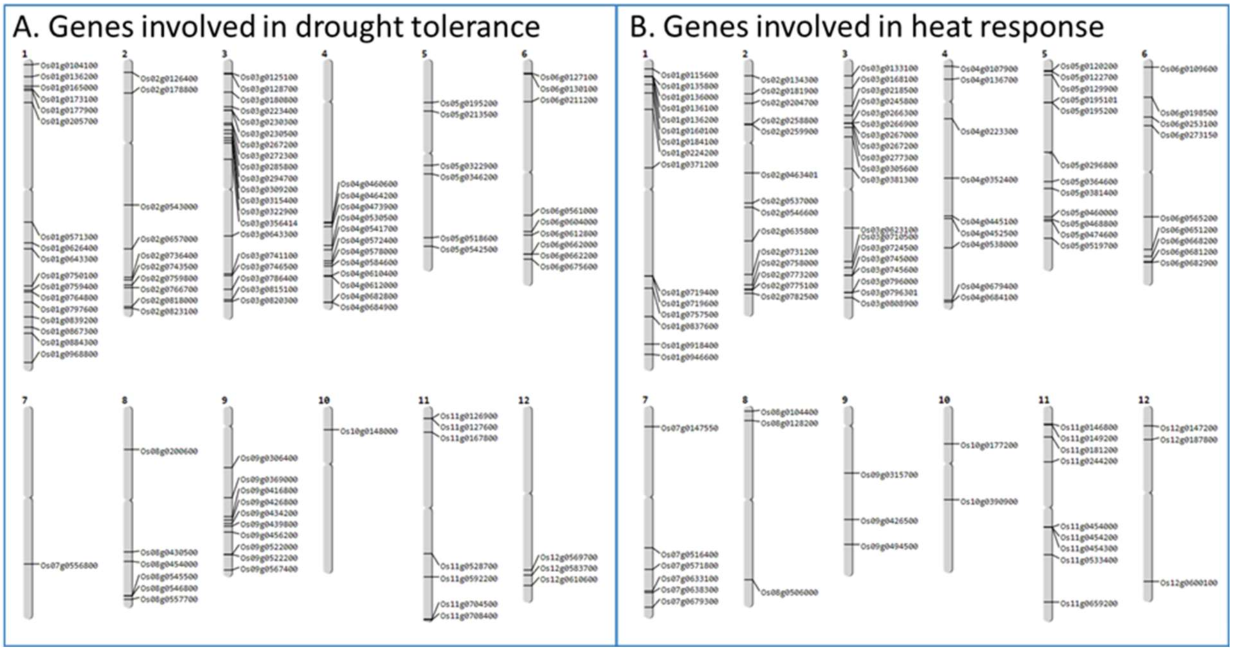

Figure 7. Genes in rice involved in drought tolerance (A) and heat response (B) are shown in their corresponding chromosome locations. Gene data on drought and high temperature were generated from QTARO database (Yonemaru et al., 2010) and from (González-Schain et al., 2016), respectively.

Moreover, the expression of HSPs is controlled by the HSFs, with a conserved DNA binding domain (central helix-turn-helix motif) that enables binding to the heat shock elements (HSEs) in the target promoter (Scharf et al., 1998, Sakurai and Enoki, 2010). Rice has 19 HSFs that have been cloned and characterized (Morimoto, 1998). Two studies on the expression analyses of HSFs in rice showed alterations in 6 HSFs under abiotic stresses (Chauhan et al., 2011, Liu et al., 2005). HsfA1a is well-studied and has been identified as a master regulator responsible for heat stress-induced gene expression including synthesis of HsfA2 and HsfB1 (Baniwal et al., 2004). HsfA2 is identified as a major factor for heat stress and is exclusively found after heat stress induction and represents the dominant HSF (Charng et al., 2007, Baniwal et al., 2004, Kotak et al., 2007). Specifically, the interaction of *OsHsfA1* and *OsHsfA2* upon heat stress increased thermotolerance of rice seedlings that was mediated by *OsHsp90* that in turn activates *OsGolS1* required for the accumulation of galactinol, an important osmoprotectant that protects the photosynthetic apparatus when plants are under oxidative stress (Hue et al., 2013). *OsHsfA2a* and *OsHSF6* were induced quickly in response to heat stress in both root and shoot tissues, while *OsHsfA3* was induced by both drought and cold stresses (Liu et al., 2005).

Regulatory mechanisms -- ROS-mediated scavenging and transcription factors (TF). When exposed to drought stress, plants also experience oxidative stress, which results in the production of reactive oxygen species, including H_2O_2 (Mittler, 2002). Previously, it has been reported that *HsfA5* is a negative regulator of the osmotic stress-responsive pathway (drought and heat), which acts in response to the attenuation of ROS signaling by forming a heterooligomer with *HsfA4* that finally inhibits the active *HsfA4a* homotrimers (Baniwal et al., 2007). Stress-induced activation of HSFs transduces the signal to downstream transcription factors like AP2/ERFs (APETALA2/ethylene response factor), ZAT (C_2H_2-type ZINC FINGER) family, WRKYs, or kinases like MAPKs (Mitogen-activated protein kinases) that finally help counteract the detrimental effects of stress. Overexpression of *HYR* (Higher Yield Rice), an AP2/ERF TF in rice, showed enhanced tolerance and yield stability by positively regulating photosynthesis and also possibly modulating signal transduction cascade via MAPKs and CRY2 photoreceptors (Ambavaram et al., 2014). A MADS-box TF *OsMADS87* has been identified, which when knocked out in rice showed tolerance to a moderate heat stress, measured in terms of seed size reduction (Chen et al., 2016). Hence, further investigations of the role of MADS box TFs in rice may detect potential candidates for enhancing thermotolerance in rice. A comprehensive analysis of *OsDREB2* (dehydration-responsive element-binding [DREB] protein) transcription factors family showed that *OsDREB2A* was induced by heat, drought, and salinity stresses (Matsukura et al., 2010). Likewise, alternative-splicing regulated *OsDREB2B* belonging to the DRE/CRT family conferred heat tolerance in transgenic Arabidopsis by regulating *OsDREB2A* (Matsukura et al., 2010).

Overexpression of *OsMYB55* in rice resulted in heat tolerance by enhancing the expression of target genes such as *glutamine synthetase* (*OsGS1/2*), *glutamine amidotransferase* (*GAT1*), and *glutamate decarboxylase 3* (*GAD3*), and also modulating other genes of the amino acid metabolism pathway (El-Kereamy et al., 2012). Overexpression of the RING zinc finger protein (RZFP) *OsRZFP34* in rice showed enhanced tolerance to HTS at the vegetative stage mediated by a cooling effect due to a larger stomatal aperture (Hsu et al., 2014). Expression analysis of the WRKY family of TFs in rice identified *OsWRKY45* and *OsWRKY11* as heat inducible, while overexpression of *OsWRKY11* under the control of *HSP101* promoter showed tolerance to HTS indicated by higher survival rates and lower leaf wilting (Wu et al., 2009). *OSWRKY47, MID1* (MYB Important for Drought Response1) were also found to contribute to drought tolerance and hence yield by modulating the expression of Calmodulin-Binding Protein (*CaMBP*) (Raineri et al., 2015, Guo et al., 2016a). Two ABA-inducible TFs: *OsAREB1* and *OsSNAC3* were also induced by heat, and when overexpressed in rice and Arabidopsis, the transgenic plants showed heat tolerance owing to efficient scavenging of ROS and regulation of genes from the ABA signaling pathway (Jin et al., 2010, Fang et al., 2015).

Likewise, the NAC family of TFs has been shown to play a critical role in drought tolerance in rice. For example, overexpression of *OsNAC6*, *OsNAC10*, *OsNAC9* and *OsNAC5* in rice improved drought tolerance through increased root number and diameter (Lee et al., 2016a). Tolerance conferred by overexpression analysis was suggested to be mediated either by nicotianamine (NA) biosynthesis, glutathione relocation, or by incorporating and controlling factors involved in cell wall biosynthesis (Lee et al., 2016a, Lee et al., 2016b, Jeong et al., 2010, Redillas et al., 2012, Jeong et al., 2013). Transgenic rice plants overexpressing SNAC1 showed drought resistance both at the vegetative and reproductive stage by reducing water loss through regulation of stomatal opening possibly by regulating the expression of *OsSRO1c* but with no compromise in photosynthesis (You et al., 2013, Hu et al., 2006).

Moreover, phytohormones such as abscisic acid (ABA), cytokinin (CK), gibberellic acid (GA), auxin, and ethylene contribute to drought tolerance and their roles in morphological adaptations were studied extensively (Basu et al., 2016). Under DS at the vegetative stage, rice plants increase ABA accumulation to prevent water loss by rapidly closing the stomata. Increased ABA content during drought at the reproductive stage led to maximum grain yields (filled grains) possibly due to the increased relocation of stored carbon from source tissue (Yang et al., 2004, Yang et al., 2003). Overexpression of *OsNAP* and *OsNAC5* TFs in rice allowed increase in drought resistance with improved yield by upregulating the expression of genes like *OsPP2C06/OsABI2*, *OsPP2C09* and *OsPP2C68* belonging to the ABA signal transduction pathway suggesting that these genes can improve grain filling through source-sink relationship manipulation (Liang et al., 2014, Chen et al., 2014, Sperotto et al., 2009).

ABA-inducible bZIP TFs were found to exert a regulatory influence on drought tolerance by binding to the ABRE elements in the promoters of ABA signaling pathway genes (Fukao and Xiong, 2013, Maruyama et al., 2014). When overexpressed in rice*, OsbZIP23, OsbZIP46* and *OsbZIP71* indicated a positive correlation with yield under drought by regulating the expression of dehydrins, membrane transporters like *OsNHX1* and *COR413-TM1*, and *ABFs* (Xiang et al., 2008, Tang et al., 2012, Liu et al., 2014). Belonging to the GRAS family, *OsGRAS23* was found to contribute to yield increase under drought through regulation of ROS scavenging (Raineri et al., 2015, Xu et al., 2015, Guo et al., 2016a).

In summary, tolerance to DS and HTS is a complicated process that is well orchestrated by the involvement of various TFs belonging to different groups. Stress response also involves hormonal crosstalk that leads to the activation of different signaling cascades predominated by TFs, which eventually leads to the secondary defense response mediated by the ROS scavenging machinery. The activation of a strong ROS scavenging machinery is mediated by the accumulation of osmolytes like polyamines, quaternary ammonium compounds or sugars and enzymatic and non-enzymatic antioxidants. Hence, to develop abiotic stress resilient crop varieties, experiments must focus on manipulation of regulatory genes identified using complementary systems biology approaches and breeding strategies and employing an enormous rice germplasm pool.

Epigenetic regulation of rice responses to DS and HTS. Epigenetic regulation is a common molecular mechanism that can facilitate efficient gene expression patterns and help plants survive in unfavorable conditions (Pikaard and Scheid, 2014). This regulatory mechanism has been observed in rice response to various abiotic stresses (Zong et al., 2013, Santos et al., 2011). Discussions below detail the roles of DNA methylation and histone modification in rice under abiotic stresses.

*DNA methylation***.** DNA methylation is a common epigenetic mark that involves addition of a methyl group at the fifth carbon position of the cytosine ring (Grativol et al., 2012). DNA methylation in rice is influenced by abiotic stresses like heat and drought (Zong et al., 2013, Folsom et al., 2014). DNA methylation in plants occurs in CG, CHG and CHH motifs (H = A, C or T) which is facilitated by families of methyl transferases: *DNA METHYLTRANSFERASE 1* (*MET1,* also known as *DMT1*) for CG, a plant-specific DNA methyltransferase

CHROMOMETHYLASE 3 (*CMT3*) for CHG, and *DOMAINS REARRANGED METHYLTRANSFERASE 2* (*DRM2*) for CHH (Santos et al., 2011, Law and Jacobsen, 2010).

CG methylation in rice is characteristic of genic regions, while non-CG methylation (CHG and CHH) is abundant in transposable elements (Zemach et al., 2010). Rate of methylation in rice with CG, CHG and CHH contexts showing 50, 27.4 and 5.2% (Zemach et al., 2010), respectively is higher compared to Arabidopsis having CG, CHG and CHH contexts at 24, 6.7 and 1.7% (Cokus et al., 2008), respectively. Drought-induced methylation accounted to 12% of the total site-specific methylation in the rice genome (Wang et al., 2010). Variant methylation frequencies were found in a genome-wide analysis of methylation pattern in three rice varieties ('IR64', stress-sensitive; 'Nagina 22', drought-tolerant; 'Pokkali', salinity tolerant) under contrasting drought and salinity response (Garg et al., 2015). The frequency of methylated cytosine (mCs) in the three rice varieties was almost similar with IR64 (11.32%) and Pokkali (11.5%) but marginally higher in 'N22' (12.30%). Methylation rate in all three rice cultivars was higher in the CG (47–49%) context followed by CHG (28–31%) and CHH (20–24%) (Garg et al., 2015) which were similar to those observed in unstressed 14-day-old rice seedlings (var. 'Nipponbare') except for a lower (5.2%) CHH context (Zemach et al., 2010).

In developing rice seeds under moderate HTS (34°C), methylation in CG and CHG motifs declined at 48 hours after fertilization (HAF) by 8.8 and 6.6%, respectively but at 72 HAF, CHG increased by 25% while CG decreased by 22% (Folsom et al., 2014). Interestingly, expression profile of *CHROMOMETHYLASE3* (*CMT3*), the methyl transferase that facilitate methylation in CHG context, only showed correlation pattern under 48 HAF. The expression of CMT3 was repressed at 48 HAF which reflects the 6.6% reduction in CHG methylation (Folsom et al., 2014).

Histone modification. In the nuclei of eukaryotic systems, chromatin is wrapped around an octamer of histones composed of 2 copies of H2A, H2B, H3 and H4 proteins (Vergara and Gutierrez, 2017). Histone modifications usually occur at the N-terminal region, called the histone tail, which is enriched in basic amino acids (lysine and arginine) (Zhou and Hu, 2010). The post-translational modifications of histone include acetylation, methylation, glycosylations, phosphorylation, carbonylations, sumoylations, and ubiquitination that can alter the activity of associated genes (Vergara and Gutierrez, 2017, Zhou and Hu, 2010).

A genome-wide analysis of histone modification in 25-day-old japonica rice var. 'ZH11' revealed H3K4me3 modification in unstressed and DS conditions by 51.1 and 48%, respectively and the observed differential H3K4me3 methylation only affected a small proportion (24-29%) of stress-responsive genes (Zong et al., 2013). Among those drought-responsive genes that were H3K4me3-methylated were *dehydrin* and dehydration-responsive element-binding protein genes *OsDREB1A* and *OsDREB1B*. The H3K4me3 methylation status of the four clustered *Dehydrin* genes in Chr. 11 increased significantly, and the *dehydrin* genes were strongly expressed (> 1,000-fold) under drought condition.

In contrast, gene expression of *OsDREB1A* and *OsDREB1B* were strongly down-regulated by drought stress and showed decreased H3K4me3 level. These findings show the potential of histone modification in enhancing gene expression under abiotic stress. Histone modification was also observed in developing rice seeds under moderate HTS, with reduction in H3K9me2 methylation compared to unstressed plants at 48 HAF (Folsom et al., 2014). HTS effect on histone modification was more profound at 72 HAF wherein H3K9me2 methylation declined by 75% in affected plants.

Histone modification also controls HTS tolerance as shown in overexpression studies with Fertilization-Independent Endosperm 1 (*OsFIE1*), a PRC2 protein. When *OsFIE1* was overexpressed in transgenic rice, plants were more sensitive to heat stress estimated from reduced seed size. It was hypothesized that sensitivity was conferred due to repression in histone modification of *OsFIE1* and reduced expression of *OsCMT3* (Folsom et al., 2014). In addition to histone modification, microRNAs were predicted to be involved in heat regulation of stress in rice. MicroRNAs *miR156*, *miR157*, *miR167*, miR168, and miR398 have been shown to be downregulated by heat stress in rice, but their roles remain limited (Sailaja et al., 2014).

8. Conclusions and future research prospects

Research studies on the effects of drought, high-temperature and their simultaneous occurrence cause significant yield reductions in rice. Such threats emphasize the need for new climate-resilient rice varieties that can sustainably increase crop productivity and withstand abiotic stresses. Understanding the multi-mechanism responses in rice plants under drought and high temperature is important for researchers to improve the resilience of rice to these stresses. Several studies have shown that some rice varieties have molecular and physiological traits that allow them to tolerate drought and high temperature. These rice germplasms can serve as potential donor parents in breeding climate-resilient varieties.

Exploring the genetic diversity of rice germplasms by ensuring the availability of genetic and phenotypic information contribute largely to their utility in breeding research. Some phenotypic traits that were identified to help make rice plants resilient to abiotic stresses through selection criteria for tolerance to these stresses include leaf size, which correlates to panicle numbers and root plasticity. Moreover, relationships among photosynthetic, stay-green and yield traits need further scrutiny as temperature changes firstly affect leaves' physiological and morphological adaptations. Early morning flowering (EMF) trait or QTL exhibited in *O. glaberrima* should be examined in rice germplasms owing to its potential in evading high-day and high night temperatures. In addition to molecular marker technology, recent advances in systems biology particularly OMICs approaches should be utilized to further elucidate the molecular mechanisms of genetic loci identified through QTL mapping and GWAS for more precise and comprehensive breeding strategies.

Additionally, profiling of transcriptional factors in rice is essential because metabolites accumulation is a common response in plants under water stress. Chemicals that are significantly distinct between tolerant and sensitive varieties are useful biomarkers in screening germplasm materials for both stresses. For instance, the accumulation of sucrose in the anthers of 'N22' rice variety was reported as a significant response of tolerance to both high temperature and drought stresses. At the molecular level, transcriptomic analyses disclosed that plants including, tobacco, Arabidopsis, and wheat have shared unique regulations in gene expression in response to both high temperature and drought stresses.

Epigenetic regulation also plays vital role in rice responses to abiotic stresses. DNA methylation can induce gene expression of stress-inducible genes as exemplified by dehydrins while repression in histone modification conferred sensitivity to stresses. In-depth studies on epigenetic regulatory mechanisms in rice are crucial. These mechanisms expediate metastable changes in gene activity as well as fine-tune gene expression patterns, thereby allow plants to endure stressful environments and continue to survive and reproduce.

Developing climate-resilient rice varieties and component cultivation strategies is a challenging task requiring a comprehensive understanding of the various morphological, biochemical, physiological, and molecular characters governing yield under drought and high temperature but possible by implementing synergistic and complementary approaches in molecular and genomics breeding and genetic engineering. Integration of various breeding approaches including genomics, transcriptomics, metabolomics, proteomics should be explored to develop novel climate-ready crops, especially rice, the main staple of populations in under-developed and developing countries. Moreover, concurrent analyses of physiological, molecular and biochemical effects of combined high temperature and drought stress must be performed in well-planned set-ups in field and growth chambers to broaden the knowledge and understanding of response mechanisms to stresses and underlying genetic traits.

Global climate change threatens crop production worldwide. Unexpected changes in weather patterns, such as high temperature and drought have dramatically affected crop yield which in turn could result in food and societal crises. It is plausible that molecular breeding strategies may speed up the traditional breeding processes to increase stress resistance in cultivars; enabling them to better cope with the changing environment. Along with the development of high-throughput DNA sequencing technology, whole genome covered markers can be produced more cost-effectively with unprecedented increases of accuracy. Meanwhile, the advances of statistical analyses for quantitative genetics provide new methodologies to dissect the genetic basis of complex traits. It can be anticipated that systematic network analyses, including genomics, transcriptomics, proteomics, metabolomics and phenomics, will provide integrative information for increased understanding of the balance between stress response and grain yield and quality. Undoubtedly, such acquired knowledge will be of great value to researchers enabling them to come up with precise molecular design and comprehensive breeding strategies of new cultivars with desired stress resistance and yield traits.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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