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Rice: Grappling with Cold under Climatic Changes, Global Impact and Counter Strategies

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AG and JS had referred the related source of information and drafted the article in final shape. Authors BAG, ABA and SB had done the proof reading. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Rice has been staple food for more than half of the world population and presently it is one of the top three crops cultivated across the world in terms of area and production. Low temperature stress has been a critical factor year after year in determining the yield globally. Since last five decades many tactics there have been developed for countering the effect of low temperature stress in rice, which includes conventional as well as molecular. Here, we have reviewed recent progress in research on cold stress-mediated physiological traits and metabolites; elaborated their roles in the cold-response network and cold-tolerance evaluation. We also have discussed criteria for evaluating

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cold tolerance, evaluated the scope and shortcomings of each application. In this review, various approaches for cold tolerance are discussed with special reference to Quantitative Trait Loci (QTL).

Keywords: Cold stress; evaluation criteria; metabolites; QTL; rice; seedling.

1. INTRODUCTION

Rice (*Oryza sp.)* belongs to the Poaceae family and have basic chromosome number n=12. It exists in two form diploid and tetraploid, the diploid (2n=24) species is known as *Oryza sativa* L. (Asian Rice) or *Oryza glaberrima* L. (African Rice) and cultivated widely across globe. The use of crop has a wide range including food like flour, snacks, cereal bran oil etc to some medicinal values. More than half of the world population depends on rice as a staple food [1]. The global area under rice cultivation is 156 million hectare which has production of 650 million tons of crop [2], while in India the area under cultivation is 44.6 million hectare and production is 103.6 million tons which is around 23 percent of global production [3]. India is also a leading exporter of rice specially the Basmati rice.

Thus, increasing rice yields to help meet and ensure world food security is a significant and pressing technological goal. However the attempts to enhancing the yield in rice is challenged by various biotic and abiotic factors, among abiotic factors temperature, salinity, rainfall, drought etc are major one. Abiotic stresses directly or indirectly affect the physiological status of rice and negatively alter its overall metabolism, often with impacts on grain yield. Among these, cold temperatures can be particularly harmful due to the tropical origin of the rice species.

Low temperatures comprise a major climatic problem for rice growing in 25 countries, including Korea and Japan [4,5]. Low temperatures can have negative impacts on rice plants during germination, vegetative growth, and reproductive stages. Yield loss due to low temperatures is a major restriction on rice cultivation not only in areas at high latitudes or high altitudes but also in tropical countries such as the Philippines and Thailand [6]. Rice is highly sensitive to cold stress during reproductive developmental stages, and little is known about the mechanisms of cold responses in rice anther. Considering the expected higher frequency of extreme temperature events in the near future, cold waves could even increase the negative impacts of low temperatures in rice production [7].

The low temperature stress has reported to account up to 45 percent of yield loss in rice due to abiotic factors and frequently occurring low temperature may cause up to 50 percent reduction in overall yield [8]. Due to diverse growing locations and climatic factors, rice cultivars face cold stress at specific growth stages [9]. Researchers have established many growth-stage specific criteria to evaluate and select cold-tolerant rice. Evaluation of rice cultivars typically takes place during seedling and reproductive stages that are critical to production of rice. However, in high-latitude or high-altitude regions, low temperatures during long, cold springs can severely inhibit germination and constrain early seedling growth. So evaluation of cold tolerance at the germination stage is especially significant for these regions.

In this review, we have discussed and clarified mechanisms and cause of low temperature stresses in rice, role of various metabolites during the response to cold stress in rice, their effect on yield, and summarize the diverse criteria that are useful for evaluating the cold tolerance of rice at different growth stages. In addition, as special reference we have discussed QTL (Quantitative Trait Loci) and markers related to cold tolerance that can be used to facilitate marker-assisted breeding through recurrent selection in rice.

2. DEVELOPMENT OF COLD STRESS AND INDICATIONS IN PLANTS

Low temperature (e.g. chilling and freezing) injury can occur in all plants, but the mechanisms and types of damage vary considerably. Many fruit, vegetable and ornamental crops of tropical origin experience physiological damage when subjected to temperatures below about +12.5°C, hence well above freezing temperatures. However, damage above 0°C is chilling injury rather than freeze injury [10]. Freeze injury occurs in all plants due to ice formation. Crop that develop in tropical climates, often experience serious frost damage when exposed to temperature slightly below zero, whereas most crops that develop in colder climates often survive with little damage if the freeze event is not too severe.

The symptoms of cold sensitivity and damage vary according to the growth stage of the rice plant [11]. In the germination stage, the most common symptoms of cold temperature damage are delayed and lower percentage of germination. At vegetative stage, chilling damage is expressed through yellowing of the leaves, lower stature, and decreased tillering of the rice plants. When cold coincides with the reproductive stage of the rice plant, sterility of the spikelets is the most common symptom of injury, but incomplete panicle exertion and spikelet abortion may also occur [12]. Spikelet sterility may result from pollen abortion due to cold during microsporogenesis, when pollen grains are being formed, at the booting stage [13].

Depending on the duration and severity of the stress, exposure to these temperatures can lead to extensive damage to the plants. Chilling sensitivity is common in plants originating from tropical and subtropical regions and the injury is mainly a consequence of destabilization of cell membranes (Levitt 1980). Some exceptions are lettuce, which originated in a temperate climate, but can be damaged at temperatures near 0°C and some subtropical fruits trees that can withstand temperatures to -5°C to -8°C. Species or varieties exhibit different frost damage at the same temperature and phenological stage, depending on antecedent weather conditions, and their adaptation to cold temperatures prior to a frost night is called "hardening".

During cold periods, plants tend to harden against freeze injury, and they lose the hardening after a warm spell. Hardening is most probably related to an increase in solute content of the plant tissue or decreases in ice-nucleation active (INA) bacteria concentrations during cold periods, or a combination. During warm periods, plants exhibit growth, which reduces solute concentration, and INA bacteria concentration increases, which makes the plants less hardy [14].

The degree of injury in rice usually depends on time of occurrence (growth stage), severity of chilling, and low temperature duration [15]. Ye and co. (2010) has shown that Low temperature has the potential to affect growth and development of rice plants during any developmental stage, from germination to grain filling. However, Yamada showed that sensitivity to cold varies between stages [16], According to his data, rice plants have a lower threshold temperature (10–13°C) for cold damage during the early stages of development (germination and vegetative), what makes them less sensitive to cold than during the reproductive stage, which has a higher threshold temperature for damage (18–20°C).

There are very typical indications when rice plant suffers low temperature stress or cold shock, for example leaves from plants injured by chilling show inhibition of photosynthesis, slower carbohydrate translocation, lower respiration rates, inhibition of protein synthesis, and increased degradation of existing proteins [17, 18]. During the early growth stages in rice, the occurrence of low-temperature stress affects seed germination that inhibits seedling establishment and eventually leads to non-uniform crop maturation [19]. One of the most common features of low temperature stuck plants is retarded height and decreased chlorophyll content in leaves. Low chlorophyll content results in varying degree of discoloration in leaves from green to brown, which can be given a score of 1-9 depending upon the degree of discoloration [20].

3. CHANGES IN MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS UNDER COLD

Low temperature stress has very clear and visible effect on crop plants especially in rice in the form of change in morphological and physiological development. Low temperature inflicts a wide range of damages to rice plants, such as low germination rate, stunted seedling growth, high death rate, and low spikelet fertility, and even lead to change in physiological functioning of plants like increased EL (electrolyte leakage)), changes in chlorophyll fluorescence, and increases in amounts of ROS i.e. reactive oxygen species, MDA (measuring malondialdehyde), proline and other metabolites etc.

The most reliable morphological parameters for assessing the cold stress in rice are seedling height, seedling colour and germination percent. The important physiological parameters which show quick response to cold stress are chlorophyll content, ABA and proline hormone, membrane fluidity, soluble sugar, channel proteins etc [21]. Liu and co. (2013) while working on oat have showed that during the cold treatment of 1°C, naked oats grew well as usual. Until 5 days later, the seedlings were always strong only except some leaf apexes began to get yellow [22] in the 7th day, most parts of seedling remained green as normal temperature. The seedling got curl after 3-4 hours after exposure to −10°C cold stress. Some leaf began to get yellow and curled seriously in the third day, while the seedling grew slowly. Most leaves showed severe rolling and wilting in the 7th day. Liu and co. (2013) observed increase in proline content upon exposure to cold stress. Compared with the control, the free proline content in seedling leaves under low temperature was obviously higher than that under room temperature. Proline plays a vital role in maintaining osmotic balance in plants. The accumulation of proline may function in preventing plants from being damaged by stress. The free proline acts as osmolytes to facilitate osmoregulation, thus protecting plants from dehydration resulting from cold stress by reducing water potential of plant cells [23]. In addition, proline can also function as a molecular chaperone to stabilize the structure of proteins as well as play a role in regulation of the antioxidant system [24,25,26]. Cook and co. (2004) while studying the effect low temperature stress on *Arabidopsis* have found that 434 metabolites monitored by GC-time-of-flight MS, 325 (75%) were increase in *Arabidopsis Wassilewskija-2 (Ws-2)* plants in response to low temperature. Of these 325 metabolites, 256 (79%) also increased in non-acclimated Ws-2 plants in response to over-expression of C-repeat/dehydration responsive element-binding factor (CBF) [27]. Worker while studying the effects of cold stress in *Arabidopsis* have found that extensive changes occur in the transcriptome of *Arabidopsis* in response to low temperature [28,29,30,31,32]) and these changes are the results of CBF cold response pathways.

4. BREEDING FOR COLD TOLERANCE IN RICE

During last six decades various approaches have been tried and tested to counter the yield loss in rice due to cold stress. During first three decades the methods were mostly relying on conventional approaches however in last three decades many molecular tools including QTLs has been used to develop cold stress tolerance in rice.

Breeding demands genetic variability. Fortunately, the rice species (*Oryza sativa* L.) has wide adaptability to cold, and cold-tolerant ecotypes are available for breeding. The cultivated species *O. sativa* L. has two subspecies: *indica* and *japonica*. The *indica* subspecies includes cultivars better adapted to tropical environments such as India, China, and Indonesia, while *japonica* cultivars are more adapted to temperate climates such as the ones in Japan, Korea, and Java. Different methodologies to screen rice genotypes for cold reaction under controlled temperature conditions at different stages of development have been used like in germination stage (germination percent, germination rate, coleoptile length) vegetative stage (survival rate 10 days after the end of the cold treatment, growth and discoloration, visual scale (1–9), survival rate after 14 days of recovery, survival percentage) and reproductive stage (percent of fertility, panicle exertion). However, the available space to grow large plant populations under controlled temperature environments is the main limiting factor.

Growth under controlled conditions leads to gain in timing and precision of the stress, but loss in the amount of populations that will be possible to test. To deal with these limitations, some rice breeding programs have implemented selection with cold water under field conditions, allowing evaluation of many different populations and thousands of plants per population Several experimental stations in Japan [33] and Korea have successfully used cold water to screen rice breeding material for cold tolerance.

Studies with large number of cultivars belonging to these two subspecies showed that *japonica* genotypes have higher degree of cold tolerance at the germination stage as well as at the vegetative and reproductive stages. da Cruz and Milach [2013] also concluded that japonica genotypes presented higher cold tolerance at the germination stage than indica genotypes, although they found variability for this trait within both subspecies. This agrees with previous reports of some indica genotypes from high-latitude regions that may present moderate level of chilling tolerance. Some javanica cultivars are also reported to be tolerant to cold. Javanica rice is considered a tropical subpopulation or an ecotype of japonica [34] and cold-tolerance genes from the javanica cultivars Silewah, Lambayque 1, and Padi Labou Alumbis were introduced into several temperate japonica breeding lines in Japan [35].

5. TOOLS AND CRITERION TO EVALUATE COLD TOLERANCE IN RICE

In mean course of time there several criterion which includes morphological, physiological and biochemical have been described for evaluation of cold stress tolerance in rice. Similarly various tools have been developed to assess the cold tolerance/resistance in rice and these tools are conventional as well as molecular in nature.

In present time the evaluation for cold stress in rice is done at three stages *viz.* germination, seedling and reproductive stages which include various parameters to be take in account.

5.1 Evaluation at the Germination Stage

The most common parameters to evaluate cold tolerance at germination stage are germination percent and rate of germination. The primary criteria in this stage are germination percent and seedling survival rate of seedlings are the two main criteria used for the evaluation of cold tolerance in rice at the germination stage. For assessing the germination percent, observations are recorded at 7 d, 11 d, 14 d, and 17 d following germination at 14°C in the dark.

> Germination vigor (%) = (Number of germinated grain /Number of total grain) × 100.

The standard assessment of whether a rice grain has germinated is determined as the point at which the bud length equals half the length of the seed, and the root length equals the seed length [36]. Gautam and co. (2016) have shown the assessment for cold tolerance in germination stage may be done by adjusting the temperature 10-12 °C in day time and 7-8 °C in night time.

5.2 Evaluation at the Seedling Stage

At seedling stage seedling colour (dark green to brown) and seedling length are the two important features that can be used reliably. Gautam and co. (2016) shown in their study that a resistant genotype shows dark green colour after cold shock treatment, while brown colour in result in cold susceptible genotypes. Similar results were found for seedling length where 11 cm was mean length for cold stress resistant genotypes and 8-9 cm was for cold stress susceptible genotypes.

The seedling survival rate for cold tolerance is evaluated as follows. When shoots are about 5 mm long, the germinated seedlings are planted in soil and are subjected to cold treatment at 2°C for 3 d, and are then moved to a sunny indoor environment where the temperature is above 20°C to ensure normal growth. The other method applied by some researcher is to treat presoaked seeds in a growth chamber at a constant temperature of 32°C for 36 h. Germinated seeds with 5 mm coleoptiles were stressed at 5°C for 10 days, and then moved to a greenhouse at 20°C for 10 days to allow seedlings to recover and resume normal growth [37]. Seedling survival rates are assessed after 7 d recovery growth and cold tolerance evaluation indices are calculated as [38]:

> Seedling survival rate (%) = surviving seedlings/budding seeds × 100

Both visual and physiological indicators are used to evaluate cold tolerance at the seedling stage in rice. Five criteria are typically used for visual assessment of cold tolerance, including fresh weight, survival rate, new leaf emergence, seedling growth, and leaf growth. While studying the cold stress at seedling stage Wang and co (2016) found that both temperate and tropical *japonica* rice cultivars are more tolerant to cold stress than *indica* and AUS cultivars [39].

5.3 Evaluation at the Reproductive Stage

Spikelet fertility is considered as the most reliable and major parameter while evaluating the stress tolerance at reproductive stage [40]. Exposure to low temperatures during the reproductive stage in rice can cause male sterility and thereby severe yield loss. Cold tolerance at reproductive stage has been evaluated by estimation of spikelet fertility using cold greenhouse cultivation (CGC) or cold deep-water irrigation (CDWI). Spikelet fertility is estimated in the rational form of filled grains to the total number of florets, under cold greenhouse cultivation (CGC) (12°C/6 d) and cold treatment under cold deep-water irrigation (CDWI) (18-19°C/~60 d).

Researchers have found that cold deep-water irrigation (CDWI) is more efficient among these two above said approaches since it exposes plants to a more moderate treatment temperature and a longer treatment period, and is conducted directly in field. So, CDWI is also helpful in evaluating cold tolerance in QTL mapping population.

6. QTLs Identified for Cold Tolerance in Rice

There have been number of QTLs identified in almost all chromosomes of rice which are contributing to the cold tolerance at different stages. It is found QTLs which are contributing 20% or more to genetic diversity are more reliable and useful assessing the cold stress tolerance in rice. Gautam et al. (2016) confirmed four QTLs namely qCSH2, qGR-1, qPSST-3, qCTS4-1 and qPSST-7 which are contributing for low temperature stress tolerance in rice. Cold tolerance in rice is a quantitative trait controlled by multiple genes. Because it is often difficult to directly associate plant phenotypes with the genes responsible for cold tolerance, marker-assisted selection is an effective means of developing cold-tolerant cultivars [41].

The development of molecular markers and linkage maps has made it possible to identify QTL that control cold tolerance in rice. QTL analyses have been carried out using rice populations with large levels of genetic variation for cold tolerance [42], Futsuhara and Toriyama in their study screened 84 SSR (simple sequence repeat) markers and validated 24 markers which are closely related to low temperature stress tolerance in rice. A single QTL for booting stage cold tolerance was reported on the long arm of chromosome 3. This QTL was named *qLTB3* and explained 24.4% of the phenotypic variance [43]. Seven SNP markers were identified in five genes within the *qLTB3* region, all of them causing amino acid substitutions. One of those SNPs (in the Os03g0790700 gene) caused a mutation in a conserved amino acid and was considered the strongest candidate for conferring cold tolerance. Shakiba and co. (2017) using Genome Wide Association (GWA) mapping have identified a total of 18 QTL in the RDP1 as a whole (*ALL*) associated with cold tolerance (measured by germination index), including nine in *INDICA* (of which four were co-located with those identified in *ALL*), 13 in *JAPONICA*, (of which three were co-located with *ALL* and one was co-located with *INDICA*), seven in *temperate japonica*, and six in *tropical japonica* [44] [45] [46] [47]. Ranawake and co. (2014) have showed after the first three-day period of cold stress, four QTLs were detected on three chromosomes 5, 6 and 11. *qCTS11(1)-1* located on the short arm of chromosome 11 and *qCTS11(1)-2* on the long arm of chromosome 11 showed 22.2% and 35.6% PVE, respectively, while the other two QTLs, *qCTS5(1)* on the long arm of chromosome 5 and *qCTS6(1)* on the short arm of chromosome 6, respectively, showed 5.8% and 8.5% PVE. *qCTS6(1)* was overlapped with *qCTG6* within the chromosomal region covered by markers Wx and RM190. Positive additive effects were conferred by HGKN alleles at all QTLs except for *qCTS5(1)* [48]. Under same study they were able to find five more QTLs located on chromosomes 2, 7, 8 and 11. Among them, *qCTS2(2)*, *qCTS7(2)* and *qCTS8(2)* showed PVE ranging from 22.9% to 35.3%, while two other QTLs, *qCTS11(2)-1* and *qCTS11(2)-2*, showed 6.5% and 12.5% PVE, respectively. A number of studies have used similar mapping populations as used by Ranawake and co (2014) and found the results in same tune [49,50].

7. CONCLUSION AND FUTURE PROSPECTS

The development of cold stress tolerance in rice is a complex phenomena which include various physical, chemical, biochemical and genetic mechanisms. Applications of genomic approaches and gene knockout strategies are beginning to accelerate efforts to assess systematically and understand complex quantitative traits such as acquired tolerance to temperature extremes. It is clear that much progress has been achieved in the understanding of cold tolerance in rice plants. However, decreased productivity caused by low temperatures remains as a problem, especially in places where *indica* rice is cultivated. Systematic studies have been carried out to improve our understanding of the physiological and genetic basis of cold tolerance in rice, which will promote the development of rice cultivars with improved cold tolerance. Cold stress interferes with metabolism and initiates changes in various physiological properties of plants.

The development of molecular markers and linkage maps has allowed detection of many QTL related to cold tolerance at various growth stages. The fact that a large number of genes identified by these studies are currently annotated with ‘‘unknown function’’ and involve new genes and new pathways indicates that our knowledge of the transcriptional control of the low temperature response is limited, and the regulation of these transcriptional responses is far more complex than previously believed.

This study provides a comprehensive overview of the recent achievement in the field of plant cold tolerance.

In the future, the integration physiological mechanistic studies of cold tolerance and QTL identification will accelerate the improvement of rice for the traits related to cold tolerance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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