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Review Article

REVIEW ON COLD TOLERANCE IN RICE GENOTYPES AT VARIOUS GROWTH STAGES

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Abstract: The study of breeding cold tolerant varieties in rice is essential to reduce yield loss, for which information on various traits that contribute to tolerance is necessary. Identification of new genetic sources for cold tolerance is very important for which effective, low cost screening techniques are essential.

Keywords: *Conventional Breeding Methods, Cold Tolerance in Rice*

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Introduction

In temperate regions and High -elevation areas, low temperature effect the growth and development of rice. Cold stress affects the crop right from germination to grain maturity causing slow growth, reduce seedling vigour and cause seedling injuries, delayed heading and yield reduction due to spikelet sterility. At seedling stage, cold stress results in low number of seedlings and reduced tillering. Tellahamsa is the ruling variety for its seedling stage cold tolerance, in the past three decades of its release and no other varieties are able to replace it. Admittedly, the progress made in this direction to tackle this problem was inadequate, due to lack of basic information on the nature and degree of cold tolerance and suitable screening technology especially during seedling to vegetative stage.

Limitations of Conventional Breeding Methods

Abiotic nature makes it unpredictable under field conditions in terms of its intensity, duration and timing what limits field selection for cold tolerance in rice [1]. In breeding programmes, where rice genotypes are to be screened, the chilling sensitivity can be estimated based on visual observations in natural field conditions. However, this type of screening is subjected to genotype x environment interactions and diurnal or random fluctuations throughout the growing season and over years. In the field, adequate cold tolerance at the seedling stage may allow early sowing, because cold tolerant seedlings should be able to survive and develop normally, thereby ensuring uniform crop establishment. Selection of cold tolerant genotypes under field conditions may not be not efficient because of the weather instability. Relatively mild, short winter seasons do not present good selection pressure [2]. The success rate of conventional methods in screening cold tolerance of important crop plants is very low. This may be due to complexity of stress tolerance traits, low genetic variance of yield components due to stress and lack of proper selection criteria [3]. There is a great need to develop alternative strategies to evaluate cold tolerant cultivars.

Cold Tolerance in Rice

Low-temperature stress is common for rice cultivation in temperate zones and high elevation environments. One of the important breeding objectives of rice cultivating areas is to develop the cultivars which can tolerate low temperature stress at critical growth stages [4]. The ability to germinate and survive under chilling temperature is the pre-requisite for success of a crop. Selection for chilling tolerance during germination and early seedling growth in rice are important breeding objectives [5]. Breeding cold tolerant varieties in rice is essential to reduce yield loss and also for sustainable productivity. Breeding demands genetic variability. Rice (*Oryza sativa* L.) shows wide adaptability to cold and cold-tolerant ecotypes are available for breeding. The cultivated species *O. sativa* L. has two subspecies, *indica* and *japonica*. 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 [6,7], as well as at the vegetative and reproductive stages [8]. *Japonica* genotypes presented higher cold tolerance at the germination stage than *indica* genotypes, although they found variability for this trait within both subspecies. Identification of genotypes with desired degree of tolerance to low temperature stress has become very important for recommendation of suitable varieties to the farmers and also for utilization in the breeding programs [9]. In some of the studies on diallel analysis of cold tolerance in rice, the low tolerance is attributed to the vegetative stage. The mode of inheritance is also very important in defining the selection strategy to be applied in a breeding program [10]. Low temperature rice is controlled by four or more dominant genes. In some cases of cold tolerance both the additive and non-additive effects are involved, though there is transgressive segregation and absence of maternal effect. In the breeding process for cold tolerance at the germination period, evaluation of the percentage of reduction in coleoptile length and of coleoptiles regrowth is indicated for the identification of cold tolerant genotypes, to be used as genitors. Low temperature resistance in plant is a very complex trait, involving many different metabolic pathways and cell compartments [11]. In order to identify the genetically best line for cold stress, environmental variations are also one of the limiting factors. It also effects the evaluation of germination trait [12]. It was also reported that diallel analysis showed that both additive and non-additive gene action were involved in cold tolerance traits at germination stage, such as percentage of reduction in coleoptile length and coleoptile growth, while the non-additive action was relatively more important for them. A good selection method to evaluate cold tolerance in segregating populations by the use of controlled air or water temperature is essential. Genetic basis of cold tolerance also effects the breeding aspect for low temperature stress cultivars. However, according to some studies, among the various growth stages of rice, the varieties with high germination and seedling vigour under low temperatures show more tolerance at booting and flowering stage [13]. Suh *et al*. (2010) [14] have developed a reliable method of phenotyping for cold tolerance by imposing cold-water irrigation on all growth stages in the field and cold-air temperature in cold tolerance. In order to overcome the limitations associated with breeding cold tolerant cultivars, some programmes have experimented selection with cold water under field conditions with many populants and thousands of plants per population were evaluated [15]. Low temperatures below 19°C at the booting stage in particular, from panicle formation to flowering, severely impacts male sterility and cause a decline in yield. Boosting the cold tolerance at the booting stage is thus one of the key targets in rice breeding [16]. In rice, if cold temperatures, coincide with the reproductive stage, it leads to seed sterility, resulting in reduced yield and decreased quality of the grain. The fertilization stage, ranging from pollen maturation to the completion of fertilization, is sensitive to unsuitable temperature. In areas with low temperatures, the main objective of the rice breeding programme is to improve cold tolerant rice cultivars at reproductive stage. Even though cold temperature affects rice growth from seed germination to seed maturity, episodes of cold temperature at the reproductive phase decrease the seed set [17]. It is reasonable to consider that tolerant genotypes, evolved under strong selection pressure, may rely on diverse gene products on different growth stages to ensure cold tolerance [18]. To overcome the problem of damage caused by low temperature, efforts are to be made by rice breeders to develop more cold-tolerant cultivars mainly at seedling stage [19]. Low temperature effects the reduction in radicle, coleoptile length, germination percent, percent decline in germination, coleoptiles growth, radical regrowth after the cold period in rice genotypes [20]. Low H_2O_2 and low MDA contents along with lower SOD and CAT activities in rice root could be the biochemical traits of cold responses in rice seedlings [21].

Screening Techniques to Measure Cold Tolerance in Rice

According to the methods of evaluation of rice genotypes for cold tolerance available in the literature, seeds are subjected to temperatures varying from 10 °C to 25 °C for periods of three to thirty-five days and characteristics most commonly measured are germination percentage, speed of germination, coleoptile length and radicle length. Cruz and Milach (2004) [2] proposed the following method for estimating the cold tolerance in two experiments. In experiment I, seeds were germinated under two conditions; 28 days in 13°C and seven days in 28°C and in experiment II, seeds were germinated at 72 hours with 28°C, 96 hours at 13°C and again 72 hours at 28°C. In experiment I, percentage of reduction in radicle and coleoptile length and germination index were measured. Sharifi (2010) [22] used the following method for evaluation of sixty-eight rice germplasm for cold tolerance at the germination stage in laboratory. Seeds of rice germplasm germinated at three different temperatures containing low temperature (constant 13°C for 28 days), alternative temperature (a temperature cycle of 12 h at 20°C and 12 h at 23°C for 14 days) and control (constant 26°C for 7 days). Temperature shows a great significant effect on germination rate, coleoptile length and radical length in Analysis of variance studies. Low temperature at various levels shown great variation in germination rate. Rice germplasm tested for low temperature tolerance shoed a reduction in germination rate, reduction in the length of coleoptile and radicle. Normal seeds germinated within 36 hours after imbibition and completed germination on the 7th day in control, but low temperatures and alternative temperatures treatments delayed the germination. When compared to the control, the low and alternative temperature treatments delay the growth of coleoptile and radicle., also the average lengths of the coleoptile and radicle strongly inhibited in all the tested germplasm. The screening of genotypes for cold tolerance at seedling stage is the primary requirement of Rabi / summer season cultivars as seedlings are raised during the cold months of December and January. Under low temperature, the rice seedling growth was slower.

Cold Tolerance and Effects of Cold in Rice

In rice, degree of injury usually depends on the time, severity and duration of the low temperatures at the growth stages. It was further reported by Ye *et al*. (2009) that low temperature has the potential to affect growth and development of rice plants during any developmental stage from germination to grain filling. In earlier studies, exposure of maize seedlings to a temperature of 15°C or below was reported to seriously reduce photosynthetic activity of the leaves [23], alter composition of leaf pigments [24] and also affect chloroplast development [25]. Spikelet sterility may result from pollen abortion due to cold during microsporogenesis, when pollen grains are being formed, at the booting stage [26]. Two oat cultivars, one tolerant and another sensitive, in relation to the physiological aspects involved during germination under cold temperature (3°C) and demonstrated that higher metabolic rates and less oxidative damage were the adaption mechanisms of the tolerant genotype to cold temperature during germination [27]. According to Cruz and Milach (2000) for transferring cold tolerance from different sources to locally adapted cultivars requires the presence of the selective agent; low temperature is essential. However, its abiotic nature makes it unpredictable under field conditions in terms of its intensity, duration and timing, limiting field selection for cold tolerance in rice. Variation in environmental effects is one of the limiting factors against evaluation of germination trait in order to identify genetically best lines for cold stress conditions. To enhance uniform establishment of rice seedlings, cold tolerance during early stage is vital [28]. Low temperature often affects plant growth and crop productivity, which causes significant yield losses in rice [29]. Low temperature stress is the major factor which affects the growth and development of rice. Cold stress results in seedling injuries, delayed heading and yield reduction. Low temperature stress at early growth stages affects seed germination, inhibits seedling establishment and leads to non-uniform crop maturation [30]. Rice growth in the temperate regions is constrained by the limited period that favours growth where it needs optimum temperature between 25°C to 35°C and temperatures below this often result in poor seedling vigour [31]. It was also reported that cold stress delays phenological development and increases spikelet sterility, resulting in low yield [32,33]. In maize (*Zea mays* L.) low temperature inhibits seedling growth, impairs chloroplast function and reduces photosynthetic capacity, leading to reduced yield [34-36]. Good cold tolerance at the seedling stage is an important character for stable rice production, especially in direct seeding fields. Coleoptile length, radicle length and germination rate seem to be the most adequate characteristics to be used to evaluate cold tolerance during the seed germination period in rice. Rice is a sensitive plant to low temperature stress, under which germination and emergence can decrease and consequently lose yield [37]. Cold stress is a major constraint in many crops of tropical origin. In rice, early season low temperatures have been reported to reduce germination [38] and stand establishment [39,40]. Rice is a cold sensitive crop that has its origin in tropical or sub-tropical areas and low temperatures dramatically reduce its production and low temperature at the early stages of development inhibits germination, seedling establishment due to which the crop establishment will be non-uniform [41]. With global climate change, most rice growing regions are experiencing more extreme environmental fluctuations and is susceptible to a variety of abiotic stresses including cold and heat [42]. This was supported by Farzin *et al*. (2013) [43] stating rice plant is sensitive to low temperature stress. The synthesis of chlorophyll in leaf of plant is an important parameter that limits the photosynthetic activity. Chilling sensitive plants response to low temperature through decreased photo synthesis [44]. Datta and Pathak (2007) evaluated 53 rice varieties and concluded that the varieties did not show tolerance to low temperature stress at different stage of growth indicating that different gene systems might be involved with the varietal response to low temperature stress at different growth stages needing use of multiple parental sources for breeding varieties with tolerance to low temperature stress at different stages of growth and all the varieties proved to be tolerant at post germination and seedling stage. Temperatures lower than 20°C decrease both the speed and percentage of germination in rice [45]. In a study on cold tolerance in rice genotypes stated that cold temperatures late in the season also have been shown to reduce yield by directly affecting reproductive processes. In rice, low temperatures after panicle initiation result in male sterility through triggering degradation of proteins in anther cells. During the grain filling stage, chilling temperature may cause delayed and incomplete grain maturation.

In a study proposed that reproductive-stage cold stress severely affects spikelet fertility of temperate rice cultivars, reduces culm length and delays heading. These are the traits which are highly correlated and required for the cold tolerant varieties to be developed in temperate regions and high-altitude regions. The crucial stage for spikelet fertility and yields is the reproductive stage. Somehow, abiotic stress conditions such as drought, salinity, low temperature effect the plant traits and the spikelets become sterile. Perfect phenotyping is also significant to identify the cold tolerant genotypes at reproductive stage [46].

Cold stress or Low temperature at reproductive stage is the main constraint of temperate *japonica* rice production and affects rice cultivars by delaying vegetative growth and heading, reducing spikelet fertility, and affecting grain quality. Low temperature stress is one of the major abiotic factors which reduce rice yield in several countries [47]. The yield losses due to cold can range from 0.5 to 2.5 t ha^{-1} [48] and grain yields can drop by up to 26%, mostly due to low temperature during the reproductive stage, even though cold temperature can be harmful during the entire developmental stage of rice plants, from germination to grain filling.

In rice, study of the early growth stages, the occurrence of low temperature stress inhibits germination, seedling establishment and eventually leads to non-uniform crop maturation. Drastic reduction in coleoptile and radicle growth was observed with decreasing temperature. Germination rate was severely limited at 13°C in comparison to the control. The low germination response under the low temperature treatment (13°C) suggests that this temperature is near the critical temperature for germination of rice. Temperature upper to 23°C was the optimum temperature for germination. Fertilization stage is known to be the most sensitive to temperature stress in several crop plants [49]. Exposure to cold temperature affects all phenological stages of rice and lower grain production and yield, too. Low temperature in vegetative stage can cause slow growth and reduce seedling vigor [50], low number of seedlings, reduced tillering, increase plant mortality [51], increase the growth period [52] and in reproductive stage, it can cause panicle sterility and lower grain production and yield. Temperature conditions place a major limit on plant productivity. Excessively hot or cold temperature conditions cause physiological disorders in plant development, which result in yield reductions. Continuous improvements in the tolerance of plants to unsuitable temperature in plant breeding programs have been performed using various strategies that have successfully produced many beneficial achievements [53,54]. However, unsuitable temperature stress still periodically decreases plant productivity. Furthermore, current speculation about climate change suggests that most agricultural regions will experience more extreme environmental fluctuations.

Abiotic stresses adversely affect growth, productivity and trigger a series of morphological, physiological, biochemical and molecular changes in plants. It is a major environmental factor that limits the agricultural productivity of plants. The responses of the rice seedlings to low temperature stress were also evaluated by measuring electrolyte leakage (EL), chlorophyll content and the concentration of four compounds associated with stress response in plants: *viz*., proline (osmoprotectant), malondialdehyde (lipid peroxidation), reduced glutathione (antioxidant) and ascorbic acid (antioxidant) also adversely affect growth and productivity [55].

Cold-temperature stress at seedling stage significantly reduced seedling vigour and dry weight and also delayed time to flowering and maturity. Cold stress interferes with photosynthesis, by inducing chlorophyll degradation or undermining chlorophyll biosynthesis. As a result, both days to flowering and maturity may be delayed [56]. Cold temperatures late in the season also have been shown to reduce yield by directly affecting reproductive processes. Moreover, low temperatures during the flowering stage also have been reported to predispose crops to other yield-limiting factors. Low temperature stress at flowering appears to be more detrimental to yield, with kernel number and kernel weight seriously affected. Future studies need to focus on examination of the specific reproductive mechanisms that are sensitive to cold stress.

In a study, screened twenty-two rice genotypes during winter for cold tolerance ability at seedling stage, and to find the morphological and biochemical parameters, which are helpful to screen rice genotypes for cold tolerance. The seedling vigour, reducing sugar, phenol content and soluble protein exhibited highly significant correlation with cold tolerance as well as among themselves [57]

Cold stress is a common problem in rice cultivation and affects global production as a crucial factor [58] and low temperature can reduce up to 25% of the final yield in rice genotypes [59]. In a study on screening cold tolerance in rice stated low temperature stress is one of the serious environmental stresses affecting plant growth and development. Germination and seedling establishment are sensitive growth stages for rice to cold stress. Even though temperature does not prevent rice germination, it delays beginning and consequently, plant emergence. On top of that, cold stress causes seedling mortality and spikelet sterility and eventually, significant yield losses. Study shoes that exposure to low non- lethal temperature usually induct a variety of biochemical, physiological and enzymatic changes in plant. This can result in an acclimation response that is characterized by a greater ability to resist injury or survive an otherwise lethal low temperature stress [60]. Cold temperature during the reproductive phase leads to seed sterility, which reduces yield and decreases the grain quality of rice. The fertilization stage, ranging from pollen maturation to the completion of fertilization, is sensitive to unsuitable temperature. Low temperatures can have negative impacts on rice plants during germination, vegetative growth and reproductive stages. It was also further stated that abiotic stresses directly or indirectly affect the physiological status of rice and negatively alter its overall metabolism, often with impacts on grain yield. Low temperatures can have negative impacts on rice plants during germination, vegetative growth and reproductive stages. 12 rice genotypes for evaluation of cold tolerance at the germination stage and the results showed that percentage of reduction in coleoptile length by cold temperature and germination index helped in better identification of tolerant genotypes and demonstrated that the influence of low temperature on germination stage occurs in the phases of coleoptile and radicle activation and growth. Rice yields are low during rabi season when compared to *kharif* due to cold effect at seedling stage especially in Telangana state and the low temperature vary from 8°C to 16°C starting from December to first fortnight of February, during which the crop will be at seedling stage, the most sensitive phase which seriously affect the yields of rice crop [61]. Some agronomic characters, such as seedling vigour (for seedling stage), tillering ability (for tillering stage) and spikelet fertility (mature grain stage) are useful for evaluating the cold tolerance of rice plant. In a study where two levels of temperature 13°C and 32°C were used, revealed that low temperature had a significant effect on yield in rice and the reduction in yield in relation to temperature stress varied significantly in tolerant and sensitive cultivars [62]. Priyanka *et al*. (2015) [63] screened 25 rice genotypes at low temperature during seedling stage. The results revealed that low temperature during seedling stage showed reduction in seedling number in the nursery. A positive correlation was obtained between the laboratory and field screening at the seedling stage indicate the cold tolerance ability of the genotypes and laboratory studies can be relied upon to screen large number of genotypes, if there is a land constraint [64-71].

Conclusion

Low temperature is a great hinderance for growth of the rice plant. Low temperature stress effects the rice plant at various stage such as germination stage, flowering and yield. So, there is a great need to develop screening technologies of rice genotypes at low temperatures. Considering the yield traits, cold tolerant varieties to be developed for timely growth, to avid damage of seedlings due to cold stress and to achieve good yields.

Application of research: Study of screening of the cold tolerant rice genotypes at different growth stages.

Review Category: Genetics and Plant Breeding

Abbreviations: CAT: Catalase, EL: Electrolyte leakage
MDA: Malondialdehyde, SOD: Superoxide Dismutase

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