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Screening techniques to measure cold tolerance in rice

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Abstract
Rice (*Oryza sativa* L.) is a major source of carbohydrate and staple food of more than 60% people in the world. It is cultivated in more than 100 countries in the world, but more than 90% of world's rice is grown and consumed in Asia. In world's increasing population and to meet the challenge of ever increasing demand for food globally, it is necessary to develop high yielding rice varieties that are tolerant to abiotic stress conditions, such as drought, salinity and cold. The productivity of rice is low in Northern districts of Telangana during Rabi. In this zone quite often the temperature drops below 10^0C during December and January resulting in poor growth of seedlings a of rice crop. Germination and seedling establishment are sensitive in rice. Cold temperature during seedling establishment (October to early November) drops to about 10 ^{o} C and such low temperature significantly reduces seedling growth and establishment (Humphreys *et al.*, 1996). For breeding cold tolerant rice variety selection of tolerance requires a suitable and cost effective evaluation and screening technology. Breeding cold tolerant varieties in rice is essential to reduce yield loss during winter or Rabi season, for which information on various traits and QTLs contributing to tolerance is necessary. Field Evaluation for cold tolerance during seedling stage under field conditions is limited by environmental variation, which makes it difficult to identify genetically superior lines. Screening for cold tolerance can be done through field evaluation, as well as laboratory screening and QTLs mapping. QTLs mapping was used to confirm the presence and absence of cold tolerant QTLs in the rice genotypes. In this SSR molecular marker was used and many QTLs have been reported which contribute tolerance against cold stress at different stages of growth viz., germination, seedling, booting, bud bursting and reproductive stages etc.

Keywords: cold tolerance, germination, screening technique, seed yields

Introduction
Rice is subjected to wide ranges of biotic and abiotic stress factors which cause a decline in yield. Low temperature stress is an important factor affecting the growth and development of rice in temperate and high-elevation areas. To meet the challenge of ever increasing demand for food globally and to achieve food security in the country, the present production level need is to be increased by two million tonnes every year. It is estimated that 106.10 million tonnes of rice will be produced by 2016 (INDIASTAT, 2016). Low temperature shows different effects on germination, seedling, vegetative, reproductive and grain maturity and weakens photosynthetic ability by inducing leaf discoloration, reduces plant height, produces degenerated spikes, delays days to heading, reduces spikelet fertility and poor grain yield. In the decade since these reviews, there has been a large volume of published work and significant progress has been made in understanding seedling cold tolerance. However, there were some potential sources of confusion that have been reported in the literature. Low temperature during the reproductive stage, even though cold temperature would be harmful during the entire developmental stage of rice plants, from germination to grain filling (Ye *et al.*, 2009) [36]. Cold temperatures late in season reduce yield by directly affecting reproductive processes (Nahar *et al.*, 2009) [19].

What is cold tolerance and how it is related to germination
Cold tolerance in rice
Nakagahra *et al.* (1997) [20] suggested that in high elevation environment and temperature zones low-temperature stress is common for rice cultivation. An important breeding objective is to develop cultivars tolerant to low temperatures at critical growth stages in this region. 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* sub species 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 (Takahashi, 1997) [33]. Rice production in Korea, air temperature is maximum 13°C for 150 days.

Too cool air and water temperatures adversely affects crop establishment, while low air temperature by itself affects the rice plant at the reproductive and grain-filling stages. Since then, it has been strengthened, particularly when the Tongil types (*Indica/Japonica* hybrids and high-yielding rice) were found to be highly susceptible to low temperatures. Research findings shows that critical low temperatures can be damage germination to maturity stage. The critical temperature for rice is usually below 20°C and varies according to growth stage, for example, for germination, the critical temperature is 10°C and for the reproductive stage, it is 17°C (Li *et al.,* 1981; Mackill and Lei, 1997) [17, 18].

Twenty four rice genotypes were studied, 12 belonging to *Japonica* and 12 to *Indica* subspecies, because they have different origins and represent a wide range of variability within the rice germplasm. These Six genotypes are (five tolerant and one sensitive) in which observation of their field performance under cold weather. The *Indica* species, six genotypes are (BR-IRGA 409, BR-IRGA 410, IRGA 416, IRGA 417, BRS 7 – Taim, and El Paso 144). Here two condition are taken: first was 13°C for 28 days (cold) and 28°C for seven days (control) and second was 28°C for 72 hours, 13°C for 96 hours and again 28°C for 72 hours, in randomized block design with a three replication. Observation were recorded, genotypes of the *Japonic*a subspecies recorded higher cold tolerance than *Indica,* but showed in variability within subspecies. The most adequate method of evaluation of cold tolerance is through percentage of reduction in coleoptile length and coleoptile regrowth. These *Japonica* genotypes likes Quilla 64117 and Diamante were highest cold tolerance than *Indica,* cultivars BR-IRGA 410 and IRGA 416 were the most cold tolerant at the germination stage. During the early growth stages of rice, the occurrence of low temperature stress reduces germination, seedling establishment and eventually leads to non-uniform crop maturation. Good cold tolerance at the seedling stage is an important character for stable rice production, especially in direct seeding fields (Cruz and Milach, 2004) [5].

The variation in environment effects is one of the limiting factors against evaluation of germination trait in order to identify genetically best lines for cold tolerance. The diallel analysis showed involvement of both additive and non-additive gene action in cold tolerance traits at germination stage, Quilla 66304 genotyes has crosses aimed at increasing cold tolerance at the germination stage because high general combining ability for both percentage of reduction in coleoptile length and coleoptile growth. Generation mean analysis was also performed for coleoptile growth in six cold-sensitive x cold-tolerant crosses and concluded that non-additive effects were due to dominance and epistatic interactions. Though broad sense heritability values were high, the relative importance of the non-additive effects suggests that selection should be applied in advanced generations of the breeding program (Cruz *et al*., 2006) [6].

A good selection method to evaluate cold tolerance in segregating populations by the use of controlled air or water temperature is essential. Besides a precise selection method, the genetic basis of cold tolerance also affects the breeding for this trait. Correlations in cold tolerance among different growth stages for rice have been reported, and it was suggested that varieties with high germination and seedling vigour under low temperature conditions are also likely to be more tolerant to low temperature exposure at the booting and flowering stages (Ye *et al.,* 2009) [36].

The 17 rice varieties were taken in this experiment found the low temperature and cold tolerance were correlation with different growth stage of varieties. This correlation suggests that varieties with high germination and seedling vigour under low temperature conditions are more tolerant to low temperature exposure at the booting and flowering stages. As it is easier to evaluate cold tolerance during the early growth stages (germination and seedling stages) than at the reproductive stages, evaluation of cold tolerance at the germination and seedling stages can be more effective mechanism to improving cold tolerant rice varieties (Suh *et al*., 2010) [14]. Shinada (2013) [28] stated that 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. Improving cold tolerance at the fertilization stage is an important objective of rice breeding program in cold temperature areas. Even though cold temperature affect rice growth from seed germination to seed maturity, episodes of cold temperature at the reproductive phase decrease the seed set. The temperature is an abiotic and unpredictable factor and, therefore, the negative effects of its occurrence on the rice are difficult to control at the management level, which makes the genetic tolerance of the cultivars extremely important to stabilize the grain yield in the areas subject to the occurrence of cold.

In Chhatisgarh, rice are grown in month of December or January in that season due to low temperature poor seedling establishment, stunting, yellowing and mortality and exhibit various symptoms of chilling injury such as chlorosis, necrosis or growth retardation. To overcome the problem breeding method are used to developed cold tolerance varieties. Evaluation cold tolerance genotypes in seeding stage (Smita *et al.,* 2014) [29].

Limitations associated with conventional breeding for cold tolerance in rice

Selection of cold tolerance varieties in field condition is difficult, need to use controlled conditions which is control over the intensity and duration of the low temperature. In addition, selection for cold tolerance will only be effective if appropriate selection pressure is used, which makes critical stress environment control (Blum, 1988) [2]. This breeding program is not accurate method to screening cold tolerance varieties in field condition, combination of breeding with molecular genetics is more reliable technique to improving cold tolerance genotypes.

To overcome cold tolerance has various approaches have been tested to produce stress tolerant plants by the use of classical genetic methods as well as improved plant breeding techniques. However, the strategy of gene transfer to crop plants from their more tolerant wild relatives using classical genetic methods has been of limited success (Flowers and Yeo, 1995) [9]. Based on this Wu and Ho (1999) [34] have proposed conventional breeding is a very slow process for generating cold tolerance crop varieties with improved tolerance to stress conditions. Incompatibility in crosses between distantly related plant species and limited germplasm resources for stress tolerance are also barriers encountered in conventional breeding. Integration of compatible molecular biological methods with conventional breeding programs accelerates the process of varietal improvement of rice.

Andaya and Mackill (2003) screening for chilling sensitivity of different rice genotypes in breeding programmes commonly relies on visual observations under 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. However, Variation in environmental effects was reported as one of the limiting factors against evaluation of germination trait in order to identify genetically best lines for cold stress conditions (Cruz *et al.*, 2006; Blum, 1988 and Revilla *et al.*, 2000) [6, 2, 23]. Review research progress on Selection of cold tolerant genotypes under field conditions is employed in countries like (Heu and Bae, 1972) [12] and United States (Carnahan *et al.*, 1972) [3], but is not efficient due to the instability of weather. Relatively mild, short winter seasons do not present good selection pressure (Cruz and Milach, 2004) [5].

Gulzar *et al.*, (2011) [11] presented that conventional breeding methods have met with limited success in improving the cold tolerance of important crop plants involving inter-specific or inter-generic hybridization. The conventional breeding approaches are limited by the complexity of stress tolerance traits, low genetic variance of yield components under stress condition and lack of efficient selection criteria. It is important, therefore, to look for alternative strategies to develop cold stress tolerant crops. Whereas, For selection of appropriate parent for cold tolerance breeding, evaluation of cold tolerance at reproductive is important due good selection approach and general combing ability to be ideal for improving tolerance varieties as relative quickly but identify cold-tolerance related QTL that performed at the reproductive stage, examination of spikelet fertility after CDWI is particularly useful because it's experiment was conducted in field. metabolic parameters are mainly responsible for cold changes in metabolism and enzyme activities are useful for revealing the role of genes that function in the cold-sensing and cold-response networks. Genetic variability for cold tolerance among wild rice lines was also studied (Suh *et al*., 1997) [31], and three QTLs (quantitative trait loci) responsible for low temperature germinability were mapped for a population resulting from a cross between a wild and a cultivated line (Suh *et al.*, 1999) [30].

Techniques to measure cold tolerance in rice

Cruz and Milach (2004) [5] proposed that evaluation of cold tolerance at germination stage, 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 was measured. In experiment II, radicle and coleoptile regrowth after the cold period were measured to identify tolerant genotypes found that the percentage of reduction in coleoptile length and coleoptile regrowth allowed a better distinction between the tolerant checks and the susceptible. This most important dequate method of evaluation of cold tolerance is through percentage of reduction in coleoptile length and coleoptile regrowth. Sharifi (2010) [27] suggested 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 13ºC for 28 days), alternative temperature (a temperature cycle of 12 hrs at 20 ºC and 12 hrs at 23ºC for 14 days) and control (constant 26ºC for 7 days). Analysis of variance revealed that temperature had a significant effect on germination rate, coleoptile length and radicle length. Germination rate was strongly affected by the low temperature and alternative treatments. Stress of low temperature at the germination stage on the tested rice germplasm caused a reduction in final germination rate and the lengths of coleoptile and radicle. The normal and healthy seeds began to germinate within 36 hrs after imbibition and germination was completed on the 7th day in the control, but delayed under the low and alternative temperature treatments. The low and alternative temperature treatments delayed the growth of coleoptile and radicle compared to the control and the average lengths of coleoptile and radicle were strongly inhibited with the decreasing temperature in all of the tested germplasm.

Sahu *et al*. (2014) [24] explained that among the tested genotype was laid RCBD with two replication and 54 treatments conducted in laboratory. Genotypes is HHZ 5-DT1- DT1 had 0.8 cm root length at 15^{0}C and its germination percentage is also 86.66 %, therefore this genotype is supposed to be good for summer paddy since it has cold tolerance. They also observed that the root and shoot length were similar in most of the genotypes at 15^0 C as well as 28 ^{0}C. The maximum shoot length recorded was 9.3 cm in HHZ 12-SAL 8-Y1-SAL1 genotype. The genotypes which showed 100 per cent germination were IR 10C132 followed by IR 10C153, HHZ 8- SAL6-SAL3-Y2 and HHZ 17-DT6-Y1-DT1. These genotypes exhibited tolerance for cold temperature for germination. The genotypes which comes under 40 to 80 per cent germination can be considered as tolerant for cold temperature. Whereas genotypes HHZ 5-Y3-Y1-DT1, HHZ 5-DT20-DT2-DT1 and HHZ 12-SAL 8-Y1-Y2 that recorded germination percentage below 40 percent are considered as susceptible for germination under cold and not recommended for growing.

Seed yield and quality components in relation to cold tolerance in rice

Satake and Hayase (1970) [26] found that cool temperature main cause sterility of the spikelets, is the most common symptom of injury, the phase of microscopic, increasing rate respiratory activity, protein and free proline has reduced due low temperature, when cold coincides with the reproductive stage of the rice plant but incomplete panicle exertion and spikelet abortion may also occur. Whereas, upper part of the panicle is more susceptive cool temperature than lower part at booting stage (Nishiyama, 1981)

Cellular membrane is considered the main target for cold damage and the primary cause of other metabolic disturbances observed within cells and the reduction in coleoptile growth during these phases may be attributed to the direct effect of cold temperature on cellular elongation and division, or to its indirect effect leading to a metabolic imbalance (Lyons, 1973). Exposure of chilling-sensitive plants to low temperatures leads to disturbances in all physiological processes – water regime, mineral nutrition, photosynthesis, respiration and metabolism. Inactivation of metabolism observed at chilling of chilling-sensitive plants is a complex function of both temperature and duration of exposure. Response of plants to low temperature exposure is associated with a change in the rate of gene transcription of a number of low molecular weight proteins, which highlighted the leading role of oxidative stress in the induction of stress response. To overcome this problem we have to improve cold tolerance considering other factors likes the thermal effect, chemical treatment and the use of gene and cell engineering (Jouyban *et al.*, 2013) [15]. The reproductive stage when evaluated under controlled temperature revealed that the effect of cold temperatures was conducted at 17°C for varying (three, five, seven and ten days) at two reproductive stages (microsporogenesis and anthesis). Cold tolerance was measured as the percentage of reduction in panicle exsertion and in spikelet fertility. Evaluating cold tolerance through the reduction in panicle exsertion found that when the reduction in spikelet fertility was considered, a minimum of seven days was required to identified the cold tolerance from cold sensitive. Genotypes was more sensitive in anthesis, screening procedure done at this stage only, since it is easier to determine. Rice cold tolerance at the reproductive stage may be characterized by the reduction in spikelet fertility due to cold temperature (17°C) applied for seven days at anthesis (Cruz *et al*., 2006) [6].

Critical stages for cold damage include germination, booting, flowering, and filling stages. Since the most sensitive stage for cold harm is the flowering stage, which occurs 10–12 days prior to heading, a study was conducted by completely randomized design (CRD) and treatment included 5 varieties with three replication and include cultivars were shirudi, fajr, local tarom, hybrid, and line 843. Two levels of temperatures (13°C, stress temperature) and (32°C, normal temperature, control) along with flowering stage were selected as two sub-factors. Three seedlings were planted in each plot. The cold stress was done in flowering stage with holding pots at 13°C for 15 days with relative humidity was maintained constantly between 70 and 80 percent by wetting bottomless sack of greenhouse. cultivars performed with least percentage yield (19%) is shirudi variety and the most sensitive one with most percentage of yield decrease (29%) was local tarom variety due less number of panicles, the length of panicle, and the number of full, empty, and total grains; as a result, yield had caused significant reduction (Ghadirnezhad and Fallah. 2014) [10].

Kaneda and Beachell (1974) [16] reported low temperature effects on seedlings can be manifested as poor germination, slow growth, discolouration or yellowing, withering after transplanting, reduced tillering and stunted growth. Peterson *et al*. (1974) [22] cold temperature is an important stress that results in delayed heading or maturation and yield reduction due to spikelet sterility. Rice cultivars vary greatly in their tolerance to low temperature. Yoshida (1981a) [37] germination is divided into three phases: imbibition, activation and post-germination growth. The effects of cold temperature during germination seem to be associated with the imbibition phase, considered the most sensitive. Cold temperature during this phase leads to increasing escape of solutes from the seeds, such as amino acids and carbohydrates, which has attributed to the incomplete plasma membrane of the dry seed and to the disturbance caused on its reconstruction during imbibition phase by cold temperature. He also demonstrated that the most influence of temperature on germination stage occurs in the phases of coleoptile and radicle activation and growth. Datta and Pathak (2007) [8] 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. They concluded that need to 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. Xu (2008) [35] suggested that temperatures lower than 20 °C decrease both the speed and percentage of germination in rice. Jena *et al.* (2010) [14] proposed that reproductive-stage cold stress severely affects spikelet fertility of temperate rice cultivars, reduces culm length and delays heading. These traits are highly correlated and are required for the development of cold-tolerant rice cultivars for temperate as well as high-altitude regions around the world. Reproductive stage is critical for spikelet fertility and ultimately yield of rice cultivars. However, during abiotic stress conditions like drought, salinity and low temperature, the major plant traits are affected and eventually the spikelet becomes infertile. Lack of perfect phenotype for cold tolerance at reproductive stage makes it difficult to correctly identify cold-tolerant genotypes.

Sarkar *et al*., (2013) [25] observed screening on 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 leads to significant yield losses. Exposure to low non- lethal temperature usually induct a variety of biochemical, physiological and enzymatic changes in plant, which can result in an acclimation response that is characterized by a greater ability to resist injury or survive an otherwise lethal low temperature stress.

Conclusion and future research need

From various researches, it can be concluded that screening cold tolerance varieties at germination stage as well as at reproductive stage were adversely affect yield attributes. In laboratory, screening is more reliable as the intensity and duration of cold stress can be adjusted and laboratory tests eliminate the chances of interference of other biotic and abiotic factors. Due Variation in weather condition is an important limiting factor in field screening. However the lab results are to be verified in the field to identify the effect of cold stress during different growth stages of rice and its effect on the seedling and subsequent stages.

Validation of cold tolerance observed in the laboratory with that of field performance for estimating early seedling cold tolerance, during *Rabi* with more number of genotypes in a wide range of temperatures. Validation of seed and seedling stage cold tolerance by expose the plants to cold sowing in late *Kharif*. Identification of QTL's for traits important during seed, seedling and reproductive stage cold tolerance and Marker Assisted Selection for hastening up breeding programme. hence cold tolerances screening techniques is most valuable and viable for validation of germination of seed and seedling.

References

1. Andaya VC, Mackill DJ. Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. Journal of Experimental Botany. 2003; 54:257-2585.
2. Blum A. Plant breeding for stress environments. Boca Raton: CRC Press, 1988, 99-132.
3. Carnahan HL, Erickson JR, Masten broek JJ. Tolerance of rice to cool temperatures–USA. In: International Rice Research Institute. Rice breeding. Los Banos: IRRI, 1972, 535-540.

4. Cruz RP, Milach SCK. Breeding for cold tolerance in irrigated rice. Cienc Rural. 2000; 30:909-917.
5. Cruz RP, Milach SCK. Cold tolerance at the germination stage of rice: methods of evaluation and characterization of genotypes. Science Agriculture. 2004; 61:1-8.
6. Cruz RPD, Milach SCK, Federizzi LC. Inheritance of rice cold tolerance at the germination stage. Genetics and Molecular Biology. 2006; 29(2):314-320.
7. Cruz RP, Sperotto RA, Cargnelutti D, Adamski JM, Terra TF, Fett JP. Avoiding damage and achieving cold tolerance in rice plants. Food Energy Secure, 2013, 96-119.
8. Dutta D, Pathak PK. Response of Assam rice varieties to low temperature stress. Journal of Plant Genetic Resources. 2007; 20(1):57-63.
9. Flowers TJ, Yeo AR. Breeding for salinity resistance in crop plants. Australian Journal of Plant Physiology. 1995; 22:875-884.
10. Ghadirnezhad R, Fallah A. Temperture effect on yield and yield componenets of different rice cultivars in flowering stage. International Journal of Agronomy, 2014, 1-4.
11. Gulzar SS, Shabir HW, Wasim H, Singh NB. Engineering Cold Stress Tolerance in Crop Plants. Current Genomics. 2011; 12:30-43.
12. Heu MH, Bae SH. Selection for lines of rice tolerant to low temperature in Korea. In: International Rice Research Institute. Rice breeding. Los Banos: IRRI, 1972, 533-534.
13. Humphreys L, Sides R, Fattore A. Rice establishment. Farmers' News Letter Large Area. 1996; 147:30-31.
14. Jena KK, Kim SM, Suh JP, Kim YJ. Development of cold tolerant breeding lines using QTL analysis in rice. Second African Rice Congress, Bamako. Innovation and partnership to realize Africa’s rice potential, 2010.
15. Jouyben KK, Kim SM, Suh JP, Lee CK, Lee JH, Kim YG. QTL mapping and development of candidate gene-derived DNAmarkers associated with seedling cold tolerance in rice (Oryza sativa L.). Molecular Genetics & Genomics. 2013; 289:333-343.
16. Kaneda C, Beachell HM. Responses of indica and japonica rice hybrids to low temperatures. SABRAO Journal of Breeding and Genetics. 1974; 6:17-32.
17. Li TG, Visperas RM, Vergara BS. Correlation of cold tolerance at different growth stages in rice. Acta Botanica Sinica. 1981; 23:203-207.
18. Mackill DJ, Lei X. Genetic variation for traits related to temperate adaptation of rice cultivars. Crop Science. 1997; 37:1340-1346.
19. Nahar k, Biswas JK, Shamsuzzaman AMM, Mirza H, Barman HN. Screening of Indica rice (*Oryza sativa* L.) genotypes against low temperature stress. Botany Research Interanational. 2009; 2(24):295-303.
20. Nakagaha JS, Seshu DV. Breeding strategy for cold tolerant rices. In: Rice Cold Tolerance Workshop, Los Banos, International Rice Research Institute, 1997, 91-99.
21. Nishiyama I. Male sterility caused by cooling treatment at the young microspore stage in rice plants. XVIII. Some enzyme activities in anthers during and after the cooling. Japanese Journal of Crop Science. 1918; 47:551-556.
22. Peterson ML, Lin SS, Jones D, Rutger JN. Cool night temperatures cause sterility in rice. i. 1974; 28:12-14.
23. Revilla P, Malvar RA, Cartea ME, Butron Aand, Ordas A. Inheritance of cold tolerance at emergence and during early season growth in maize. Crop Science. 2000; 40:1579-1585.
24. Sahu A, Verma RK, Sarawgi AK, Verulkar SB, Guhey A, Saxena RR. Impact of low temperature tolerance at seedling stage on rice genotypes. Journalof Rice Research. 2014; 7(1-2):36-44.
25. Sarkar BC, Haque MM, Bashar MA, Roy B, Rahman MS. Physiological responses of rice sedling towards screening out cold tolerant rice cultivars in Northwest Bangaldesh. Asian Journal of Experimental Biological Sciences. 2013; 4(4):623-628.
26. Satake T, Hayase H. Male sterility caused by cooling treatment at the young microspore stage in rice plants. Estimation of pollen developmental stage and the most sensitive stage to coolness. Proceedings of the Crop Science Society of Japan. 1970; 39:468-473.
27. Sharifi P. Evaluation on sixty-eight rice germplasms in cold tolerance at germination stage. Rice Science. 2010; 17(1):77-81.
28. Shinada H, Iwata N, Takashi S, Kenji F. Genetical and morphological characterisation of cold tolerance at fertilization stages in rice. Breeding science. 2013; 63:179-204.
29. Smita R, Verma RK, Ruplata G. Identification of Rice Genotypes Tolerant to Cold Temperature at Seedling Stage. Trends in Bioscience. 2014; 7(23):3833-3837.
30. Suh HS, Suh JP, Ahn SN, Moon HP. QTL analysis oncold tolerance at seedling stage of Korean weedy rice. Korean Journal of Breeding Science. 1999; 31:434-439.
31. Suh JP, Ahn SN, Choi IS, Cho YC, Hwang HG, Suh HS. Identification of QTLs for cold tolerance at seedling stage in Korean weedy rice (Oryza sativa L.). Korean Journal of Breeding Science. 1997; 35:96-101.
32. Suh JP, Jeung JU, Lee JI, Choi YH, Yea JD, Virk PS. Identification and analysis of QTLscontrolling cold tolerance at the reproductive stage and validation of effective QTLs in cold-tolerant genotype of rice (*Oryza sativa* L.). Theoretical and Applied Genetics. 2010; 120:985-995.
33. Takahashi N. Inheritance of seed germination and dormancy. In: Science of the rice plant: Genetic, Food and Agriculture. Matsuo, T. Fatsuhara, Y. Kikuchi F and Yamaguchi H (Eds.). Policy Research Center, Tokyo, Japan, 1997, 34-59.
34. Wu RJ, Ho THD. Production of water stress or salt stress tolerant transgenic cereal plants, US patent issued on November- Decmber 9,http:/WWW.docstoc.com/ 52272202/ Production-Of-Water-Stress-Or-SaltStress-Tolerant-Transgenic-Cereal-Plants, 1999.
35. Xu LM, Zhou L, Zeng YW, Wang FM, Zhang HL, Shen SQ *et al*. Identification and mapping of quantitative trait loci for cold tolerance at the booting stage in a Japonica rice near isogenic line. Plant Science. 2008; 174:340-347.
36. Ye C, Fukai S, Godwin I, Reinke R, Snell P, Schiller J, Basnayake J. Cold tolerance in rice varieties at different growth stages. Crop and Pasture Science. 2009; 60:328-338.
37. Yoshida S. Fundamentals of rice crop science. International Rice Research Institute, 1981a, 65-110.