

STUDIES ON SEEDLING COLD TOLERANCE AND VALIDATION OF IDENTIFIED QTLs IN RICE GENOTYPES

BY

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B.Sc. (Ag.)

**THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR
TELANGANA STATE AGRICULTURAL UNIVERSITY IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF THE DEGREE OF**

**MASTER OF SCIENCE IN AGRICULTURE
(SEED SCIENCE AND TECHNOLOGY)**

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2016

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I, **SARITA PRADHAN**, hereby declare that the thesis entitled “**STUDIES ON SEEDLING COLD TOLERANCE AND VALIDATION OF IDENTIFIED QTLS IN RICE GENOTYPES**” submitted to **Professor Jayashankar Telangana State Agricultural University** for the degree of **Master of Science in Agriculture** is the result of the original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

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Ms. SARITA PRADHAN has satisfactorily prosecuted the course of research and that the thesis entitled **“STUDIES ON SEEDLING COLD TOLERANCE AND VALIDATION OF IDENTIFIED QTLS IN RICE GENOTYPE”** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

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This is to certify that the thesis entitled “**STUDIES ON SEEDLING COLD TOLERANCE AND VALIDATION OF IDENTIFIED QTLS IN RICE GENOTYPES**” submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Professor Jayashankar Telangana State Agricultural University, Hyderabad is a record of the bonafide research work carried out by **Ms. SARITA PRADHAN** under our guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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ACKNOWLEDGEMENTS

*First and foremost, I offer my obeisance to the ‘Almighty’ for his boundless blessing, which accompanied me in all the endeavors. Words are not enough to express my whole-hearted and affectionate gratitude to my beloved parents **Sri. Sundarmani Pradhan** and **Smt. Kumudini Pradhanm**, my sister **Sasmita and Pranmita**, my brother **Nebedit** and **my all family members** for their blessings, encouragement and moral support throughout my educational carrier.*

*I am pleased to place my profound etiquette to **Dr. K. Jhansi Rani**, Associate Professor and University Head, Department of Seed Science and Technology Agricultural College, Rajendranagar, Hyderabad and esteemed Chairperson of my Advisory Committee for her learned counsel, unstinted attention, arduous and meticulous guidance, keen interest, patience, amiable dealing, constant supervision, warmer affection, mental support, immeasurable help and inspiration given to me throughout my work which has made it possible to bring out this thesis.*

*I sincerely extend my profound gratitude and appreciation to the member of my advisory committee to **Dr. M. S. Madhav**, Principal Scientist (Biotechnologist), Indian Institute of Rice Research, Rajendranagar, Hyderabad, Co- chairman of my Advisory committee for his munificent acquiescence and meticulous reasoning to refine this thesis and most explicitly to reckon with set standards. Ineffable in my gratitude and sincere thanks to him for his transcendent suggestions and efforts to embellish the study*

*I deem it my privilege in expressing my fidelity to **Dr. Ch. Damodar Raju** member of my Advisory Committee. Senior Scientist (Plant Breeding), Rice Section, ARI, Rajendranagar, Hyderabad for his valuable guidance, untiring interest, cooperation and technical, scientific views, cherishable counseling, moral support and also his help in bringing out this thesis.*

*I sincerely extend my profound gratitude to **Dr K. Keshavulu**, Director of Telangana State Seed Certification Agency, Hyderabad, for his valuable counsel, note-worthy guidance and cordial co-operation during the course of investigation.*

*I extend my deep sense of reverence and gratitude to **Dr Razia Sultana**, Associate Professor, and **Dr. P. Sujatha** Assistant Professor, Department of Seed*

Science and Technology Agricultural College, Rajendranagar, Hyderabad. for their support and encouragement throughout my career.

*I am grateful to **Supriya mam, Deepti mam, Umakant sir, Vshlaxmi mam** and all other staff of Indian Institute of Rice Research, Rajendranagar, Hyderabad, for providing me the facilities for carrying out my work and all the technical help in completing my study in time.*

*My principle indebtedness to my beloved classmate **Aditya, Joseph, Nagesh Viran, Manju, Jagadeesh, Hari, Ram sir** and my loving & caring juniors **Samph** and **Prasanth** for their abundant love, support, help and continuing encouragement during completion of my research work.*

*I wish to remember and never forget in my life the company of my amazing friends **Deepak, Sanghamitra, Sheileja, Twinkle, Priyanka, Khirod, Priyabrat, and Pritam** for their unconditional affection and homely atmosphere which never allowed me to feel away from my parents during my stay at Hyderabad.*

I humbly prostrate to all the teachers right from Kindergarten whose dedicated efforts shaped my career and brought out the best in each of my endeavours.

I am thankful to the authorities of Professor Jayashankar Telangana State Agricultural University and Government of Odisha State for the financial help in the form of stipend during my study period.

Finally, I wish my humble thanks to one and all who have directly or indirectly contributed to the conduct of the study.

Date:

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Place: Hyderabad

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
σ	:	Standard deviation
$^{\circ}\text{C}$	:	Degree Celsius
ANOVA	:	Analysis of variance
C.D	:	Critical difference
cm	:	Centimetre
C.R.D	:	Completely Randomized Design
ml	:	Mililiter
<i>et al.</i>	:	And others
etc.	:	etcetera
mM	:	mili molar
pM	:	Pico molar
GA	:	Genetic advance
GCV	:	Genotypic coefficient of Variation
GI	:	Germination Index
h	:	Hours
Fig.	:	Figure
g m^{-2}	:	Gram per square meter
g	:	Gram
g/l	:	Gram per litre
h^2b	:	Heritability in board sense
ha	:	Hectare
hrs	:	Hours
kg	:	Kilogarm
Kg ha^{-1}	:	Kilogarm per hactare
DNA	:	Deoxyribonucleic acid
MAS	:	Marker assisted selection
TBE	:	Tris Borate EDTA
CTAB	:	Cetyltrimethylammonium bromide
TRIS	:	tris (hydroxymethyl) aminomethane
SDS	:	Sodium dodecyl slfate
EDTA	:	Ethylene Diamine Tetra Acetic acid
PCR	:	Polymerase chain reaction

PERCOL	:	Coleoptiles superior to 5 mm
RFLP	:	restriction fragment length polymorphism
SSR	:	simple sequence repeats
RAPD	:	random amplified polymorphic DNA
REDCOL	:	Percentage of reduction in coleoptiles length
QTL	:	quantitative traits loci
PJTSAU	:	Professor Jayashankar Telangana State Agricultural University
IRRI	:	International Rice Research Institute
SES	:	standard evaluation system
<i>viz.</i>	:	Namely
yield ha ⁻¹	:	Yield per hectare

Author : SARITA PRADHAN
Title of the thesis : STUDIES ON SEEDLING COLD TOLERANCE AND
VALIDATION OF IDENTIFIED QTLS IN RICE
GENOTYPES
Degree : MASTER OF SCIENCE IN AGRICULTURE
Faculty : AGRICULTURE
Department : SEED SCIENCE AND TECHNOLOGY
Chairperson : Dr. K. JHANSI RANI
University : PROFESSOR JAYASHANKAR TELANGANA STATE
AGRICULTURAL UNIVERSITY
Year of submission : 2016

ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important food crops and primary source of food for more than half the world's population. Among the biotic factors affecting rice productivity cold is the major factor which results in low productivity and delay maturity especially during post rainy season. The problem is more pronounced in Northern districts of Telangana state. The low temperature prevailing during December and January results in poor germination and establishment. To breed cold tolerant rice cultivars, effective screening techniques are essential. As field evaluation for cold tolerance is less accurate due to environmental variation, screening under control temperature in the laboratory would be more reliable. Hence, 39 rice genotypes were evaluated for cold tolerance at germination and early seedling growth stage at normal and 15⁰C temperature.P

Germination was significantly high in RNR 21579, Sathya, MTU 1010 and Krishna. Germination index was high in Pusa 1121, Krishna and KNM 110. The percentage of seeds with coleoptiles superior to 5 mm was high in Krishna, JGL 11118, Bhadrakali and Varalu and percentage of reduction in coleoptiles length was high in MTU 1010, Varalu and JGL 11470. The difference between germination under control and cold temperature was low in Tellahamsa, Pusa 1121, KNM 110 and Taramathi, which have capacity to survive under cold temperature. Highest difference in germination percentage under control and cold temperature are RNR 21579, RNR 21588, MTU 1010, RNR 17420 and MTU 1075, this may be due to cold susceptibility.

The same set of genotypes under field evaluation during late *kharif* planting showed differential response, when the crop was subjected to cold at reproductive stage. The none of the genotypes were superior to checks for field emergence. The seed yield was high in Taramati, RNR 21582 WGL 44, compared to checks Sheetal, Tellahamsa and JGL 3844.

The confirmation of QTLs presence among genotypes against cold tolerance was done through the genetic level using SSR markers. The molecular profiling of these genotypes was done for presence of already known QTLs with available linked markers using Sheetal and JGL 3844 as positive control and MTU 1010 as a susceptible control. Among the 48 genotypes 9 genotypes showed resistance at genetic level by showing QTLs. When the same genotypes were observed for cold resistance at phenotypic level only two genotypes viz., WGL 32100 and RNR 15048 showed resistance, whereas RNR 174 20 was resistant at molecular level but susceptible to phenotypic level.

Chapter I

INTRODUCTION

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. Rice occupied 43.95 million hectares area with production 105.48 million tonnes in India, during 2014-2015. In Andhra Pradesh, area under rice was 36.28 lakh hectares along with production of 115.11 lakh tonnes and productivity of 3173 kg ha⁻¹ (INDIA STAT, 2013).

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). The production of rice was low during *Rabi* (84.78 million tonnes), compared to *Kharif* (102.59 million tonnes) in Andhra Pradesh, during 2012-2013, (INDIASTAT, 2013). The productivity of rice is low in Northern districts of Telangana during *Rabi*. In this zone quite often the temperature drops below 10⁰C during December and January resulting in poor growth of seedlings of rice crop. Low temperature shows different effects on germination, seedling emergence, 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 ultimately leading to poor grain yield (Thippeswamy *et al.*, 2015). Decline in rice production may lead to famine like condition especially in Asia countries. Therefore, addressing the challenges facing the production, productivity and nutritional adequacies of rice is of vital importance to the future of India and world. Extreme cold at reproductive stage results in reduction of grain yield to the extent of 26 % (Lee, 2001).

Rice suffers stress condition caused by various biotic factors *viz.*, bacteria, fungi, Mycoplasma like organism, virus etc. and abiotic factors *viz.*, heat, temperature, moisture, salinity, and cold. Among abiotic factors, the yield loss in rice due to cold or low temperature stress is about 45% at global level (Lafitte *et al.*, 2004). Cold temperatures late in season reduce yield by directly affecting

reproductive processes (Nahar *et al.*, 2009). Siezo (1966) reported that the optimum day and night temperatures for rice are 31°C and 21°C respectively, before heading and 8 °C to 16 °C during the period for 15 days after heading stage. With global climate change, most rice growing regions are experiencing more extreme environmental fluctuations (Solomon *et al.*, 2007).

Low temperature is probably one of the most common environmental stresses encountered during germination by summer crops sown early in the season (Blum, 1988). Yoshida (1981) concluded that sensitivity to cold varies between stages, Rice plant has a lower threshold temperature(10–13°C) for cold damage during the early stages of development(germination and vegetative), which makes it less sensitive to cold than during the reproductive stage, which has a higher threshold temperature for damage (18–20°C).Ye *et al.* (2009) reported rice under low temperature cultivation has the potential to affect growth and development during any developmental stage, from germination to grain filling.

Low temperature stress at the early growth stage of rice inhibits seedling establishment and gradually leads to stunted growth and delayed heading or incomplete grain filling (Sthapit and Witcombe, 1998; Andaya and Tai, 2006). Among the two rice sub species, *Indica* cultivars are cold sensitive, grown mainly in tropical and subtropical regions, while *Japonica* cultivars are more tolerant to low temperatures, and have adapted to temperate climates or higher altitudes (Oka, 1958).

One of the most common feature of low temperature during seedling growth has a notable effect on mean seedling height (Satya, 2010). Another important feature of low temperature stuck plant is retarded height and decreased chlorophyll content in leaves from green to brown, which can be given a score of 1-9 depending upon the degree of discolouration. Low temperature during panicle initiation has an adverse effect on number of spikelet per panicle (Nahar *et al.*, 2009).

Low temperature leading to spikelet sterility during reproductive stage, has the highest impact in the final yield of rice. Cold weather and cold irrigation water can damage the rice plant during any developmental stage germination, grain maturity (Majumder *et al.*, 1989; Andaya and Mackill, 2003a; Ji *et al.*, 2008; Xu *et al.*, 2008). Spikelet It was hypothesized that rice with low temperature adaptation may maintain a greater capacity for phosphorylation of the light-harvesting protein

complex of PSII (Gesch and Heliman, 1999). Screening for chilling sensitivity of different rice genotypes in breeding programs commonly relies on visual observation under field conditions. This type of screening is subjected to genotype x environment interaction and diurnal or random fluctuation throughout the growing season and over years. Methods were also developed to assess cold tolerance under controlled conditions like chlorophyll fluorescence analysis (Sthapit *et al.*, 1995).

For breeding cold tolerant rice variety selection of tolerance requires a suitable and cost effective evaluation and screening technology. Evaluation for cold tolerance during seedling stage under field conditions is limited by environmental variation, which makes it difficult to identify genetically superior lines. Evaluation of cold tolerance during germination under controlled temperature conditions may be performed by assessing percentage of reduction in coleoptile length and coleoptile growth (Cruz *et al.*, 2006). Even after three decades of the release of variety Tellahamsa, a ruling variety for its cold tolerance during seedling stage, no other varieties developed are able to replace it and progress was inadequate in this direction to tackle this problem. This may be attributed to lack of basic information on the nature and degree of cold tolerance, especially during seedling to vegetative stage.

Molecular marker class of genetic markers based on difference in the DNA nucleotide sequences of chromosome of different individuals. These difference are referred as polymorphism at DNA level and they arise as result of insertions, deletions, duplications, substitution of nucleotides. A variety of marker system has been developed to visualize these DNA polymorphism (Peterson, 1996). The most common molecular markers currently in use are restriction fragment length polymorphism (RFLP), single nucleotide polymorphism(SNP), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA(RAPD).

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., The small size rice genome viz.,

3.9 x 10⁸bp or 28, 236 genes is point of convenience while applying some molecular approach.

QTLs against cold stress have been reported in all chromosomes in rice, except for chromosome 10. Among these QTLs the most important reported at reproductive stage in rice, are *q PSST-3*, *q PSST-7* and *q PSST-9*. These were identified to provide tolerance against high male sterility due to low temperature during reproductive stage. These QTLs are present on chromosome number 3, 7 and 9 respectively. Their contribution to variability was recorded as high as 27.4% (Jena, 2010).

Some other important QTLs which have been reported seedling stage in rice, are *qSCT1a*, *qSCT2*, *qSCT10*, *qSCT11*. Their contribution to cold tolerance genotypic variability was recorded as 10.6%-16.5% . These QTLs are present on chromosome number 1,2,10 and 11 respectively (Jena, 2014) and other QTLs which have been reported high contribution to cold tolerance genotypic variability and these was *qCTS3.1*, *qCTS3.3*, *qCTS7.1*, *qCTS7.2*, *qCTS8*, *qCTS12.1*, *qCTS12.2* and *qCTS12.3*. These QTLs are present on chromosome number 3, 7, 8 and 12 with contribution to cold tolerance genotypic variability found to be in a range of 17.6%-29.7% (Chen, 2014). The QTLs for cold tolerance at different growth stages have been reported in all chromosome of rice genome.

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 is limited by environment variation, hence Marker Assisted Selection can be employed for identification of superior genotypes. Keeping in view of the above problem, the present study is proposed with the following objectives.

OBJECTIVES

1. Evaluation of rice genotypes for cold tolerance based on seed and seedling quality parameters
2. Validation of tolerant genotypes by the identified QTL's for cold in rice
3. To study relationship between seedling cold tolerance and reproductive stage cold tolerance

Chapter II

REVIEW OF LITERATURE

Rice is cultivated round the year in many parts of the country with a wide range of ecological diversity. India is second in population at global level but have land share around 2% only. This massive increase in population and substantial income growth require an extra food grain annually. Future increase in production of rice to meet the growing demand will have to be essentially achieved through increase in productivity as the scope of area is minimal.

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. 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. One important strategy for increasing crop productivity is to minimize losses due to abiotic and biotic stresses by developing more stress tolerance varieties. Identification of new genetic sources for cold tolerance is very important for which effective, low cost screening techniques are essential.

The present review is an attempt for documentation of existing knowledge regarding this research, classified in

2.1 Cold tolerance in rice

2.2 Limitations associated with conventional breeding for cold tolerance in rice

2.3 Screening techniques to measure cold tolerance in rice

2.4 Seed quality components in relation to cold tolerance and effects of cold on rice at different stages

2.5 QTLs for cold tolerance

2.6 QTLs with high contribution to variability for cold tolerance

2.1 COLD TOLERANCE IN RICE

Ratho and Pradhan (1991) identified a male sterile line with high cold tolerance at the germination stage, having cytoplasmic inheritance.

Nakagahra *et al.* (1997) 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). Studies with large number of cultivars belonging to these two sub species showed that Japonica genotypes have higher degree of cold tolerance at the germination stage (Lee, 2001; Mertz *et al.*, 2009), as well as at the vegetative and reproductive stages (Li *et al.*, 1981; Mackill and Lei, 1997). Cruz and Milach (2004) 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.

Prianka *et al.* (2000) stated that genetic variability in 26 rice genotypes to identify traits associated with cold tolerance and Indicated high heritability for germination percentage and moderate heritability for shoot root ratio.

As per Revilla *et al.* (2000), cold tolerance at the germination stage in maize under controlled temperature and allowed the identification of additive-dominant gene action for emergence percentage and initial growth.

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).

As per Cruz and Milach (2004), cold tolerance is different among genotypes, but only the percentage of reduction in coleoptile length and coleoptile regrowth showed a better differences between the tolerant and the sensitive genotypes.

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, *viz.*, percentage of reduction in coleoptile length and coleoptile growth, while the non-additive action was more important for them (Cruz *et al.*, 2006).

Singh *et al.* (2005) reported in Australia, rice farmers have suffered losses ranging from 0.5 to 2.5 t/ha in 75% of the years due to low temperature during the reproductive stage .

Two genotypes of rice having dark green seedlings and light green seedlings may be used as donor for cold tolerance in breeding programs for boro situation. Any variety to be ideally fitted in the boro rice season must have cold tolerance at the vegetative stage of growth (Dutta and Pathak, 2007).

As per Ye *et al.* (2009) 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.

Suh *et al.* (2010) 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.

Shinada (2013) 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.

Nishiyama *et al.* (2013) reported that 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.

While the minimum temperature is low, its results in 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 of damage caused by low temperature, rice breeders are giving efforts to develop more cold-tolerant cultivars at seedling stage (Smita *et al.*, 2014). Evaluation of cold tolerance at the reproductive stage is important (Zhang *et al.*, 2014).

2.2 LIMITATIONS ASSOCIATED WITH CONVENTIONAL BREEDING FOR COLD TOLERANCE IN RICE

Blum (1988) reported that rice cold tolerance at the germination stage has been studied under controlled temperature conditions, which allows adjusting intensity and duration, as well as greater precision resulting from the absence of other abiotic and biotic factors that may interfere in field data.

According to Kotaka and Abe (1988) improving the low temperature tolerance of varieties has been difficult, due to a lack of clarity of the genetic basis to low temperature tolerance.

Flowers and Yeo (1995) stated that 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.

Wu and Ho (1999) reported that conventional breeding is a slow process for generating 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.

Cruz and Milach (2000) reported that transferring cold tolerance from different sources to locally adapted cultivars requires the presence of the selective agent, which is low temperature in this case. Its abiotic nature makes it unpredictable under field conditions in terms of its intensity, duration and timing, which limits field selection for cold tolerance in rice.

As per 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.

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).

Selection of cold tolerant genotypes under field conditions is employed in countries like (Heu and Bae, 1972) and United States (Carnahan *et al.*, 1972), 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).

According to Snell *et al.* (2008) 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. Phenotyping of segregating population and breeding lines for cold tolerance and their association with molecular marker is the major limitation to developing against cold tolerant rice cultivars with high spikelet fertility.

As per Gulzar *et al.* (2011) 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.

Seedling cold tolerance was evaluated based on visual ratings, measurements of growth and various physiological Indicators of stress Kim and Tai, (2011) and showed that some *Indica* species provide useful genes for improving seedling cold tolerance in *Japonica*.

Cruz *et al.* (2013) reported the importance of the non-additive effects in cold tolerance and suggested that selection should be applied in advanced generations of breeding programs (F₄ or F₅), when dominance effects have been decreased due to the increase in homozygosity of the individuals.

According to Moffat *et al.* 2012 and Nishiyama *et al.* 2013 tolerant genotypes, evolved under strong selection pressure, may rely on diverse gene products on different growth stages to ensure cold tolerance. Tolerance to environmental stresses often comprises redundant components, ensuring species survival even if one or two tolerance genes are lost.

Thippeswamy *et al.* (2015) concluded that efforts to develop rice cultivars which have impact on yield with improved cold tolerance typically focus on seedling and reproductive traits. Since natural incidence of low temperatures is erratic and environmental trait, screening the rice genotypes against cold stress obviously be difficult and such results need further confirmation.

2.3 SCREENING TECHNIQUES TO MEASURE COLD TOLERANCE IN RICE

Cruz and Milach (2004) 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. In experiment II, radicle and coleoptile regrowth after the cold period were measured to identify tolerant genotypes.

Sharifi (2010) 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 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). 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 h 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.

Ranawake and Nakamura (2011) followed protocols for screening rice germplasm for cold tolerance and evaluated the germination stage cold tolerance 2 – day cold stress at 20°C followed by a 12- day cold stress at 15°C and continuously in dark condition is appropriate for a population derived from two genotypes. A cold stress for 5 days at 4° C for 4-day germinated seedling at 35°C are suitable for the evaluation of post-germination stage cold tolerance and 3-day cold stress at 4 °C followed by a 5 day recovery period was suitable for scoring the level of cold tolerance in the seedling stage.

Sahu *et al.* (2014) explained that among the tested genotype, HHZ 5-DT1-DT1 had 0.8 cm root length at 15°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. Observed that the root and shoot length were similar in most of the genotypes at 15° C as well as 28 °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.

Smita *et al.* (2014) screened genotypes under field condition on seedling stages during December-January and the minimum temperature which retard seedling growth was below 10°C at least for a 12 days. The screening of cold tolerant genotypes at seedling stages is main requirement for *Rabi*/summer season.

2.3 SEED YIELD AND QUALITY COMPONENTS IN RELATION TO COLD TOLERANCE IN RICE

According to Satake and Hayase (1970) sterility of the spikelets is the most common symptom of injury, when cold coincides with the reproductive stage of the rice plant but incomplete panicle exertion and spikelet abortion may also occur.

Two stages of the reproductive phase in rice which are known to be the most sensitive to cold temperature mainly booting stage (Satake and Hayase, 1970) and fertilization stage (Satake and Koike, 1983).

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).

Kaneda and Beachell (1974) 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.

According to Peterson *et al.* (1974) 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 (1981) reported that optimum temperature for normal germination and early seedling growth in rice varieties is between 20°C to 35°C and critical temperature for germination is 10 °C.

As per Yoshida (1981a) 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.

Miedema (1982) suggested that reduction in coleoptile length was one of the best characteristic for better distinction between the genotypes sensitivity to cold stress. Comparison of coleoptile growth under normal temperature in relation to cold temperature showed the genotypes response to cold stress effectively.

According to Murata and Yamaya (1984) differences in the composition of the lipid layer were pointed out as responsible for the greater tolerance to the phase transition under cold temperature, in such a way that the greater the lipid insaturation degree, the larger the cold tolerance, because the phase transition would occur at a lower temperature.

Krishnasamy and Seshu (1989) stated that good performance during germination is important to guarantee fast establishment and uniform crop stand in rice. It was also reported that there is a positive correlation between rice seedling emergence in the field and the germination rate measured by germination index.

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 (Nie *et al.*, 1992), alter composition of leaf pigments (Haldimann, 1998) and also affect chloroplast development (Robertson *et al.*, 1993).

Spikelet sterility may result from pollen abortion due to cold during microsporogenesis, when pollen grains are being formed, at the booting stage (Mackill *et al.*, 1996).

Massardo *et al.* (2000) studied 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.

As per Cruz and Milach (2000) transferring cold tolerance from different sources to locally adapted cultivars requires the presence of the selective agent, in this case the low temperature. Its 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.

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 (Cruz *et al.*, 2006; Blum, 1988 and Revilla *et al.*, 2000).

To enhance and uniform establishment of rice seedlings in the seedling stage, cold tolerance during this time is vital (Sato *et al.*, 2001).

As per Shimono *et al.* (2002), low-temperature stress is an important factor affecting the growth and development of rice (*Oryza sativa* L.) in temperate and high-elevation areas. Cold stress may cause various seedling injuries, delayed heading and yield reduction due to spikelet sterility. 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.

Reyes *et al.* (2003) found that rice growth in the temperate regions is constrained by the limited period that favour growth where it needs optimum temperature between 25 °C to 35 °C and temperatures below this often result in poor seedling vigour.

Pathak *et al.* (2003) reported that low temperature during seedling stage causes poor germination, stunted seedling growth and seedling mortality which directly affects the yield.

Low temperature injury in rice results in poor germination of seeds, poor establishment of seedling in the field and yellowing of leaves *viz.*, leaf bleaching and tissue necrosis (Abdelkhalik *et al.*, 2010), Leaf bleaching and tissue necrosis which directly impair photosynthesis (Smillie *et al.*, 1988) leads to reduced seedling growth. Synthesis of intracellular components, in particular of key proteins required

for photosynthesis is specifically susceptible to low temperature stress during development of rice leaves (Maruyama *et al.*, 1990).

Andaya and Mackill (2003) suggested that low-temperature stress is an important factor affecting the growth and development of rice (*Oryza sativa* L.) in temperate and high-elevation areas. Cold stress may cause various seedling injuries, delayed heading and yield reduction due to spikelet sterility. Rice plants are injured at the seedling stage when they are grown in early spring in temperate or subtropical environments.

Cruz and Milach (2004) reported that 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.

According to Cruz and Milach (2004), Jiang *et al.* (2008) and Sharifi (2010) rice is a sensitive plant to low temperature stress, under which germination and emergence can decrease and consequently lose yield.

Cold stress is a major constraint in many crops of tropical origin. In rice, early season low temperatures have been reported to reduce germination (Basnayake *et al.*, 2003) and stand establishment (Shimono *et al.*, 2004 and Shimono *et al.*, 2007).

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. Xu (2008) suggested that temperatures lower than 20 °C decrease both the speed and percentage of germination in rice.

Nahar *et al.* (2009) 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 (Ye *et al.*, 2009).

Jena *et al.* (2010) 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 become infertile. Lack of perfect phenotyping for cold tolerance at reproductive stage makes it difficult to correctly identify cold-tolerant genotypes.

According to Gulzar *et al.* (2011) 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.

Kim and Tai (2011) reported that 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 (osmo-protectant), malondialdehyde (lipid peroxidation), reduced glutathione (anti-oxidant) and ascorbic acid (anti-oxidant) also adversely affect growth and productivity.

According to Maulana and Teso (2011) 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. 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 pre-dispose 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.

Parmhansh *et al.* (2011) screened twenty two genotypes of rice during winter season to study their tolerance ability against low temperature stress at seedling stage and to find out suitable morphological and biochemical parameters, which may help in screening for cold tolerance at seedling stage. Out of ten morphological and biochemical parameters, seedling vigour, reducing sugar, phenol content and soluble protein exhibited highly significant correlation with cold tolerance as well as among themselves.

Cold stress is a common problem in rice cultivation and affects global production as a crucial factor Zhou *et al.* (2012) and according to Lima *et al.* (2012) low temperature can reduce up to 25% of the final yield in rice genotypes.

Sarkar *et al.* (2013) 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. Exposure to low non-lethal temperature usually induce 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.

Shinada *et al.* (2013) stated that the degradation of mature pollen grains, the inhibition of pollen germination and pollen tube elongation by cold temperature at the fertilization stage was observed.

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 (Shinada *et al.*, 2013).

Cruz *et al.* (2013) experimentally proved that low temperature can have negative impacts on rice plants during germination, vegetative growth and reproductive stages. 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. 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.

Farzin *et al.* (2013) studied 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* when compared to *kharif* due to cold effect at seedling stage especially in Telangana state and the low temperature varies 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 affects the yields of rice crop (Prasad *et al.*, 2013).

According to Smita *et al.* (2014) 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.

Ghadinezhad and Fallah (2014) 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.

Priyanka *et al.* (2015) 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.

Sharifi (2010) studied inheritance and heritability of cold tolerance at the germination stage in rice genotypes and reported highly significant differences among the replications, genotypes, General Combining Ability (GCA) and Specific Combining Ability (SCA) for percentage of reduction in radicle length (RL),

percentage of reduction in plumule length (PL) and percentage of reduction in germination measure at percentage (GP). For all of three traits the higher magnitude of the dominance variances showed that non-additive gene action was predominant to additive one.

2.4 QTLs FOR COLD TOLERANCE

Nagamine (1991) reported that chilling injury expressed on leaf withering is controlled by a major gene designated *cts2* (t) and expression of leaf chlorosis by a gene designated *cts1*(t) . *cts1* (t) is a single dominant gene controlling resistant to leaf yellowing that was identified earlier using 10 days old seedling screened at 12°C for 10 days (Kwak et al., 1984).

According to Misawa *et al.* (2000) QTLs analysis for cold tolerance at seedling stage by subjecting plant to a temperature of 4 °C for 3 days and identified five QTLs that were associated with cold temperature response, one each on chromosome 1,9 and 11, and two on chromosome 3. QTLs for cold sensitivity were identified on rice chromosome 5 (Kim *et al.*, 2000).

Foolad and Lin (2001) identified cold tolerant RILs population possessing QTLs for seedling as well as reproductive stage cold tolerance.

In rice low temperature can retard growth lead to poor stand establishment as well as delayed and non-uniform crop maturation. Seedling cold tolerance is of particular important to direct seedling culture system as planting typically coincide with the lowest temperature of the season. Genetic analysis of seedling cold tolerance has resulted in the identification of a number of single gene and QTLs that were involved in different response to cold stress (Andaya and Mackill 2003; Jiang *et al.*, 2008; Lou *et al.*, 2007; Suh *et al.*, 1999, 2003 and Zhang *et al.*, 2005).

QTLs for cold tolerance at the early seedling stage were located on chromosomes 3, 7 and 11 based on seedling survival percentage after cold treatment for 10 and 13 days at 10°C and one QTLs on chromosome 11 designated as qSCT-11 explained 30% of the phenotypic variation (Zhang *et al.*, 2005).

As per Jiang *et al.* (2008) the three QTLs affecting seedling mortality due to cold stress at 6°C for 7 days on chromosomes 1, 5 and 6 respectively, which were

confirmed by analysis of four lines derived from IR 24 chromosome segment substitution lines (CSSL).

To determine the basis for the difference in low temperature tolerance between *Japonica* and *Indica*, Andaya and Mackill (2003) developed a recombinant inbred line mapping population using the cold tolerant California temperate *Japonica* cultivar M 202 and the cold sensitive *Indica* cultivar IR 50. Analysis of this population for seedling cold tolerance led to the identification of a number of M 202-derived QTLs that confer tolerance to cold –induced wilting and necrosis observed in the cold sensitive *Indica*, IR 50. These loci a major QTLs affecting cold –induced wilting and necrosis, designated as *q CTS12* and which accounted for more than 40% of the phenotypic variation was mapped to the short arm chromosome 12.

Suh *et al.* (2010) reported that low temperature or cold stress at reproductive stage is the main constraint to temperate *Japonica* rice production and affects rice cultivars by delaying vegetative growth and heading, reducing spikelet fertility and affecting grain quality.

In order to find QTLs for cold tolerance between Geumoby eo and IR66160-121-4-4-2, recombinant inbred lines(RILs) mapping population using a cold-tolerant temperate *Japonica* cultivar, Geumoby eo, and a cold sensitive tropical *Japonica* breeding line, IR66160-121-4-4-2 were phenotyped for cold tolerance or sensitivity based on the degrees of cold tolerance as cold tolerance indices at the seedling stage. Two QTLs (*qCTS4a* and *qCTS4b*) associated with cold tolerance at the seedling stage were identified on the long and short arms of chromosome 4 with 8.3 and 7.8% respectively of the total phenotypic variation (Jena *et al.*, 2012).

Liu *et al.* (2013) analysed QTLs for cold tolerance at the early seedling stage and seven cold-tolerant QTLs were detected on chromosomes 1, 2, 5, 6, 7 and 10. Each QTL could explain 8%-20% of total phenotypic variation. With the exception of *qCST6*, the additive effect of the other QTLs was positive, which suggested that the IL112-derived alleles could increase the cold tolerance of the population.

Park *et al.* (2013) evaluated 80 recombinant inbred lines (RILs) from an inter sub-specific cross between Milyang23 (*O. sativa* ssp. *Indica*) and Hapcheonaengmi3, a *Japonica* weedy rice and the parents for leaf discoloration and SPAD value of

seedlings. A total of four QTLs for SPAD were identified and the phenotypic variance explained by each QTL ranged from 5.4 to 16.0%. Two QTLs detected in the control condition were located on chromosomes 2 and 5 respectively. Two QTL on chromosomes 1 and 4 were detected at the low-temperature condition and Hapcheonaengmi3 alleles increased the SPAD values at these loci.

Chen *et al.* (2014) stated 13 QTLs for seedling cold tolerance were identified in Dongxiang wild rice (DXWR). The total phenotypic variance (PVE) explained by the thirteen QTLs ranged from 17.6 - 29.7%.

Jena *et al.* (2014) concluded that F₇ recombinant inbred line (RIL) population using the cold-tolerant *Japonica* cultivar Jinbu and the cold-susceptible *Indica* cultivar BR29 for QTL mapping. Six QTLs including two on chromosome 1 and one each on chromosome 2, 4, 10 and 11 for seedling cold tolerance were identified with phenotypic variation (R^2) ranging from 6.1 to 16.5 %. The QTL combinations (*qSCT1* and *qSCT11*) were detected in all stable cold-tolerant RIL groups, which explained the critical threshold of 27.1 % for the R^2 value determining cold tolerance at the seedling stage. Two QTLs (*qSCT1* and *qSCT11*) on chromosomes 1 and 11 respectively were fine mapped.

Ranawake *et al.* (2014b) performed combining the phenotypic data and the linkage map, composite interval mapping to identify QTLs associated with CTG and CTS. For CTG, five QTLs were detected on chromosomes 6, 7, 8 and 11 among which *qCTG7-1* and *qCTG7-2* were overlapped between markers RM125 and RM214 on the short arm of chromosome 7. The total phenotypic variance (PVE) explained by the five QTLs ranged from 5.7 to 9.3%. At the three QTLs on chromosomes 6 and 7 alleles from the cold tolerant *Japonica* parent HGKN gave negative additive effects. At the two other QTLs on chromosomes 8 and 11 on the other hand, HGKN alleles gave positive additive effects and thus contributed to increase the level of CTG.

2.5 QTLs WITH HIGH CONTRIBUTION TO VARIABILITY FOR COLD TOLERANCE

There have been many QTLs which contribute for cold tolerance at different stages of growth in rice. Those QTLs which have a high contribute for genetic variability against low temperature stress are particularly more useful in a breeding programme to develop cold tolerant varieties. Those QTLs which contribute more than 20% to genetic variability are considered good for breeding programme. Among the surveyed QTLs, some are very excellent in performance against the low temperature stress.

Yan *et al.* (2002) identified QTL, *Cts-7* in marker interval G379b-RG4 on chromosome 7. This QTL has a high variability account of 39% and confers cold tolerance at seedling stage of rice. Another QTL, *q CTS-2* was reported in *Japonica* cultivars AAV002863 have cold tolerance at seedling stage and contribution to genetic variability was 27.42%.

Andaya and Mackill (2003) stated that *q CTS12a* accounts total 41% of genetic variability to cold tolerance, which is present on chromosome 12 and mapped between the two flanking markers in a distance of 2.37 Mb. This QTLs confers cold tolerance at seedling stage as well as reproductive stage. The another QTLs *q CTS4-1* mapped on chromosome on 4 between the SSR markers RM335-RM261 and this has been identified in donor parent M 202. This is located in a region of 5.9 Mb between the two markers.

The QTLs *q RCT7* provides stress tolerance against low temperature at reproductive stage which was identified in a *Japonica* cultivar Kunmingxiaobaigu and its contribution to genetic variability has been up to 20.6% (Dai *et al.*, 2004).

The most important QTLs, *q GR-1* was reported in a *Japonica* cultivar Daguandao. It is present on a chromosome 1, between RM9-RM7075 and confers cold tolerance at germination stage with an excellent contribution of 68.5% to genetic variability. Another QTLs, *q GI-11* was identified in *Japonica* cultivars Daguandao, has a high contribution to genetic variability for cold tolerance as high as 54.9%, was mapped between two flanking marker RM3428-RM6391 on chromosome 11. It has been reported to provide cold tolerance at the initial stage of germination in rice (Wang *et al.*, 2010-11).

Wang *et al.* (2011) reported *qCTS8.1*, which was mapped on chromosome 8 and provided cold tolerance at germination stage contribute 22.4% for variability. This located in marker interval of RM6356-RM22491 which is 2.94 Mb. A QTLs identified in cold tolerant donor parent M202 is located between RM101-RM292.

Jena *et al.* (2014) concluded that *qSCT1a* is most important QTLs, which was mapped on chromosome 1 and provided cold tolerance at seedling stage contributing 10.6% for variability . This was located in marker interval of RM3602–RM5310 and cold tolerant donor parent was *Japonica* cultivars Jinbu and cold susceptible *Indica* cultivar BR 29 Another important QTLs were *qSCT11* and *qSCT4*, which were present on chromosome 11 and 4 with cold tolerant donor parent was *Japonica* cultivars Jinbu. The phenotypic variability on chromosome 11 and 4 was 16.5% and 12.0% respectively.

Chen *et al.* (2014) reported that *qCTS7.1* was mapped on chromosome 7 and provided cold tolerance at seedling stage contributing 27.0% for the variability and located in marker interval of MK89914 MK84046. This was identified in cold tolerant donor parent Dongxiang wild rice (DXWR) .

Chapter III

MATERIALS AND METHODS

The present study entitled “Studies on seedling cold tolerance and validation of identified QTLs in rice genotypes” was carried out during 2015-16 at the Department of Seed Science & Technology, College of Agriculture, Professor Jaya Shankar Telangana State Agricultural University, Rajendranagar, Hyderabad. Three experiments were conducted and the material used for the study and the methods followed are presented here.

3.1 EXPERIMENT 1: EVALUATION OF RICE GENOTYPES FOR COLD TOLERANCE BASED ON SEED AND SEEDLING QUALITY PARAMETERS

3.1.1 Material

The experiment was conducted at Department of Seed Science and Technology, College of Agriculture, Rajendranagar by using thirty nine rice genotypes comprising of cold tolerant varieties, advance generation breeding lines and few pre released varieties and released varieties obtained from Agricultural Research Institute, Rajendranagar and Regional Agricultural Research Station, PJTSAU, Jagitial, Karimnagar district, Telangana State. The released varieties namely Tellahamsa, JGL 3844 and Sheetal were used as tolerant checks. The list of genotypes are presented in Table 3.1

3.1 List of rice genotypes used for the study

S. No.	Genotypes	Parentage	Remark
1	RNR 15038	MTU 1010 / JGL 385	Advance generation breeding line
2	Satya	Tellahamsa / Rasi	Released variety
3	Bhadrakali	Phalguna / IR 36	Released variety
4	Sumathi	Chandan / Pak Basmati	Released variety
5	MTU 1075	PLA 1100 / MTU 1010	Released variety
6	Taramati	BPT 5204 / Tellahamsa	Released variety
7	Pusa1121	Pusa 614-1-2/ Pusa 614-2-4-3	Released variety
8	NLR 33359	Selection from IR 50	Advance generation breeding line
9	JGL 17004	WGL 14377 / JGL 3855	Released variety
10	WGL 44	BPT 5204 / ARC 5984 // Kavya / BPT 5204	Released variety
11	JGL 11118	IET 8585 / JGL 1798	Advance generation breeding line
12	WGL 14	BPT 5204 / ARC 5984 // BPT 3291	Released variety
13	MTU 1010	MTU 2077 / IR 64	Released variety
14	Varalu	WGL 20471 / CR 544-1-2	Released variety
15	BPT 5204	GEB 24 / TN 1 // Mashuri	Released variety
16	RNR 15048	MTU 1010 / JGL 3855	Pre-released culture
17	Sheetal	Chaitanya / Tellahamsa	Released variety
18	Tellahamsa	HR 12 / TN 1	Released variety
19	WGL 32100	Chaitanya / Tellahamsa	Released variety
20	JGL 11470	WGL 14377 / JGL 3855	Released variety
21	MTU 1001	MTU 5249 / MTU 7014	Released variety
22	IR 64	IR 5857-33-2-1/ IR 2061-465-1-5-5	Released variety
23	RNR 17420	MTU 1010 / JGL 3855	Advance generation breeding line
24	KNM 118	MTU 1010 / JGL 13595	Pre-released culture
25	Krishna	Chandan / BPT 5204	Released variety

26	RNR 27572	MTU 1081/ Tellahamsa	Advance generation breeding line
27	RNR 2354	Early samba / RNR 19994	Advance generation breeding line
28	KNM 110	MTU 1010 / JGL 13595	Advance generation breeding line
29	Swarna	Vasishta / Mashuri	Released variety
30	JGL 3844	BPT 5204 / ARC 5984 // Kavya	Released variety
31	RNR 21583	MTU 1010 / JGL 3855	Advance generation breeding line
32	RNR 21582	MTU 1010 / JGL 3855	Advance generation breeding line
33	RNR 21579	MTU 1010 / JGL 3855	Advance generation breeding line
34	ErraMallelu	BC 5-55 / W 12708	Released variety
35	JGL 18047	MTU 1010 / JGL 13595	Advance generation breeding line
36	RNR 21567	MTU 1010 / JGL 3855	Advance generation breeding line
37	RNR 21588	MTU 1010 / JGL 3855	Advance generation breeding line
38	Sugandha samba	Early samba / RNR 19994	Released variety
39	RNR 21564	MTU 1010 / JGL 3855	Advance generation breeding line

3.1.2 Methods

The rice genotypes were evaluated for cold tolerance based on seed and seedling parameters as per Cruz and Milach (2004). Seeds were germinated under two conditions *i.e* 28 °C for 7 days (control) and 15 °C for 28 days (cold), in a germinator in the laboratory.

3.1.3 Experimental design

The treatments were arranged in Completely Randomized Design (CRD) with three replications for germination studies at cold and control temperature.

3.1.4 Observations recorded

The following seed and seedling quality observations were recorded at different temperature levels (28 °C and cold 15 °C) and data were recorded as detailed below.

3.1.4.1 Germination (%)

The laboratory test for germination was conducted as per the ISTA rules (ISTA, 1985) by adopting between paper method. Four replications of hundred seeds each in a treatment were used for the germination test that were placed in seed germinator and maintained at constant temperature of 28 ± 0.5 °C and more than 90 per cent relative humidity. The first and the final counts were taken on fourth and seventh day, respectively. On the day of final count, the number of seeds germinated was counted and the per cent germination was calculated as follows

$$\text{Germination \%} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds planted}} \times 100$$

3.1.4.2 Germination index (GI)

The germination index was calculated by using the formula given by Cruz and Milach (2004).

$$\text{GI} = (\text{N14} + \text{N21}/2) / 20 \times 100$$

Where,

N14 = number of germinated seeds 14 days after the beginning of the cold treatment,

N21 = number of germinated seeds 21 days after the beginning of the cold treatment,

20 being the total number of seeds per genotype per replication.

For the calculation of GI only the seeds presenting coleoptile and radicle were considered.

3.1.4.3 Percentage of seeds with coleoptile superior to 5 mm (PERCOL)

This was obtained by considering all the germinated seeds 28 days after the beginning of the cold treatment and by verification of the percentage that presented coleoptile length superior to 5 mm, according to the formula given by Cruz and Milach (2004).

$$\text{PERCOL} = (\text{Number of seeds with coleoptile} > 5\text{mm}) \times 100/20$$

3.1.4.4 Percentage of reduction in coleoptile length (REDCOL)

This was obtained through comparison of average coleoptile length 28 days after germination at 13 °C (cold treatment) with that obtained 7 days after germination at 28 °C (control) and calculating the percentage of reduction in coleoptile length by germination under cold temperature, according to the formula given by Cruz and Milach (2004).

$$\text{REDCOL} = [(\text{coleoptile length under cold temperature} \times 100) / \text{coleoptile length under control}] - 100$$

Where coleoptile length is the average of the 20 seeds evaluated per replication per genotype.

3.1.4.5 Shoot and root length (cm)

Ten normal seedlings in each treatment were randomly selected from the germination test for measuring the shoot and root length on 7th day of germination test. The shoot length was measured from the cotyledonary node to the tip of the apical bud and the root length was measured from the collar region to the tip of the root respectively. The mean of shoot and root length of ten seedlings were computed and expressed in centimeters.

3.1.4.6 Seedling length (cm)

Ten normal seedlings in each treatment were randomly selected from the germination test for measuring the seedling length on 7th day of germination test. The seedling length was measured from the root tip to the shoot tip. The mean of seedling length of ten seedlings were computed and expressed in centimeters.

3.1.4.7 Seedling dry weight (mg)

Ten normal seedlings used for shoot and root length measurement were taken from each replication in butter paper bags and kept in an oven maintained at 80 ± 1 °C for 24 h. Then these seedlings were kept in a dessicator for cooling. The weights of dried seedlings were recorded and mean weight was calculated per seedling and was expressed in milligrams per seedling.

3.1.4.8 Seedling vigour indices

The Seedling vigour indices were calculated as per the method suggested by Abdul-Baki and Anderson (1973) as given below and expressed in whole number.

Seedling vigour index I = Germination (%) x Seedling length (cm)

Seedling vigour index II = Germination (%) x Seedling dry weight (mg)

3.1.5 Analysis of variance

The analysis of variance was done as suggested by panse and Sukatme (1985) in the following form

Analysis of variance table for Completely Randomize Design (CRD)

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F – Calculated value
Treatments (t)	(t-1)	Tr. S.S.	$TMS = Tr. S.S / (t-1)$	$F1= \frac{TMS}{EMS}$
Replications(r)	(r-1)	R.S.S	$RMS= R.S.S/ (r-1)$	
Error	(tr-1)-(t-1)	ESS	$EMS = ESS / (tr-1)-t-1$	
Total	tr-1			

3.2 EXPERIMENT II: TO STUDY RELATIONSHIP BETWEEN SEEDLING COLD TOLERANCE AND REPRODUCTIVE STAGE COLD TOLERANCE

3.2.1 Material

All the genotypes were evaluated to assess cold tolerance based on seedling and reproductive character in the field, under cold during late *Kharif*, 2015-2016 at Rice Research Scheme, Agricultural Research Institute, Rajendranagar.

3.2.2 Experimental design

The experiment was conducted in Randomized Block Design with three replications with spacing of 15 cm between rows and 15 cm between plants in a plot size 200 cm x 15 cm.

3.2.3 Sowing and cultural practices

Nursery bed was raised in the third week of July 2015. The maximum and minimum temperatures at the sowing time were 12.3°C and 30°C (Annexure I). Transplanting was done in August. The basal dose of 30 kg N, 45 kg P₂O₅ and 30 kg K₂O per hectare was applied at the time of sowing. The usual cultural practices like weeding, irrigation were followed during the crop growth, as per recommendations.

3.2.4 Observations recorded

Following observations were recorded on five randomly selected plants from each treatment in each replication and mean was worked out. The mean of five plants for all the characters except days to 50% flowering, complete flowering and maturity which were utilized for statistical analysis, were recorded replication wise on whole plot basis.

3.2.4.1 Field emergence (%)

Field emergence of seeds was assessed by sowing 400 seeds in four rows of 100 seeds each per treatment with three replications in raised beds. The seedlings were considered emerged when the plumules were just visible on the soil surface. The emergence of the plumule was recorded at the end of every 24 h from 3rd day to 10th day after sowing. Field emergence was calculated by adding the quotients of daily count divided by the number of days taken for germination.

3.2.4.2 Seedling length (cm)

It is measured after establishment of seedlings in the main field one week after transplanting by uprooting five randomly selected seedlings from each treatment and each replication. The length is measured on a linear scale and is expressed in centimeters.

3.2.4.3 Days to 50% flowering

The number of days required from sowing to the day on which 50 per cent of the plants flowered in a treatment was recorded as days to 50 per cent flowering.

3.2.4.4 Days to complete flowering

The number of days required from sowing to the day on which complete flowering of the plants was recorded as days to complete flowering.

3.2.4.5 Days to maturity

The number of days required from date of sowing to the date on which maturity of the plants noticed was recorded as days to maturity.

3.2.4.6 Panicle exertion (%)

It Indicates the extent of panicle that comes out from the boot leaf of rice plant. It is calculated as the percentage of actual length of the panicle that exerted out.

3.2.4.7 Plant height (cm)

Plant height was measured from ground level to the tip of the plant at maturity on five randomly selected plants using a linear scale and is expressed in centimeters.

3.2.4.8 Number of tillers plant⁻¹

Total number of tillers per plant at maturity were counted on five randomly selected plants from each genotype and replication.

3.2.4.9 Number of productive tillers plant⁻¹

The number of tillers in a plant, which bear panicles, were recorded as number of productive tillers per plant at the time of maturity on five randomly selected plants.

3.2.4.10 Panicle length (cm)

It is measured as length from the panicle base node to the tip of last spikelet, prior to harvesting and expressed in centimeters.

3.2.4.11 Pollen viability (%)

Pollen grains were collected and were tested for pollen viability using iodine-potassium iodide solution (I-KI) (Charles and Harris, 1972). This solution was prepared using 1g potassium iodide (KI) + 0.5g iodine (I) dissolved in 100 ml distilled water. The

pollen counts were made few minutes after pollen grains were placed on Iodine- Potassium Iodide (IKI) solution under low power (10X) of a microscope. The viable pollen turned to dark blue colour, where as non viable pollen remained yellow. Three slides were prepared for each replication and for each slide three microscopic fields were identified and the total number of viable and non viable pollen grains (in each field) were recorded and percentage was calculated.

3.2.4.12 Spikelet sterility (%)

Number of filled and unfilled grains in each panicle was counted and sterility percentage was calculated by dividing the unfilled grains with total number of grains per panicle and expressed in percentage.

3.2.4.12 Test weight (g)

One thousand well filled grains were counted at random from each genotype per replication and weighed after thorough drying at 13% moisture content and recorded in grams with the help of electronic balance.

3.2.4.13 Seed yield ha⁻¹ (kg)

Panicles from a single plant were harvested at maturity, threshed, cleaned and seeds are dried to a safe moisture level of 13 per cent. Weight of seed of each plot was recorded separately and expressed as seed yield in kg ha⁻¹.

3.2.5 Statistical Analysis

The mean values of three randomly selected observational plants for fifteen different traits were used for statistical analysis. The following statistical measures/parameters were worked out for presentation of the data on different quantitative attributes.

3.2.5.1 Analysis of variance (ANOVA)

The analysis of variance was done as suggested by Panse and Sukhatme (1985) in the following form.

Source of variation	Degree of Freedom	MSS	Expected mean sum of squares
Replication	$r - 1$	Mr	$\sigma_e^2 + t \sigma^2_r$
Treatment	$t - 1$	Mt	$\sigma_e^2 + r. \sigma^2_t$
Error	$(r - 1) (t - 1)$	Me	σ^2_e
Total	$(rt - 1)$	-	

Where,

r = number of replications

t = number of treatments

3.2.5.2 Genotypic coefficients of variation (GCV)

It was estimated as per the formula suggested by Burton (1952)

$$GCV = \sqrt{\sigma^2_g} / \bar{X} \times 100$$

Where,

σ^2_g = Genetic variance

$\bar{X}$ = General mean of the character

3.2.5.3 Phenotypic coefficient of variation (PCV)

It was estimated as per the formula suggested by Burton (1952)

$$PCV = \sqrt{\sigma^2_p} / \bar{X} \times 100$$

Where,

σ^2_p = Phenotypic variance

$\bar{X}$ = General mean of the character

3.2.5.4 Heritability

Heritability in broad sense was calculated as per the formula given by Burton (1952), and expressed as percentage

$$h^2 (b s) = V_g / V_p \times 100 \quad \text{or} \quad h^2 (b s) = \sigma^2_g / \sigma^2_p \times 100$$

Where,

h^2 (b s) = Heritability broad sense

σ^2_g (V_g) = Genotypic variance

σ^2_p (V_p) = Phenotypic variance

3.2.5.5 Genetic advance

Genetic advance was calculated by the formula given by Johnson *et al.* (1955)

$$G.A = K \times (\sigma^2_g / \sigma^2_p) \times \sigma_p \quad \text{or} \quad G.A. = K \times h^2 \times \sigma_p$$

Where,

K = Selection differential which is 2.06 at 5 per cent selection intensity

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

σ_p = Phenotypic standard deviation

h^2 = Heritability (broad sense)

3.2.5.6 Estimation of correlation co-efficients

Correlation coefficients were calculated at genotypic and phenotypic level using the formulae suggested by Falconer (1964)

$$\text{Genotypic coefficient of correlation } (r_g) = r(x_i, x_j)_g = \frac{\text{Cov. } (x_i, x_j)_g}{\sqrt{V(x_i)_g \cdot V(x_j)_g}}$$

Where,

$r(x_i, x_j)_g$ is genotypic correlation between i^{th} and j^{th} characters

$\text{Cov. } (x_i, x_j)_g$ is genotypic covariance between i^{th} and j^{th} characters

$V(x_i)_g$ is genotypic variance of i^{th} character

$V(x_j)_g$ is genotypic variance of j^{th} character

$$\text{Phenotypic coefficient of correlation } (r_p) = r(x_i, x_j)_p = \frac{\text{Cov. } (x_i, x_j)_p}{\sqrt{V(x_i)_g \cdot V(x_j)_g}}$$

Where,

$r(x_i, x_j)_p$ is phenotypic correlation between i^{th} and j^{th} characters

$\text{Cov. } (x_i, x_j)_p$ is phenotypic covariance between i^{th} and j^{th} characters

$V(x_i)_p$ is phenotypic variance of i^{th} character

$V(x_j)_p$ is phenotypic variance of j^{th} character

To test the significance of correlation coefficients, the estimated values were compared with the table values of correlation coefficients (Fischer and Yates, 1967) at 5 per cent and 1 per cent level of significance with $n-2$ degrees of freedom, where 'n' is the number of genotypes used in the experiment.

3.3 EXPERIMENT III: VALIDATION OF TOLERANT GENOTYPES BY THE IDENTIFIED QTL'S FOR COLD IN RICE

3.3.1 Isolation of genomic DNA

Seedlings of each genotype were grown separately in field and laboratory as material for the DNA isolation. Samples of 10-15 grams were collected from the 10 days old seedlings for each genotype in early hours of day and were used for extraction of DNA. Genomic DNA was extracted from the samples taken from the seedling using cetyltrimethylammoniumbromide (CTAB) method in detail is as described below.

- 1-2 grams of sample taken from fresh seedlings was taken into a well of spot test plate (Thomas Scientific) and added 350 μl of CTAB extraction buffer. Then the sample was ground using a polished glass rod. Grinding was done until the buffer turned green like an evenly distributed solution. This is an Indication of cell breakage and release of chloroplast and cell contents. Another 350 μl of the extraction buffer was added and mixed into the well by pipetting.
- Now the total amount of lysate was transferred to the 1.5ml labeled centrifuge tube (eppendorf).

- Now the lysate was added with equal volume of chloroform (CHCl_3) *i.e* 700 μl and mixed well for 5 minutes.
- In next step the lysate was spun at 13,000 rpm for 15 minutes, after spinning collected 200-400 μl of supernatant into a new labeled centrifuge tube.
- An equal volume of prechilled isopropanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$) added, mixed gently and kept at 4⁰ C for 15 minutes.
- Now centrifuge the sample at 13,000 rpm for 10 minutes to spin down the DNA, a colourless supernatant appears, discard this.
- Obtained DNA pellet was washed two times with 100 μl of 70% ethanol ($\text{C}_2\text{H}_5\text{OH}$).
- DNA was air dried till the complete removal of ethanol traces and resuspended in 50 μl of T₁₀-E₁ buffer (10 Mm Tris-HCL pH 8.0, 1 Mm EDTA pH 8.0).
- This DNA extraction was done in 10 replications and after adding T₁₀-E₁ buffer, the sample were pooled in a labeled centrifuge tube and stored at -20⁰ C for any further use.
- While using any next time, the DNA samples were subjected to short spin before use.

3.3.2 Quality check for DNA / Quantification

Extracted DNA samples were run through 0.8% agarose gel (0.5XTBE) and after series of dilution all samples were brought to uniform concentration of 20-25ng/ μl , then these samples were tested for amplification using primers and run through 1% agarosegel (0.5XTBE).

3.3.3 Screening of resistant genotypes

Fourteen QTLs were released from our survey, for most of them check were not available. For this, we had run the DNA of 2 genotypes Sheetal and JGL 3844 (on the basis of results of phenotyping) were run with flanking markers of respective QTLs, using 3% agarose gel. Those which were showing amplification with both flanking marker and

having polymorphism were selected as reference check for other. To confirm the presence of QTLs on particular chromosome this reference check was also run with interval marker.

3.3.4 Polymorphism in Genotypes

The reference check was now run with all resistant genotype (on the basis of results of phenotyping) using 3% agarose gel to identify the presence of cold tolerance QTLs on particular chromosome. Total 51 SSR markers were used for checking polymorphism. The PCR protocol is as follow.

Table 3.2 PCR components (1X)

S.No.	Component	Volume (µl)
1	Deionised distilled water	7.0
2	10XPCR buffer	1.5
3	dNTP(mix)(10mMeach)	1.0
4	Primer forward(5Mm)	1.0
5	Primer reverse (5Pm)	1.0
6	Taq Polymerase(5U/ µl)	0.5
7	DNA template (20-25ng/L)	3.0

Table 3.3 Details of PCR cycles

Step	Sub-step	Temperature	Time
Initial denaturation		94 ⁰ C	5 min
Cycle 1-35	Denaturation	94 ⁰ C	30 seconds
	Primer annealing	55 ⁰ C	1 min
	Primer extension	72 ⁰ C	1 min
Final primer extension		72 ⁰ C	1 min

3.3.5 Selection of SSR interval marker between flanking region

1-5 internal SSR markers were selected between the flanking region of all the QTLs. For this propose Rice TOGO Browser software was used, this software exposes all the available SSR and RLPs for a given flanking region on a chromosome. These internal markers were if more than one, the selected at uniform distances between the two flanking markers. Wherever the physical distance between flanking markers was less than 1Mb, in that case only one interval marker was selected at intermediate distance.

3.3.6 Visualization of amplified product in agarose gel

Separation and visualization of PCR products was done on 3% agarose gel electrophoresis (AGE). Agarose was casted in 3% gel in TBE buffer (0.5%). Slabs were casted in a horizontal gel frame and products were visualized by incorporating 10 µl ethidiumbromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide) per 10 ml of gel solution and viewed in a gel documentation system.

The procedure of gel electrophoresis is as follow.

- The Perspex try and combs were cleaned thoroughly with 70% alcohol with tissue paper.
- The ends of Perspex try were sealed with cellophane tape.
- Agarose gel (3%) was prepared by adding 9 mg. agarose powder in 300 ml of TBE (0.5X) buffer (EDTA 0.5M at pH8.0).
- The solution was boiled by putting the flask in microwave oven and cooled at room temperature when solution is clear like water.
- Ethidium bromide (10 µl of conc.10mg/ml) was added to the gel and mixed gently and carefully to avoiding bumping.
- The gel was poured into try from one corner to ensure even distribution and air bubbles were removed by using a pipette. After the gel was completely set, tapes were removed and the gel was placed into the electrophoresis tank.
- 0.5X TBE buffer was poured into the electrophoresis tank enough to cover the gel to a depth of 5 mm.

- Combs were removed carefully after solidification completed.
- 5 µl of loading dye (3X) bromo-phenol blue was added to PCR products and mixed well by gentle tapping.
- PCR sample were loaded into wells and the power supply of 120volts for first 10 minutes and 160 volts after 0 minutes onward was provided to run the gel.
- The power supply was switched off when the gel has run about more than the $\frac{3}{4}$ th of total length and gel was removed from gel apparatus.
- The gel was viewed and documented by gel documentation system (Alpha innotech, USA).

Table 3.4 Survey of QTLs for cold stress tolerance in rice

S.N	QTL	Parents		Marker Interval	Chromosome No.	Stage	Contribution (%)	Reference
1	<i>qCSDW1</i>	Milyang23 (<i>Indica</i>)	Jileng1 (<i>Japonica</i>)	RM129- RM9	1	Seedling	16.2	Journal of Genetics and Genomics (Formerly <i>Acta Genetica Sinica</i>), 2007. 34(3):239-246.
2	<i>qGR-1</i>	Daguando (<i>Japonica</i>)	IR28 (<i>Indica</i>)	RM9- 7075	1	Germination	68.5	Journal of Zhejiang University Biomed and Biotechnology, 2010. 11(12):958-964.
3	<i>qSCT-1</i>	Asominori (<i>Japonica</i>)	IR24 (<i>Indica</i>)	XNpb87- 2-C955	1	Seedling	24.51	Journal of Cereal Science, 2008. 148 (1):173-179.
4	<i>q SPA-1</i>	Milyang23 (<i>Indica</i>)	Hapcheonaengmi3 (<i>Japonica</i>)	RM428- RM323	1	Seedling	7.5	Plant Breeding Biotechnology, 2013. 1 (1):1-8.
5	<i>q CSTI-1</i>	IL122	Guichao2 (<i>Indica</i>)	RM282- RM3426	1	Early seedling	12	Molecular Plant Breeding, 2013. 6(3):757-767.
6	<i>q CSTI-2</i>	IL122	Guichao2 (<i>Indica</i>)	RM1- RM2220	1	Early seedling	8	Molecular Plant Breeding, 2013. 6(3):757-767

7	<i>qSCT1a</i>	Jinbu (<i>Japonica</i>)	BR26 (<i>Indica</i>)	RM3602– RM5310	1	Seedling	10.6	Molecular Genetics and Genomics, 2014. 289:333–343.
8	<i>qCTS-1-b</i>	AAV00283 (<i>Indica</i>)	Zhejnshan97B (<i>Indica</i>)	RM493- RM9	1	Seedling	11.31	Euphytica, 2007. 158:87-94.
9	<i>qSCT1b</i>	Jinbu (<i>Japonica</i>)	BR26 (<i>Indica</i>)	RM3602– RM8236	1	Seedling	8.4	Molecular Genetics and Genomics, 2014. 289:333–343
10	<i>q CST2</i>	IL122	Guichao2 (<i>Indica</i>)	RM341- RM6318	2	Early seedling	2	Molecular Plant Breeding, 2013. 6(3):757-767.
11	<i>qCTS2.1</i>	DXWR	XieqingzaoB (<i>Indica</i>).	MK178244- MK192291	2	Seedling		Theoretical and Applied Genetics, 2015. 128:1359–1371.
12	<i>q CTB2a</i>	M202 (<i>Japonica</i>)	IR-50 (<i>Indica</i>)		2	Booting	17	Theoretical and Applied Genetics, 2003. 106(6):1084-90.
13	<i>q CTS-2</i>	AAV002863 (<i>Japonica</i>)	Zhejnshan97B (<i>Indica</i>)	RM561- RM341	2	Seedling	27.42	Euphytica, 2007. 158:87-94.
14	<i>q CSH2</i>	Milyang 23 (<i>Indica</i>)	Jileng1 (<i>Japonica</i>)	RM262- RM263	2	Seedling	16.6	Journal of Genetics and Genomics, (Formerly <i>Acta Genetica Sinica</i>) 2007. 34(3):239-246.

15	<i>qSCT2</i>	Jinbu (<i>Japonica</i>)	BR26 (<i>Indica</i>)	RM1211– RM2634	2	Seedling	6.5	Molecular Genetics and Genomics, 2014. 289:333–343 -9.
16	<i>qCTS3.1</i>	Daguando (<i>Japonica</i>)	IR28 (<i>Indica</i>)	RM717- RM282	3	Seedling	15.7	Journal of Zhejiang University (Biomed and Biotechnology), 2010. 11(12):958-964.
17	<i>qCTS3.1</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK160280- MK137366	3	Seedling	17.6	Theoretical and Applied Genetics, 2015. 128:1359–1371.
18	<i>qCTS3.3</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK149624- MK137032	3	Seedling	18.7	Theoretical and Applied Genetics, 2015. 128:1359–1371.
19	<i>qSCT3-1</i>	DP15and DP30(comm on wild)	9311	RM1501- RM340	3	Seedling	8-74	Chinese Journal of Rice Science, 2011. 25 (1):52-58.
20	<i>q LTB3</i>	Ukei840	Hilomebore	RM3719- RM7000	3	Booting	24.4	Theoretical and Applied Genetics, 2012. 124:937-946.
21	<i>q CTB3</i>	M-202 (<i>Japonica</i>)	IR-50 (<i>Indica</i>)	-	3	Booting	17	Theoretical and Applied Genetics, 2003. 106(6):1084-90.
22	<i>q PSST-3</i>	IR66160- 121-4-4-2	Geumobyao	RM569- M231	3	Reproductive	7.1	Molecular Genetics and Genomics, 2014. 289:333–343.

23	<i>qSCT4</i>	Jinbu (<i>Japonica</i>)	BR26 (<i>Indica</i>)	RM3648– RM1113	4	Seedling	12.0	Plant Breeding and Biotechnology, 2013. 1 (1):1-8.
24	<i>q SPA-4</i>	Milyang23 (<i>Indica</i>)	Hapcheonaengmi3 (<i>Japonica</i>)	RM335- RM518	4	Seedling	16.0	Plant Science, 2008. 174 (3): 340-347.
25	<i>q CTB-4-1</i>	Kunmingxia obaigu (<i>Japonica</i>)	Towada (<i>Japonica</i>)	RM518- RM6770	4	Booting	14.81	Euphytica, 2012. 184:101-108.
26	<i>q CTS4a</i>	Geumobyeo (<i>Japonica</i>)	IR66160- 121-4-4-2	RM3648- RM2799	4	Seedling	8.3	Euphytica, 2012. 184:101-108.
28	<i>q CTS4b</i>	Geumobyeo (<i>Japonica</i>)	IR66160- 121-4-4-2	RM3375a- RM558	4	Seedling	7.8	Journal of Experimental Botany, 2003. 54 (392) :2579-2585.
29	<i>q CTS4-1</i>	M-202 (<i>Indica</i>)	IR50 (<i>Indica</i>)	RM335 -RM261	4	Seedling and late vegetative stages	20.8	Molecular Plant Breeding, 2013. 6(3):757-767.
30	<i>q CST5</i>	IL122	Guichao2 (<i>Indica</i>)	RM305- RM3620	5	Early seedling	8	Molecular Plant Breeding, 2013. 6(3):757-767.
31	<i>q CST6</i>	IL122	Guichao2 (<i>Indica</i>)	RM162- RM340	6	Early seedling	8	Journal of Zhejiang University- (Biomed and Biotechnology), 2010. 11(12):958-964
32	<i>q GP-6</i>	Daguandao (<i>Japonica</i>)	IR28 (<i>Indica</i>)	RM528- RM340	6	Germination	24.0	Journal of Cereal Science, 2008.

								48 (1): 173-179.
33	<i>q SCT-6</i>	Asominori (<i>Japonica</i>)	IR24 (<i>Indica</i>)	G2028- XNpb386	6	Seedling	11.06	Biotechnology and Biotechnological Equipment, 2014. 28(6):989-998.
34	<i>q CTG6</i>	HGKN (<i>Japonica</i>)	HOK (<i>Indica</i>)	MGR3332- Wx	6	Germination and early seedling stage	2.6	Theoretical and Applied Genetics, 2015. 128:1359–1371.
35	<i>qCTS7.1</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK89914- MK84046	7	Seedling	27.0	Theoretical and Applied Genetics,2015.128:1359 –1371.
36	<i>qCTS7.2</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK80004- MK80344	7	Seedling	20.4	Molecular Plant Breeding, 2013. 6(3):757-767.
37	<i>q CST7</i>	IL122	Guichao2 (<i>Indica</i>)	RM6081- RM3826	7	Early seedling	12	Theoretical and Applied Genetics,2010. 120 (5):985-995.
38	<i>q PSST-7</i>	IR66160- 121-4-4-2	Geumobyeo	RM1377- RM3767	7	Reproductive stage	10.9	Chinese Journal of Rice Science,1999. 13(3): 134-138.
39	<i>q Cts7</i>	Balilla (<i>Japonica</i>)	Nanjing11 (<i>Indica</i>)	G379b-RG4	7	Early Seedling	39	Rice Science,2011. 18 (1):71-74.
40	<i>q CTB-7</i>	9311 (<i>Indica</i>)	Nipponbare (<i>Japonica</i>)	RM11- RM2752	7	Bud Bursting	-	Breeding Science,2004 54:253-258.
42	<i>q RCT7</i>	Kunmigxiao baigu	Towada (<i>Japonica</i>)	RM182- RM1789	7	Reproductive	20.6	Breeding Science, 2004 54:253-258.

		(<i>Japonica</i>)						
43	<i>q CT-7</i>	Kunmigyao baigu (<i>Japonica</i>)	Towada (<i>Japonica</i>)	RM182- RM1789	7	Reproductive	22.1	Theoretical and Applied Genetics, 2010. 120 (5):895-905.
44	<i>q CTB7</i>	ZL1929-4	Towada (<i>Japonica</i>)	RI02905- RM21862	7	Booting	9-21	
45	<i>q CTS8.1</i>	Daguandao (<i>Japonica</i>)	IR28 (<i>Indica</i>)	RM6356- RM22491	8	Germination	22.45	Euphytica, 2011. 181:405-413.
46	<i>q CTS-8</i>	AAV002863 (<i>Japonica</i>)	Zhejnshan97B (<i>Indica</i>)	RM506- RM152	8	Plumule and 3-Leaf seedling	7.4	Euphytica, 2007. 158:87-94.
47	<i>qCTS8</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK36963- MK25274	8	Seedling	16.1	Theoretical and Applied Genetics, 2015. 128:1359–1371.
48	<i>q PSSN-8</i>	IR66160- 121-4-4-2	Geumobyao	RM3553- RM5485	8	Reproductive stage	9.3	Theoretical and Applied Genetics,2010. 120 (5): 985-995.
49	<i>q PSST-9</i>	IR66160- 121-4-4-2	Geumobyao	RM24545	9	Reproductive stage	8.7	Theoretical and Applied Genetics,2010. 120 (5): 985-995.
50	<i>q CST10</i>	IL122	Guichao2 (<i>Indica</i>)	OSR33	10	Early Seedling	12	Molecular Plant Breeding,2013.6(3):757- 767.
51	<i>qSCT10</i>	Jinbu (<i>Japonica</i>)	BR26 (<i>Indica</i>)	RM1347– RM590	10	Seedling	6.1	Molecular Genetics and Genomics, 2014. 289:333–343.

52	<i>q Gl-11</i>	Daguandao (<i>Japonica</i>)	IR28 (<i>Indica</i>)	RM3428- RM6091	11	Germination	54.9	Journal of Zhejiang University- Biomed and Biotechnology, 2010.11(12):958-964.
53	<i>qSCT11</i>	Jinbu (<i>Japonica</i>)	BR26 (<i>Indica</i>)	RM1341– RM4112	11	Seedling	16.5	Molecular Genetics and Genomics, 2014. 289:333–343.
55	<i>q SCT-11</i>	-	-	RM202	11	Early seedling	26-30	Plant Science, 2005. 23 (2):116-120.
56	<i>q CTP11</i>	A58	W107	RM209- RM144	11	Plumule	22.2	Euphytica, 2009 165:459-470.
57	<i>qCTS11.1</i>	Daguandao (<i>Japonica</i>)	IR28 (<i>Indica</i>)	RM5704- RM3701	11	Seedling	9.0	Euphytica, 2011.181:405-413.
58	<i>q PSSN11</i>	IR66160- 121-4-4-2	Geumobyeo	RM287- RM5857	11	Reproductive stage	7.4	Theoretical and Applied Genetics,2010. 120 (5): 985-995.
59	<i>qCTS12.1</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK240278- MK253183	12	Seedling	22.1	Theoreticaland Applied Genetics, 2015.128:1359–1371.
60	<i>qCTS12.2</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK244291- MK238924	12	Seedling	34.3	Theoreticaland Applied Genetics, 2015. 128:1359–1371.
61	<i>qCTS12.3</i>	DXWR	Xieqingzao B (<i>Indica</i>)	MK238050- MK251857	12	Seedling	41.6	Theoretical andApplied Genetics, 2015. 128:1359–1371.

62	<i>q CTS2a</i>	M202 (<i>Japonica</i>)	IR50 (<i>Indica</i>)	RM101- RM292	11	Seedling & late vegetative stage	41	Jouranal of Experimental Botany,2003. 54(392):2579-2585.
63	<i>qCTS12b</i>	M202 (<i>Japonica</i>)	IR50 (<i>Indica</i>)	RM101- RM292	12	Seedling & late vegetative stage	41.7	Jouranal of Experimental Botany, 2003.54(392):2579-2585.
64	<i>q CTP12</i>	A58	W107	C62896- K22A	12	Plumule	27.0	Euphytica, 2009. 165:459-470
65	<i>qCSH12</i>	Milyang 23	Jileng1	RM270- RM17	12	Seedling	17.9	Journal of Genetics and Genomics, (Formerly <i>ActaGeneticaSinica</i>) 2007.34(3):239-246.
66	<i>qSDW12</i>	Milyang 23 (<i>Indica</i>)	Jileng1 (<i>Japonica</i>)	RM19- RM270	12	Seedling	15.9	Journal of Genetics and Genomics, (Formerly <i>ActaGeneticaSinica</i>) 2007. 34(3):239-246.

Table 3.5 Selected QTLs from survey, their donor parent, flanking marker, chromosomal location

S.N	QTL	Parents		Markers Interval	Chr.No.	Physical Location	Gap mb	Contributing parent
1	<i>q GR-1</i>	Daguandao	IR 28	RM 9 RM 7075	1	2332 15.10	8.2	Daguandao
2	<i>q CSH2</i>	Asominori	IR 24	RMXNpb87-2C 955	2	-	-	Asominori
3	<i>q CTS-2</i>	Milyang 23 (<i>Indica</i>)	Jileng1 (<i>Japonica</i>)	RM 262 RM 263	2	21.6 26.7	5.1	Jileng1 (<i>Japonica</i>)
4	<i>q LTB3</i>	Ukei840	Hilomebore	RM 3719 RM7000	3	32.42 33.58	1.16	Ukei840
5	<i>q PSST-3</i>	IR66160-121-4-4-2	Geumobyeo	RM 569 RM231	3	18.89 24.53	5.64	IR66160-121-4-4-2
6	<i>q CTS4-1</i>	M-202 (<i>Japonica</i>)	IR50 (<i>Indica</i>)	RM 335 RM261	4	0.6 6.5	5.9	M-202
7	<i>q CTS4a</i>	Geumobyeo	IR66160-121-4-4-2	RM 3648 RM2799	4	33.34 34.35	1.01	Geumobyeo
8	<i>q GP-6</i>	Daguandao	IR 28	RM 528 RM340	6	26.17 28.21	2.04	Geumobyeo
9	<i>q CTB7</i>	ZL1929-4	Towada	RI 02905 RM 21862	7	- 23.32	-	Towada
10	<i>q PSST-7</i>	IR66160-121-4-4-2	Geumobyeo	RM 1377 RM 3767	7	13.44 9.06	4.37	Geumobyeo
11	<i>q CTS8.1</i>	Daguandao (<i>Japonica</i>)	IR 28 (<i>Indica</i>)	RM 6356 RM 22491	8	1.55 4.49	2.94	Daguandao
12	<i>q PSST-9</i>	IR66160-121-4-4-2	Geumobyeo	RM 24427 RM24545	9	17.05 18.87	1.82	IR66160-121-4-4-2
13	<i>q SCT-11-1</i>	IR 28	Daguandao	RM 26281 RM 3701	11	6.47 8.10	1.63	IR 28
14	<i>q CTS12a</i>	M-202	IR50	RM 5746	12	5.09	2.37	M-202

		<i>(Japonica)</i>	<i>(Indica)</i>	RM 3103		7.46		<i>(Japonica)</i>
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Chapter IV

RESULTS AND DISCUSSION

Rice is a staplefood for half of the world population. 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.

Selection or screening for cold tolerance in field is not always efficient due to weather instability. Screening in laboratory is more reliable as the intensity and duration of cold stress can be adjusted. The interference of other biotic and abiotic factors is avoided in laboratory hence accurate results can be obtained.

Some efforts have been initiated to achieve cold tolerance in rice through breeding. These include the major tools used for evaluating cold tolerance in rice plants, the discovery of quantitative trait loci (QTLs) and genes related to this tolerance, and genetic transformation of rice plants with potential cold-tolerance genes. Although much progress has been achieved, joint efforts from breeders and plant biologists could speed up the production of cold-tolerant rice plants (Cruz *et al.*, 2013).

Hence the present study on rice genotypes for cold tolerance using seed, seedling and reproductive traits was carried out at the Department of Seed Science and Technology, College of Agriculture, PJTSAU, Rajendranagar and the experimental results obtained from this investigation are presented under the following headings:

1. Evaluation of rice genotypes for cold tolerance based on seed and seedling quality parameters
2. To study relationship between seedling cold tolerance and reproductive stage cold Tolerance
3. Validation of tolerant genotypes by the identified QTL's for cold in rice

4.1 EVALUATION OF RICE GENOTYPES FOR COLD TOLERANCE BASED ON SEED AND SEEDLING QUALITY PARAMETERS

4.1.1 Analysis of variance for seed and seedling parameters of rice genotypes under

control temperature

Analysis of variance showed highly significant differences among the genotypes for the traits *viz.*, germination percentage, seedling length and seedling vigour indices (Table 4.1). The significant difference was reported among the genotypes for the characters like germination percentage, seedling vigour and rate of germination (Kaw, 1991). Dutta and Pathak (2007) reported significant differences among the rice genotypes for seedling length under cold. Roy and Challa (2012) reported highly significant differences among the genotypes for shoot length, shoot fresh weight, shoot dry weight, days to 50% flowering and yield. Fatima *et al.* (2012) stated that significant differences among the genotypes were detected for radicle length under cold stress and percentage of reduction in radicle length.

4.1.2 Effect of control temperature on rice genotype for seed and seedling parameters

The mean values for seed and seedling quality parameters under control are given in Table 4.2.

4.1.2.1 Germination (%)

There was a significant difference among the genotypes for this parameter (Table 4.2, plate 4.1). The germination percentage was high in RNR 21579 (97%), followed by Satya (94%), MTU1001 (94%) and Krishna (94%) which were significantly superior to the cold tolerant varieties Tellahamsa (85%), Sheetal (89%) and JGL 3844 (90%). Yoshida (1981) reported that optimum temperature for normal germination and early seedling growth in rice varieties is between 20 to 35°C and critical temperature for germination is 10 °C. Priyanka *et al.* (2015) reported that among 25 rice genotypes used for study, Jaya (82.3%) recorded the highest germination followed by Gautam (78.1%) and Krishna Hamsa (76.4%), whereas IR 64 recorded lowest germination (10%).

Table 4.1 Analysis of variance for seed and seedling parameters of rice genotypes under control temperature

S. No.	Character	Mean Sum of Squares	
		Treatments (d. f=38)	Error (d. f =78)
1	Germination (%)	159.63**	11.08
2	Root length (cm)	7.02**	0.99
3	Shoot length(cm)	1.99**	0.19
4	Seedling length(cm)	9.62**	2.57
5	Dry weight (g)	0.003**	0.000
6	Seedling vigour index-I	112884.43**	18409.75
7	Seedling vigour index-II	27.3**	2.18

** Significant at 1 per cent level

4.1.2.2 Shoot length (cm)

Shoot length showed significant difference among the genotypes (Table 4.2). The shoot length was high in JGL 17004(15.5cm) followed by Bhadrakali (15.4 cm) and WGL 14 (15.4 cm) which were significantly superior to the cold tolerant checks Sheetal (13.8cm), Tellahamsa (15.1 cm) and JGL 3844 (13 cm). Zhang *et al.* (2005b) reported that shoot length was considered as an important character associated with seedling vigour. Roy and Challa (2012) suggested that the seedling height was affected by the low temperature during seedling stage directly influenced the seedling fresh and dry weights. Sahu *et al.* (2014) concluded that among 50 rice genotypes used for the experiment, HHZ 12-Y4-Y1-DT1, HHZ 5-SAL 14-SAL 2-Y2, IR 64197-3B-15-2 and HHZ 5-SAL 14-SAL 2-Y1 have shown 8.5cm shoot length and maximum shoot length recorded was 9.3 cm in HHZ 12-SAL 8-Y1-SAL1.

4.1.2.3 Root length (cm)

Root length showed significant difference among the genotypes. The root length was high in Tellahamsa (23.3 cm) followed by NLR 33359 (22.5cm), MTU-1010 (22.3 cm), Bhadrakali (22.2 cm) and Taramati (22.2 cm) which were significantly superior to the checks Sheetal (14.5 cm) and JGL 3844 (13.1cm). Sharifi (2010) stated that early and rapid elongation of roots were leading to resistance to abiotic stresses such as drought and cold.

4.1.2.4 Seedling length (cm)

In relation to the seedling length, the genotypes varied significantly. The seedling length was significantly higher in Bhadrakali (27.6 cm) and Taramati (27.4 cm) which were significantly superior to the cold tolerant varieties Tellahamsa (26.7 cm), Sheetal (25.6 cm) and JGL 3844 (14.7 cm). Tests based on seedling length performed were adequate indicators of field performance for the number of days to emergence, emergence percentage and emergence index as mentioned by Peterson and Jones (1974). The coleoptile length was correlated to seedling establishment (Ogiwara and Terashima, 2001).

4.1.2.5 Seedling dry weight (mg)

There was a significant difference among the genotypes for seedling dry weight (Table 4.2). The seedling dry weight was high in MTU-1010 (3.23 mg), Varalu (3.21 mg) followed by Sumathi (3.11 mg), MTU-1075 (3.09 mg) and Pusa-1121 (3.08

mg) which were significantly superior to the cold tolerant varieties Sheetal (1.30 mg), Tellahamsa (2.81mg) and JGL 3844 (2.04 mg). Zhang *et al.* (2005) suggested that decrease in temperature up to 15°C was directly proportional to the decrease in the seedling dry weight.

4.1.2.6 Seedling Vigour Index I (SVI-I)

The seedling vigour index-I was high in JGL-17004(1918), RNR-21567(1910), RNR-21579(1909) followed by Bhadrakali (1891), RNR-17813(1719) and KNM-110(1652) which were significantly superior to the cold tolerant varieties Sheetal (1266), Tellahamsa (1456) and JGL 3844 (1237) (Table 4-2). Peterson *et al.* (1978) reported that seedling vigour as the most important parameter to evaluate plant establishment under any kind of stress condition. Redona and Mackill (1996) evaluated 27 rice cultivars and concluded that the length of the primary leaf was considered as the best and least variable trait for field predictions of seedling vigour, independently of the screening temperature and had strongest correlation with vigour. At the germination stage, chilling can cause slow growth and severely reduce seedling vigour (Ali *et al.*, 2006). Prianka *et al.* (2000) studied genetic variability in 26 rice genotypes to identify traits associated with cold tolerance. Seed germination rate, mean germination time and germination index have been widely used to evaluate seed vigour (Krishnasamy and Seshu, 1989; Ranal and Santana, 2006).

4.1.2.7 Seedling Vigour Index II (SVI-II)

The genotypes showed significant difference for the seedling vigour index II (Table 4.2). The seedling vigour index II was high in KNM-118(25), RNR 21564 (24) followed by NLR 33359 (22), JGL18047(22) and RNR 21567 (21) which were significantly superior to the cold tolerant varieties Sheetal (14), Tellahamsa (19) and JGL 3844 (20). Redona and Mackill (1996) suggested that shoot weight, shoot length and coleoptile length were considered determinants to seedling vigour. Foolad *et al.* (2007) reported that seeds with strong vigour impact to speed and uniformity of seed germination, field emergence, crop performance and high yield. Rangle *et al.* (2006) reported that evaluation of vigor under controlled conditions must be performed with seedlings developed in the dark and measurements of primary leaf and root length. Germination rate and post germination early seedling growth were the two major traits directly related to seedling vigour (Ranawake *et al.*, 2014)

4.1.3 Analysis of variance for seed and seedling parameters of rice genotypes under cold temperature

Analysis of variance showed highly significant differences among the genotypes for most of the traits under cold *viz.*, germination percentage, germination index, PERCOL, REDCOL and rate of germination (Table 4.3). Cruz and Milach (2004) concluded that there were highly significant differences among genotypes for all the traits like germination index, reduction in radicle and coleoptile length and radicle and coleoptiles regrowth. Fatima *et al.* (2012) reported the significant differences among the genotypes for percentage of reduction in coleoptile length and percentage of reduction in radicle length. Priyanka *et al.* (2015) stated that significant differences among the rice genotypes were recorded with respect to mean germination index under cold.

4.1.4 Effect of cold temperature on rice genotype for seed and seedling parameters

The mean values for seed and seedling quality parameters under cold was given in Table 4.4.

4.1.4.1 Germination (%)

Germination percentage showed significant differences among the genotypes. The germination percentage was high in Tellahamsa (85%) followed by Sheetal (82%), JGL 3844 (77%) and Krishna (71%). Yoshida (1981a) reported the greatest influence of temperature on germination. Blum (1988) suggested high germination percentage is important for early season cold tolerance in rice. Fujino *et al.* (2008) suggested that QTLs may not be involved in the response to low temperature, but rather in seed germination itself, under different kinds of stress. Temperature lower than 20°C decrease both the speed and percentage of rice seed germination (Xu, 2008). Cruz *et al.* (2013) reported that low temperature stress may affect rice seed germination, avoiding development to the seedling stage. In direct seedling cultivation under the low temperature, germination and early growth of seedlings take place rapidly, (Suh *et al.*, 2003). Germination percentage of rice genotypes under normal and cold temperature are compared (Table 4.5). The germination percentage under cold was reduced to the extent of more than 50% in the genotypes Pusa 1121, NLR 33359, JGL 17004, KNM 110, Swarna and RNR 21582 which indicated cold susceptibility at germination rate. Sharifi (2010) evaluate 68 rice germplasms for cold tolerance at germination stage and showed that germination rate, radicle and coleoptile length were reduced under cold. The low germination under cold may be due to less metabolic activity and inactive

Table 4.3 Analysis of variance for seed and seedling parameters of rice genotypes under cold temperature

S. No.	Character	Mean sum of squares	
		Treatments (d. f=38)	Error (d. f =78)
1	Germination index (%)	1275.03**	50.92
2	Germination (%)	1549.39**	39.46
3	PERCOL(%)	43.30**	4.22
4	REDCOL(%)	403.87**	6.00

PERCOL: Percentage of seeds with coleoptile superior to 5 mm

REDCOL: Percentage of reduction in coleoptile length

4.4 Effect of cold temperature on seed and seedling parameters of rice genotypes

S. No.	Genotypes	Germination (%)	Germination index (%)	PERCOL (%)	REDCOL (%)
1	RNR-15038	26	35.3	5.3	53.3
2	Satya	30	36.3	12.7	47.6
3	Bhadrakali	27	31.3	17.3	57.3
4	Sumathi	22	27.3	9.3	58.3
5	MTU-1075	21	29.3	10.7	61.3
6	Taramati	29	32.3	14.0	60.2
7	Pusa-1121	61	81.0	10.3	51.4
8	NLR-33359	27	23.3	6.0	54.6
9	JGL-17004	60	63.3	10.0	15.3
10	WGL-44	69	65.7	4.7	47.9
11	JGL-11118	49	45.7	18.0	58.9
12	WGL-14	51	53.0	12.7	61.4
13	MTU-1010	22	48.3	9.3	83.8
14	Varalu	75	56.7	16.7	80.6
15	BPT-5204	12	23.0	6.7	70.8
16	RNR-15048	66	31.7	13.3	64.6
17	Sheetal (c)	82	40.0	10.0	66.5
18	Tellahamsa (c)	85	55.7	7.3	69.4
19	WGL-32100	64	47.0	8.0	57.0
20	JGL-11470	59	55.0	15.3	76.6
21	MTU-1001	17	17.3	11.3	75.6
22	IR-64	56	57.7	7.3	52.8
23	RNR-17420	21	27.7	10.0	57.6
24	KNM-118	66	60.0	10.7	65.3
25	Krishna	71	76.3	18.0	59.4
26	RNR-27572	25	24.7	6.7	48.2
27	RNR-2354	68	39.3	6.0	62.6
28	KNM-110	68	73.7	10.7	67.6
29	Swarna	63	66.0	14.7	54.2
30	JGL-3844 (c)	77	78.0	12.7	59.9
31	RNR-21583	17	19.0	6.0	67.1
32	RNR-21582	26	22.0	6.7	64.6
33	RNR-21579	15	4.0	9.3	64.7
34	Erramallelu	34	30.0	16.7	76.4
35	JGL-18047	42	10.3	10.7	59.5
36	RNR-21567	26	9.3	6.7	56.6
37	RNR-21588	15	9.3	6.7	57.5
38	Sugandha samba	37	40.0	7.3	50.9
39	RNR-21564	47	46.0	11.7	50.8
	Mean	44.30	44.30	10.44	60.21
	S.E.D	5.106	5.827	1.678	1.999
	C.D 5%	10.148	11.622	3.347	3.988

PERCOL : Percentage of seed with coleoptile superior to 5 mm

REDCOL : Percentage of reduction in coleoptiles length

Table 4.5 Comparison of germination (%) of rice genotypes under control and cold temperature

S. No.	Genotype	Germination		Difference
		Control (28 ⁰ C)	Cold (15 ⁰ C)	
1	RNR-15038	93	26	67
2	Satya	94	30	64
3	Bhadrakali	93	27	66
4	Sumathi	75	22	53
5	MTU-1075	91	21	70
6	Taramati	88	29	59
7	Pusa-1121	62	61	1
8	NLR-33359	79	27	52
9	JGL-17004	75	60	15
10	WGL-44	84	69	15
11	JGL-11118	85	49	36
12	WGL-14	87	51	36
13	MTU-1010	90	22	68
14	Varalu	87	75	12
15	BPT-5204	84	12	72
16	RNR-15048	89	66	23
17	Sheetal	89	82	7
18	Tellahamsa	85	85	0
19	WGL-32100	92	64	28
20	JGL-11470	83	59	24
21	MTU-1001	94	17	77
22	IR-64	86	56	30
23	RNR-17420	91	21	70
24	KNM-118	85	66	19
25	Krishna	94	71	23
26	RNR-27572	92	25	67
27	RNR-2354	89	68	21
28	KNM-110	72	68	4
29	Swarna	75	63	12
30	JGL-3844	90	77	13
31	RNR-21583	91	17	74
32	RNR-21582	77	26	51
33	RNR-21579	97	15	82
34	Erramallelu	90	34	56
35	JGL-18047	89	42	47
36	RNR-21567	84	26	58
37	RNR-21588	93	15	78
38	Sugandha samba	85	37	48
39	RNR-21564	81	47	34

enzymes that play key role in germination. The genotypes PUSA 1121 and check JGL 3844 recorded low difference showing germination stage cold tolerance (Table 4.5, Fig. 4.1, Plate 4.1 and plate 4.2).

4.1.4.2 Germination index (%)

With respect to the germination index there was a significant difference among the genotypes. The germination index was high in Pusa 1121 (81%) followed by JGL 3844 (78%), Krishna (76%) and KNM110 (73%) which were significantly superior to the cold tolerant varieties Tellahamsa (55.7%) and Sheetal (40%) (Table 4.4 and Fig 4.3). Germination speed is important for crop establishment; however, it does not necessarily hold any relation with the ability of a genotype to elongate coleoptile and radicle under cold temperature (Krishnasamy and Seshu, 1989 and Souza, 1990). Germination speed and consequently germination index are related to high seed vigour and the better performance. (Krishnasamy and Seshu, 1989 and Souza, 1990). Cruz and Milach (2004) suggested that germination index expresses the germination speed under cold temperature. Germination index can be used in combination with germination rate and mean germination time, to more accurately determine the cold tolerance of rice varieties at the germination stage (Ye *et al.*, 2009). The genotypes Pusa 1121, as well as KNM 110 exhibited high germination percentage during under cold hence can be recommended in areas having low temperature at the time of sowings and seedling establishment in the nursery.

4.1.4.3 Percentage of seeds with coleoptile superior to 5 mm (PERCOL)

In relation to the PERCOL there was a significant difference among the genotypes. The PERCOL was high in Krishna (18%), JGL 11118 (18%) followed by Bhadrakali (17.3%) and Varalu (16.7%) which were significantly superior to the cold tolerant varieties JGL 3844 (12.7%), Sheetal (10%) and Tellahamsa (7.3%) (Table 4.4 and Fig 4.4). Yoshida (1981a) demonstrated that the most influence of temperature on germination stage occurs in the phases of coleoptiles and radicle activation and growth. Maya (1988) concluded PERCOL as a good criterion to distinguish cold-tolerant from cold-sensitive rice genotypes. Fourteen genotypes presented more than 70% of the seeds with coleoptile length superior to 5 mm, including the tolerant checks (Cruz and Milach, 2004) who stated that this characteristic reflects the coleoptile elongation capacity under cold temperature important for early seedling establishment and growth.

4.1.4.4. Percentage of reduction in coleoptile length (REDCOL)

The genotypes showed significant difference for REDCOL. The REDCOL was high in MTU 1010 (83%) followed by Varalu (80%), JGL 11470 (76.6%) and Erramallelu (76.4%) (Table 4.4 and Fig 4.5). The high REDCOL indicates susceptibility of rice seedlings to low temperature at germination stage. The other genotypes which showed significantly low REDCOL values compared to checks are RNR 15038, Pusa 1121, WGL 44, RNR 27572, Sugandasamba and RNR 21564 which are to be further evaluated in field for cold tolerance of seedling stage. The lowest value for this trait was recorded in JGL 17004 (15.3%) indicating tolerance to cold at germination and seedling growth. This genotype also recorded high PERCOL (10.0%) high germination (60.0%) under cold hence can be recommended for *Rabi* in Telangana. The reduction in coleoptile growth during these phases may be attributed to the direct effect on cellular elongation and division, or to its indirect effect leading to a metabolic unbalance (Lyons, 1973). In this study, reduction in coleoptiles length was one of the best characteristic for better distinction between the genotypes sensitively to cold stress. REDCOL was the one that allowed for a better distinction between the tolerant and sensitive checks (Cruz *et al.*, 2004). The decrease in radicle length was more pronounced compared to that in coleoptile length in the low temperature (Sharifi, 2010). The reduction in the length of the coleoptile under cold temperature of 15° C helped in distinguishing the tolerant genotypes and sensitive ones (Farzinet *al.*, 2013). Priyanka *et al.* (2015) evaluated cold tolerance of rice genotypes in terms of REDCOL. Cruz and Milach (2004) suggested that both REDCOL and COLREG seem to be the most adequate characteristics to be used to evaluate cold tolerance during the germination period in rice.

4.2 STUDY OF RELATIONSHIP BETWEEN SEEDLING COLD TOLERANCE AND REPRODUCTIVE STAGE COLD TOLERANCE

The same set of 39 rice genotypes were evaluated for cold tolerance under field conditions during late *Kharif*, 2015-16, where in the reproductive stage was coincided with low temperature during months of November and December

4.2.1 Analysis of variance

Analysis of variance showed highly significant differences among the genotypes for most of the traits *viz.*, field emergence, seedling length, days to 50% flowering, days to complete flowering, pollen viability, days to maturity, plant height, total number of tillers per plant, productive tillers per plant, panicle length, spikelet fertility, panicle exsertion, test weight, seed yield per plant and seed yield per ha (Table 4.6). The similar results were reported by Roy and Challa, 2012 and Shiva Prasad *et al.* 2013.

4.2.2 Genetic parameters

The genetic parameters *viz.*, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance and genetic advance as per cent of mean were estimated for various traits (Table 4.7 and Fig. 4.6.).

The highest PCV (23.5) and GCV (22.4) were recorded for number of tillers per plant followed by yield per hectare (PCV – 21.5 and GCV 21.1). Days to 50 % flowering had lowest PCV (2.5) and GCV (2.4). Environmental factors influence the yield attributing traits, for this reason phenotypic coefficient of variation (PCV) was higher than that of genotypic coefficient of variation (GCV) (Barbora and Hazarika, 1998 and Kumar *et al.*, 2005). Heritability (%) was high for seedling length (99) and days to maturity (99) followed by field emergence (98), days to complete flowering (98), panicle exsertion (98) and seed yield ha⁻¹ (98). High heritability estimates for panicle exsertion and seed yield under cold can be relied upon for selection potential genotypes similar to the reports of Prianka *et al.* 2000. Contrary, the heritability of the traits evaluated under cold conditions was lower than that of parameters under optimal temperature (Fatima *et al.*, 2012). Jin (1990) reported that cold tolerance at the germination stage for rice is a dominant trait with high heritability. The genetic advance was high for yield ha⁻¹ (2438.8%) followed by number of tillers per plant⁻¹ (29 %) and lowest was days to maturity (1.2%). The genetic advance as per cent of mean has high for yield ha⁻¹ (43.4%) followed by number of productive tillers per plant (39.6%) and lowest was

Table 4.6 Analysis of variance for seedling and reproductive components of rice genotypes

S. No.	Character	Mean sum of square		
		Replication (d. f=2)	Treatments (d. f=38)	Error (d. f=76)
1	Field emergence (%)	1.36	263.03**	2.59
2	Seedling length(cm)	0.46	16.70**	0.55
3	Days to 50% flowering	1.64	210.23**	2.57
4	Days to complete flowering	12.21	288.12**	3.96
5	Pollen viability(%)	8.98	87.49**	19.38
6	Days to maturity	51.46	384.20**	2.61
7	Panicle exertion (%)	1.44	240.68**	0.69
8	Plant height(cm)	24.79	206.35**	14.25
9	No. of tillers plant ⁻¹	0.92	13.72**	1.88
10	No. of productive tillers plant ⁻¹	1.70	12.84**	2.63
11	Panicle length (cm)	4.67	12.02**	2.95
12	Spikelet fertility(%)	9.94	49.74**	13.194
13	Test weight(g)	0.26	35.02**	1.30
14	Seed yield plant ⁻¹ (g)	0.10	27.75**	1.78
15	Seed yield ha ⁻¹ (kg)	823.21	5475423.00**	335105.90

** Significant at 1 per cent level

Table 4.7 Genetic parameters for seedling and reproductive components of rice genotypes

S.No.	Traits	Mean	Range		Std. Error (±)	PVC	GCV	h ² (b) (%)	GA (%)	GAM (%)
			Min.	Max.						
1	Field emergence (%)	71.53	55	98	0.92	8.01	8.0	98	14.31	16.26
2	Seedling length(cm)	24.43	20.4	30.0	0.42	14.6	13.9	99	14.26	42.85
3	Days to 50% flowering	91.38	73.7	99.7	0.92	2.5	2.4	97	6.84	5.02
4	Days to complete flowering	95.64	64.6	113.6	1.15	2.8	2.6	98	7.22	5.50
5	Pollen viability (%)	94.83	71.9	99.2	2.54	10.5	10.2	97	17.23	21.42
6	Days to maturity	118.30	87.3	136.6	0.93	4.6	1.48	99	1.28	1.34
7	Panicle exertion (%)	58.28	46.0	99.0	0.79	10.3	10.1	98	12.26	21.44
8	Plant height(cm)	88.17	72.3	108.3	2.17	9.2	9.1	94	16.47	17.31
9	No. of tillers plant ⁻¹	10.56	7.3	16.0	0.79	16.9	16.2	96	2.93	32.62
10	No. of productive tillers plant ⁻¹	8.98	5.6	13.6	0.93	23.5	22.4	84	2.52	39.88
11	Panicle length (cm)	23.95	19.3	30.3	0.99	9.1	8.8	94	4.01	15.84
12	Spikelet fertility(%)	93.54	81.3	98.6	2.09			97		
13	Test weight(g)	17.54	11.86	24.7	0.65	18.1	17.8	87	4.72	29.98
14	Seed yield plant ⁻¹ (g)	12.06	5.7	19.6	0.77	19.5	18.2	96	5.36	27.80
15	Seed yield ha ⁻¹ (kg)	5796.00	2522.6	8729.3	334.21	21.5	21.1	98	2438.8	43.43

PVC: Phenotypic coefficient of variation

GCV: Genotypic coefficients of variation

h²(b): Heritability in broad sense

GA: Genetic Advance

GAM: Genetic Advance as per cent of mean

recorded days to maturity (1.3%) indicating with influence of environment on days for maturity.

4.2.3 Mean performance

4.2.3.1 Field emergence (%)

The data pertaining to field emergence was represented in Table 4.8 and depicted in figure 4.7. Field emergence differed significantly among the genotypes and was high in cold tolerant varieties JGL 3844 (99%), Tellahamsa (98%) followed by Sheetal (88%) whereas significantly low field emergence was recorded in WGL 14 (55%) with a mean of 71.53%. Early seedling development will overcome cold condition and tolerant seedlings should be able to survive and develop normally, thereby ensuring uniform crop establishment (Andaya and Mackill, 2003). However, the field emergence has to be evaluated during winter under cold for reliable data.

4.2.3.2 Seedling length (cm)

The data pertaining to seedling length was represented in Table 4.8 and depicted in figure 4.8. The genotypes showed significant difference for seedling length. The seedling length was high in JGL 11118 (30 cm), MTU 1075 (29.5 cm) followed by RNR 15048 (27.8 cm) which were significantly superior to the cold tolerant varieties Sheetal (25.9 cm), Tellahamsa (24.4 cm) and JGL 3844 (24.1 cm) whereas significantly low seedling length was recorded in Swarna (20.5 cm) with a mean of 24.53 cm. Prasad *et al.* (2013) reported the adverse effect of cold on the growth, development and establishment.

4.2.3.3 Days to 50% flowering

The data pertaining to days to 50% flowering was represented in Table 4.8 and depicted in figure 4.9.

The genotypes showed significant difference for days to 50% flowering. The days to 50% flowering was high in BPT 5204 (106 days), Swarna (104 days), JGL 11470 (103 days) and Sheetal (101 days) which recorded significantly more number of days to 50% flowering compared to the cold tolerant varieties Tellahamsa (84 days) and JGL 3844 (96 days) whereas significantly lower days to 50% flowering was recorded in RNR 21582 (73 days) with a mean of 91.38. Suhet *et al.* (2010) proposed that low temperature at the seedling stage delayed flowering and maturity. Similar results were

4.8 Mean performance for seedling and yield components among rice genotypes

S.NO.	Genotypes	Field emergence (%)	Seedling length (cm)	Days to 50% flowering	Days to complete flowering	Pollen viability (%)	Days to maturity	Panicle exertion (%)
1	RNR-15038	72	26.0	96.7	104.0	97.0	127.3	66.0
2	Satya	65	25.4	99.7	106.7	96.9	128.7	66.1
3	Bhadrakali	66	24.4	94.7	101.0	96.8	129.0	56.9
4	Sumathi	65	28.1	95.0	99.0	99.7	128.3	70.9
5	MTU-1075	75	29.5	97.3	101.7	96.2	127.7	60.4
6	Taramati	74	25.4	97.7	101.7	94.7	126.3	54.2
7	Pusa-1121	76	24.5	90.3	97.0	95.4	117.7	99.0
8	NLR-33359	75	22.9	96.0	100.3	97.4	127.3	58.4
9	JGL-17004	66	26.2	78.3	85.3	92.2	108.3	66.0
10	WGL-44	63	26.6	97.0	98.7	94.6	119.0	55.8
11	JGL-11118	65	30.0	89.7	96.3	93.8	118.7	51.8
12	WGL-14	55	21.3	97.0	102.0	98.5	128.3	51.0
13	MTU-1010	56	21.8	81.3	88.0	92.3	107.7	46.0
14	Varalu	61	21.6	76.3	64.7	97.2	87.3	60.9
15	BPT-5204	63	23.3	106.7	113.3	96.4	136.3	48.1
16	RNR-15048	65	27.8	88.3	91.7	97.6	118.0	57.1
17	Sheetal (c)	88	25.9	101.7	107.3	95.7	127.3	56.6
18	Tellahamsa(c)	98	24.4	84.0	88.7	96.8	107.7	55.2
19	WGL-32100	65	24.2	99.0	106.0	95.0	129.0	57.7
20	JGL-11470	69	22.8	103.0	104.7	98.0	124.7	55.1
21	MTU-1001	70	22.1	95.3	98.3	98.9	118.3	46.0

Table 4.8 Contd....

22	IR-64	75	28.0	94.7	96.7	94.3	109.0	65.3
23	RNR-17420	74	25.8	80.0	87.3	95.9	108.3	63.0
24	KNM-118	75	22.9	93.3	96.7	75.2	118.3	52.2
25	Krishna	79	26.3	93.3	96.0	96.3	117.3	51.1
26	RNR-27572	67	26.0	90.0	96.0	97.9	119.0	55.3
27	RNR-2354	67	24.1	90.3	95.3	97.1	102.0	50.0
28	KNM-110	64	24.9	95.3	98.0	91.5	112.3	55.4
29	Swarna	70	20.5	104	113.7	98.6	136.7	58.9
30	JGL-3844 (c)	99	21.5	96.0	95.7	99.2	118.0	51.0
31	RNR-21583	86	22.7	83.7	90.7	96.8	117.7	53.5
32	RNR-21582	75	24.7	73.7	80.3	92.7	104.0	52.0
33	RNR-21579	75	25.6	91.0	74.7	71.9	99.3	53.3
34	Erramallelu	67	26.9	74.3	81.0	92.7	103.3	52.5
35	JGL-18047	66	23.5	89.0	96.0	95.5	118.0	50.7
36	RNR-21567	75	23.0	93.3	99.3	95.0	128.0	56.0
37	RNR-21588	75	22.3	78.7	85.3	95.0	113.7	52.9
38	Sugandha samba	72	21.3	88.0	96.7	94.9	128.0	59.9
39	RNR-21564	75	22.4	88.0	94.7	96.8	118.0	52.8
	Mean	71.53	24.53	91.38	95.64	94.83	118.30	58.28
	C.V.	2.250	3.025	1.755	2.083	4.642	1.366	1.459
	S.E.D	1.314	0.606	1.309	1.626	3.594	1.319	0.680
	C.D 5%	2.623	1.20	2.613	3.246	7.173	2.633	1.356

Table 4.8 Contd...

S.NO.	Genotype	Plant height (cm)	No. of tillers plant⁻¹	No. of productive tillers plant⁻¹	Panicle length(cm)	Spikelet fertility(%)	Test weight (g)	Seed yield plant⁻¹(g)	Seed yield kg ha⁻¹
1	RNR-15038	108.3	10	7	30.3	92.0	15.5	14.6	4297
2	Satya	86.3	13	11	25.3	87.3	24.0	13.8	4048
3	Bhadrakali	96.3	9	7	24.3	90.7	18.3	17.0	7605
4	Sumathi	93.7	13	11	25.0	93.0	18.5	22.3	6438
5	MTU-1075	81.3	12	12	24.7	82.7	18.4	27.1	4447
6	Taramati	83.3	7	6	23.7	89.3	17.7	12.6	8729
7	Pusa-1121	100.0	15	13	25.0	94.7	21.2	14.5	4253
8	NLR-33359	94.3	8	8	25.7	94.7	17.6	20.4	5984
9	JGL-17004	75.3	10	9	21.3	93.3	14.2	11.5	3432
10	WGL-44	78.3	13	12	22.7	90.7	14.8	13.6	8455
11	JGL-11118	82.7	12	10	24.7	95.7	15.5	13.8	5613
12	WGL-14	87.0	9	8	22.3	98.0	15.1	18.1	5309
13	MTU-1010	87.0	9	8	21.7	94.3	24.7	22.6	6644
14	Varalu	85.7	12	10	23.7	98.0	17.7	18.4	5426
15	BPT-5204	75.0	13	11	21.7	95.3	14.3	16.6	4869
16	RNR-15048	91.0	10	8	23.7	92.7	13.2	19.5	5720
17	Sheetal (c)	92.0	8	8	24.0	95.7	17.5	18.7	6555
18	Tellahamsa (c)	91.3	15	14	25.7	97.3	20.3	16.5	7161
19	WGL-32100	77.0	11	10	24.0	91.3	14.9	20.8	6130
20	JGL-11470	89.0	9	8	24.7	92.7	14.7	12.2	2522
21	MTU-1001	91.3	12	11	22.7	97.0	17.7	13.7	4033
22	IR-64	79.0	10	9	22.3	94.0	24.4	19.1	5485
23	RNR-17420	90.3	9	7	25.0	92.0	17.8	16	4429
24	KNM-118	92.7	9	8	24.0	92.0	22.7	23.5	6761
25	Krishna	80.0	9	7	24.3	91.3	14.6	18	7445

Table 4.8 Contd . . .

26	RNR-27572	98.0	10	8	28.0	96.7	13.3	20	5852
27	RNR-2354	86.3	10	9	25.0	95.3	14.4	16	4693
28	KNM 110	85.0	9	8	24.0	98.7	11.9	18.1	5338
29	Swarna	72.3	11	9	19.3	97.0	17.7	20.1	6204
30	JGL-3844 (c)	79.0	7	6	21.3	96.0	15.2	13.8	7145
31	RNR-21583	94.3	11	8	22.7	94.7	22.7	20.7	6087
32	RNR-21582	96.0	16	14	23.7	95.7	23.7	35.0	8653
33	RNR-21579	100.0	9	8	25.3	97.7	19.5	19.1	5617
34	Erramallelu	81.3	12	10	25.0	98.0	18.9	17.0	3740
35	JGL-18047	97.0	11	9	26.7	97.7	20.9	26.2	7700
36	RNR-21567	95.7	9	8	22.0	88.7	15.2	14.3	4092
37	RNR-21588	82.3	13	11	24.3	81.3	20.8	25.5	7509
38	Sugandha samba	84.0	9	7	20.7	97.3	15.0	18	5309
39	RNR-21564	99.3	8	7	24.0	87.7	17.4	21.4	6307
	Mean	88.17	10.56	8.98	23.95	93.54	17.74	18.22	5796.00
	C.V.	4.281	13.007	18.086	7.176	3.883	6.432	6.15	9.389
	S.E.D	3.082	1.122	1.327	1.404	2.966	0.932	1.23	444.321
	C.D 5 %	6.151	2.239	2.647	2.801	5.919	1.860	2.24	886.701

reported by Peterson *et al.* (1974). The increase in duration of varieties was done because delayed maturity due to cold temperature. Those genotypes were taking more days for 50 % flowering may mature late in season or may reduce yield due to high temperature. Days taken for 50% flowering is an important maturity indices and in cold susceptible varieties of rice more number of days will be recorded for 5% flowering thereby increasing the duration. However, the comparison should be between varieties under similar duration group for drawing reliable conclusion on prolongation of vegetative stage under cold.

4.2.3.4 Days to complete flowering

Days to complete flowering showed significant difference among the genotypes. (Table 4.8) The days to complete flowering was high in BPT 5204 (113 days) followed by Swarna (111 days), Sheetal (107 days), Satya (106 days) and WGL 32100 (106 days) which recorded significantly more number of days to complete flowering compared to the cold tolerant varieties JGL 3844 (96 days) and Tellahamsa (88 days). Significantly lower days to complete flowering was recorded in Varalu (64 days), RNR 21579 (74 days), RNR 21582 (80 days), Erramallelu (81 days), JGL 17004 (85 days), RNR 21588 (85 days), RNR 17420 (87 days) and MTU 1010 (88 days) with a mean of 95.64. These genotypes are to be evaluated for grain yield during late *Kharif* and *Rabi*, for recommending to the areas where cold temperature prevail at flowering.

4.2.3.5 Pollen viability (%)

Pollen viability showed significant difference among the genotypes. (Table 4.8 and Figure 4.1) Pollen viability was high in Sumathi (99.7%), JGL 3844 (99.2%) followed by MTU 1001 (98.9%), Swarna (98.6%) and WGL 14 (98.5%) which were significantly superior to the cold tolerant varieties Tellahamsa (96.8%) and Sheetal (95.7%). Cold stress at flowering leads to pollen sterility, which in turn leads to spikelet sterility and low yield in susceptible genotypes, high pollen viability in these genotypes may result in high seed set hence useful under cold temperature tolerance at flowering. Significantly lower pollen viability was recorded in KNM 118 (75.2 %) and RNR 21579 (71.9%) with a mean of 94.83. Gulzaret *al.* (2011) reported that cold tolerance at reproductive stage is expressed as improved seed set and pollen fertility. It is largely a function of floral structure and function under stress.

4.2.3.6 Days to maturity

In relation to the days to maturity there was a significant difference among the genotypes (Table 4.8). The days to maturity was high in BPT 5204 (136 days), WGL 32100 (129 days) and Bhadrakali (129 days) followed by Satya (128 days), Sumathi (128 days) and RNR 21567 (128 days) which were significantly superior to the cold tolerant varieties Sheetal (127 days), JGL 3844 (118 days) and Tellahamsa (107 days) whereas significantly lower days to maturity was recorded in RNR 21579 (99 days) and Varalu (87 days) with mean of 118.30%. Sabouriet *al.* (2008) reported variations in days to maturity in different genotypes of rice. Early, maturity varieties escape cold injury during grain filling and development stages, at flowering and pollination stage under late *Kharif* and during grains filling and development stage in *Rabi*.

4.2.3.7 Panicle exertion (%)

The data pertaining to panicle exertion was represented in Table 4.8 and depicted in figure 4.11. Panicle exertion showed a significant difference among the genotypes and was high in Pusa 1121 (99%) followed by Sumathi (70%) which were significantly superior to the cold tolerant varieties Sheetal (56 %), Tellahamsa (55 %) and JGL 3844 (51%). Despite delayed panicle exertion or heading some of the genotypes had their panicle initiation during the low temperature period at 10°C (Naharet *al.*, 2009). Andaya and Mackill (2003) reported that cold stress caused delayed panicle exertion of rice. Cold stress significantly reduced panicle emergence which is one of the most important criterion of cold tolerance as reported by Lee *et al.* 1979; Farrell *et al.* 2006 and Hamdani *et al.* 1979. Sanghera *et al.* (2011) reported that cold tolerance is associated with panicle exertion under temperate conditions. Complete panicle exertion under cold contribute to high spikelet fertility and more no. of filled grains/ panicle.

4.2.3.8 Plant height (cm)

Plant height showed a significant difference among the genotypes (Table 4.8 and figure 4.12). The plant height was high in RNR 15038 (108 cm) followed by Pusa 1121 (100 cm) and RNR 21579 (100 cm) which were significantly superior to the cold tolerant varieties Sheetal (92 cm), Tellahamsa (91 cm) and JGL 3844 (79 cm). Cruz *et al.* (2013) reported that low temperature retards growth of rice plants. Low temperature exposure of seeds reduced stem elongation and this was reflected in reduction in plant height and panicle length (Ye *et al.*, 2009). Levitt (1980) reported that low temperature

stress during the growing season, retarded plant growth and development in rice genotypes.

4.2.3.9 Number of tillers plant⁻¹

The data pertaining to number of tillers per plant was represented in Table 4.8.

Number of tillers per plant showed significant difference among the genotypes and was high in RNR 12582 (16), Tellahamsa(15) and Pusa 1121(15) followed by Satya, Sumathi, WGL 44, BPT 5204 and RNR 12588 (13) which were significantly superior to the cold tolerant varieties Sheetal (8) and JGL 3844 (7). Ye *et al.* (2009) proposed that reduction rate in response to low temperature for the number of leaves and number of tillers. Tahir *et al.*(2004) stated that relative stability of this character and that it is less prone to varying environmental conditions.

4.2.3.10 Number of productive tillers plant⁻¹

The data pertaining to number of productive tillers per plant was represented in Table 4.8 and depicted in figure 4.13.

Number of productive tillers per plant showed a significant difference among the genotypes. Number of productive tillers plant⁻¹ was high in Tellahamsa (14) and RNR 21582 (14) followed by Pusa 1121 (13) which were significantly superior to the cold tolerant varieties Sheetal (8) and JGL 3844 (6).Cruz *et al.* (2013)reported that low temperature decreased tillers of rice. Low temperature in vegetative stage can reduce tillering (shimono *et al.*, 2002). The percentage productive tillers of total tillers was high in RNR 21582 (76%) and Pusa 1121 (81%), hence effect of cold on no. of panicle bearing tillers was negligible which is a reliable selection criteria for high yield under low temperature.

4.2.3.11 Panicle length (cm)

Panicle length showed significant difference among the genotypes (Table 4.8 and figure 4.14),which was high in RNR 15038 (30 cm) followed by JGL 18047 (26.7 cm), Satya (25.3 cm) RNR 21579 (25.3 cm) and were significantly superior to the cold tolerant varieties Tellahamsa (25.7cm), Sheetal (24 cm) and JGL 3844 (21.3 cm).Low temperature was reflected in reductions in plant height and panicle length (Ye *et al.*, 2009).Ahmad *et al.* (2014) reported the effect of low temperature on panicle length in rice genotypes.

4.2.3.12 Spikelet fertility (%)

The data pertaining to spikelet fertility was represented in Table 4.8 and depicted in figure 4.15. Spikelet fertility showed a significant difference among the genotypes, was high in KNM 110 (98.7 %) followed by WGL 14, Varalu and Erramallelu (98 %) which were significantly superior to the cold tolerant varieties Tellehamsa (97.3 %), JGL 3844 (96 %) and Sheetal (95.7%). Lower number of spikelet m^{-2} might be affected primarily at the differentiation stage then at the reduction division stage (Nahar *et al.*, 2009). Cruz *et al.* (2006) found that the reduction rate in spikelet fertility in response to low temperature can be used for evaluating cold tolerance. Spikelet fertility was widely used as a character for evaluating the cold tolerance of rice varieties at both the booting and flowering stages (Ye *et al.*, 2009). Sanghera *et al.* (2011) reported that cold tolerance is associated with high spikelet fertility (>90%). The genotypes found to have high spikelet fertility may either be recommended for *rabi* areas having temperature below optimum at flowering and seed development stage or they may be used as donor for breeding cold tolerant varieties.

4.2.3.13 Test weight (g)

The data pertaining to test weight was represented in Table 4.8 and depicted in figure 4.16. Which showed a significant difference among the genotypes. Test weight was high in MTU 1010 (24.7 g), IR 64 (24.4 g) and Satya (24 g) which were significantly superior to the cold tolerant varieties Tellehamsa (20.3 g), Sheetal (17.5 g) and JGL 3844 (15.2 g). Nahar *et al.* (2009) reported that lower number of thousand-grain weight due to lower percentage of fertile grain. Low temperature exposure reduced grain yield by reducing spikelet fertility and grain weight in rice (Ye *et al.*, 2009). Environmental factors such as temperature, season and time and soil fertility have also an effect on grain size and weight (Ahmad *et al.*, 2014).

4.2.3.14 Seed yield plant⁻¹ (g)

The data pertaining to seed yield per plant was represented in Table 4.8 and depicted in figure 4.17, recorded significant difference among the genotypes. Seed yield plant⁻¹ was high in RNR 21582 (35.0 g) followed by MTU 1075 (27.1 g), JGL 18047 (26.2 g) and RNR 21588 (25.5 g) which were significantly superior to the cold tolerant varieties Tellehamsa (16.6 g), JGL 3844 (13.8 g) and Sheetal (18.7 g). Exposure to cold temperature affect slower grain production, yield and tillering (Shimono *et al.*, 2002).

4.2.3.15 Seed yield ha⁻¹ (kg)

Seed yield per hectare showed a significant difference among the genotypes. 15 Seed yield ha⁻¹ was high in Taramati (8729 kg), RNR 21582 (8653kg) and WGL 44 (8455 kg) which were significantly superior to the cold tolerant varieties Tellehamsa (7161 kg), JGL 3844 (7145 kg) and Sheetal (6555 kg). Low temperature during the reproductive stages, can cause serious yield and yield components losses (Farrell *et al.*, 2006). Exposure to cold temperature affects all phenological stages of rice and lower grain production and yield (Ahmad *et al.*, 2014). Biswass *et al.* (1998) reported that higher number of effective tillers per hill and higher number of grains per panicle showed higher yield in rice. Tahir *et al.* (2002) reported that the environmental coefficient of variation (ECV) was the highest for grain yield/plant and grains/panicle showing these characters were sensitive to environment. The genotypes which recorded higher yield than the cold tolerant checks can be recommended for late *kharif* planting in which crop is likely to be exposed to low temperature at flowering and grain filling stage in certain areas.

4.3 CORRELATION COEFFICIENT ANALYSIS

The traits exhibiting high PCV, GCV, heritability and genetic advance respond to the selection. But the most efficient selection scheme needs to account for correlation among the traits. The association between laboratory and field parameters under cold temperature aids in selection of genotypes for cold tolerance. Hence, correlation analysis was carried out among seed, seedling and yield components and the results are presented in (Table 4.7).

4.3.1 Germination (%)

Germination percentage had positively significant correlation with germination index (0.7773**) at 1% level. It showed positive non-significant correlation with PERCOL (0.250), field emergence (0.223), days to 50% flowering (0.049), days to complete flowering (0.032), pollen viability (0.058), panicle exertion (0.101), number of productive tillers per plant (0.003), spikelet fertility (0.271) and yield (0.101). Germination index play vital role in chilling tolerance mechanism of rice plant (Sthapit and Witcombe, 1988). Jiang *et al.* (2004) reported that low temperature affects positive correlations between the germination and seedling stages. The low positive correlation between germination percentage and yield and yield component can be utilized for selection of cold tolerance genotypes based on lab studies, however more number of

Table 4.9 Phenotypic correlation coefficient among seed, seedling and reproductive components under cold in rice genotypes

[illegible]

genotypes at more precise temperature requires are to be screened for finding out significant correlation if any.

4.3.2 Germination index (%)

Germination index recorded a negative significant correlation with plant height (-0.393*) at 3% level. It recorded a non-significant positive correlation with PERCOL (0.305), field emergence (0.082), seedling length (0.032), days to 50% flowering (0.176), days to complete flowering (0.134), panicle exertion (0.291), spikelet fertility (0.178) and yield (0.003) and non-significant negative for REDCOL (-0.145), days to maturity (-0.057), plant height (-0.393), number of tillers per plant (-0.072), number of productive tillers per plant (-0.028), panicle length (-0.281) and test weight (-0.181). Krishnasamy and Seshu (1989) reported that there is a positive correlation between rice seedling emergence in the field and the germination rate measured by germination index, in the laboratory. The positive correlation between germination indeed and yield may further be confirmed by growing large no. of genotypes over seasons and locations.

4.3.3 Percentage of seeds with coleoptile superior to 5 mm (PERCOL)

Percentage of seeds with coleoptile superior to 5 mm (PERCOL) recorded a negative non-significant correlation with field emergence (-0.155), days to maturity (-0.034), panicle exertion (-0.067), plant height (-0.219), number of productive tillers per plant (-0.227), panicle length (-0.106), test weight (-0.103) and yield (-0.060). It showed a positive non-significant correlation with REDCOL (0.215), pollen viability (0.062) and spikelet fertility (0.074). PERCOL was a good criterion to distinguish cold tolerant from cold sensitive rice genotypes (Maya, 1988). The positive association between PERCOL and pollen viability, spikelet fertility under cold stage be used for selection of cold tolerant rice genotypes at reproductive.

4.3.4 Percentage of reduction in coleoptile length (REDCOL)

Percentage of reduction in coleoptile length (REDCOL) registered positive significant correlation with none of the characters. It recorded a negative significant correlation with panicle exertion (-0.425**) at 1% level. It showed positive non-significant correlation with days to 50% flowering (0.118), plant height (0.091), number of productive tillers per plant (0.064), panicle length (0.017), spikelet fertility (0.272), test weight (0.187) and yield (0.043). REDCOL recorded highly negative correlation with coleoptile length and radicle length (Fatima *et al.*, 2012). Correlation between the REDCOL and COLREG was negative

by lower reduction in coleoptile length due to cold (Cruz and Milach, 2004). Panicle exertion under cold is an important attribute for selection of cold tolerant genotypes. As per results of this study lower the reduction in coleoptile length under cold higher the panicle exertion, which is an useful selection criterion.

4.3.5 Field emergence (%)

The field emergence registered positive significant correlation with none of the characters. It showed positive non-significant correlation with yield (0.225) and negative non-significant for seedling length (-0.140), pollen viability (-0.014), number of productive tillers per plant (-0.018), spikelet fertility (-0.047). Priyanka *et al.* (2015) reported that germination and field emergence was is highly significant and positive correlation between the laboratory and field screening.

4.3.6 Seedling length (cm)

The seedling length had no positive correlation with any of the characters. It recorded positively non-significant correlation with pollen viability (0.059), panicle exertion (0.175), number of tillers per plant (0.076), number of reproductive tillers per plant (0.061), panicle length (0.266) and yield (0.016). Laxuman *et al.* (2011) reported that significant positive correlation between the panicle exertion and seedling length.

4.3.7 Days to 50% flowering

Days to 50% flowering recorded positive significant correlation with days to complete flowering (0.919**), pollen viability (0.416**) and days to maturity (0.699**) at 1% level. It showed positive non-significant correlation with panicle exertion (0.025) and negative non-significant for spikelet fertility (-0.120), test weight (-0.232) and yield (-0.117). The duration of rice genotypes under cold increase in terms of days taken for 50%, complete flowering and maturity.

4.3.8 Days to complete flowering

Days to complete flowering obtained positive significant correlation with pollen viability (0.489**) and days to maturity (0.808**) at 1% level. It recorded positive non-significant correlation with panicle length (0.052) and negative non-significant for plant height (-0.146), spikelet fertility (-0.202) and yield (-0.025).

4.3.9 Pollen viability (%)

Pollen viability exhibited significant positive correlation with days to maturity (0.334) at 3 % level. It showed a positive non-significant correlation with panicle exertion (0.111), number of tillers per plant (0.120) and number of productive tillers per plant (0.072) and negative non-significant for yield (-0.076). Sandra (2005) reported that non-significant correlation with pollen viability.

4.3.10 Days to maturity

Days to maturity had no positive correlation with any of the characters. It showed a positive non-significant correlation with panicle exertion (0.077) and negative non-significant for yield (-0.006).

4.3.11 Panicle exertion (%)

Panicle exertion showed non-significant positive correlation with plant height (0.196), number of tillers per plant (0.297), number of productive tillers per plant (0.254), panicle length (0.180) and test weight (0.135). It recorded non-significant negative correlations with spikelet fertility (-0.105) and yield (-0.292). Nanda and Seshu (1979) reported that panicle exertion positively correlated with the number of productive tillers per plant.

4.3.12 Plant height (cm)

Plant height recorded significant positive correlation with panicle length (0.638**) at 1% level. It showed positive non-significant correlation with spikelet fertility (0.057) and test weight (0.203) and negative non-significant correlations yield (-0.010). Correlations were observed between the reduction rate of plant height and panicle length (Ye *et al.*, 2009). Zahid *et al.* (2005) reported that plant height has a negative correlation with yield. Ranawake and Amarasinghel (2014) reported had positive non-significant correlation of plant height with yield.

4.4.13 Number of tillers plant⁻¹

Number of tillers per plant exhibited positively significant correlation with test weight (0.336*) at 5 % level. Positive significant correlation was recorded with number of productive tillers per plant (0.944**) at 1 % level. It showed a positive non-significant correlation with panicle length (0.114) and yield (0.015) and negative non-significant for

spikelet fertility (-0.008). Laxuman *et al.* (2011) exhibited positive significant of this trait correlation with number of productive tillers per plant.

4.4.14 Number of productive tillers plant⁻¹

Number of productive tillers per plant exhibited positive correlation with test weight (0.325*) at 5 % level. It showed a positive non-significant correlation with panicle length (0.053) and yield (0.015) and negative non-significant correlation with spikelet fertility (-0.043).

4.4.15 Panicle length (cm)

Panicle length had not registered positive correlation with any of the characters but a non-significant positive correlation was found with test weight (0.011) and negative non-significant for spikelet fertility (-0.092) and yield (-0.060). Ranawake and Amarsingh (2014) stated that it recorded non-significant positive correlation with yield.

4.4.16 Spikelet fertility (%)

Spikelet fertility had no positive correlation with any of the characters. It showed negative non-significant correlation with spikelet fertility (-0.133) and yield (-0.079). Zhang *et al.* (2005) reported that germination and seedling length was positively correlated with spikelet fertility at the booting stage.

4.3.17 Test weight (g)

Test weight recorded no positive correlation with any of the characters. It registered non-significant positive correlation with yield (0.229).

4.3.18 Yield ha⁻¹ (kg)

Yield ha⁻¹ recorded non-significant positive correlation with germination (0.101), germination index (0.003), REDCOL (0.043), field emergence (0.225), seedling length (0.0163), number of tillers per plant (0.0152), number of productive tillers per plant (0.0157) and test weight (0.229). It showed a negative non-significant correlation with PERCOL (-0.060), days to 50 % flowering (-0.177), days to complete flowering (-0.025), pollen viability (-0.076), days to maturity (-0.006), panicle exertion (-0.292), plant height (-0.010), panicle length (-0.060) and spikelet fertility (-0.079). The low positive correlation of laboratory parameters (germination and germination index) with seed yield per hectare can

be utilized for selection of cold tolerant genotypes in terms of yield. The laboratory screening is more precise and less expensive. These results are in accordance with Ye *et al.*, (2009) who reported correlation of cold tolerance at different stages in rice. However, more number of genotypes are to be screened at different temperatures in the laboratory and in the field screening the variation in temperature is to be minimized by providing controlled environmental facility for establishing significant correlation between lab and field parameters for selection of cold tolerant genotypes.

4.5 VALIDATION OF TOLERANT GENOTYPES BY THE IDENTIFIED QTLs FOR COLD IN RICE

One of the objectives of this study is to validate of rice genotypes for cold tolerance with reported QTLs for cold tolerance in rice. Thirty nine genotypes of rice were morphologically analyzed for their cold tolerance in both laboratory and field condition. It was also necessary to confirm their cold stress tolerance ability at molecular level, therefore, molecular profiling of these genotypes was carried out for presence of already known QTLs for cold with available linked markers using Sheetal and JGL 3844as positive control and MTU 1010 as a susceptible control. In this analysis 2 QTLs and 4 SSR markers were used in 48genotypes which also include the set of 39 genotypes used in lab and field evalua

Table 4.10 List of rice genotypes used for validation of QTLs.

S. No.	Genotype
1	MTU 1010
2	Sheetal
3	WGL 32100
4	Swarna
5	KNM 110
6	JGL 18047
7	KNM 118
9	Sugandhasambha
10	MTU 1075
11	RNR 15048
12	JGL 11118
13	WGL 14
14	JGL 11470
15	Pusa 1121
16	JGL 17004
17	RNR 17420
18	Sumathi
19	Krishna
20	WGL 44
21	JGL 3844
22	Bhadrakali
23	Taramathi
24	RNR 2354
25	RNR 21277
26	RNR 21278
27	RNR 21279
28	RNR 21297
29	RNR 21281
30	RNR 21275
31	RNR 21263
32	RNR 15038
33	RNR 21291
34	RNR 21292
35	RNR 21583
36	RNR 21261
37	RNR 21271
38	RNR 21564
39	RNR 21579
40	RNR 27572
41	RNR 21219
42	RNR 21258
43	NLR 33359
44	RNR 21588
45	RNR 21582
46	RNR 21264
47	RNR 21259
48	RNR 21260

4.5.1 Cold relation of rice genotypes based on phenotypic data

Based on three parameters is difference in germination (%) under normal and cold temperature, PERCOL and REDCOL, the 39 genotypes were scored as tolerant (RNR 15038, Satya, Bhadrakali, NLR – 33359, JGL 17004, WGL 44, KNM 110, Swarna, JGL 3844, RNR 21579, RNR 21564 and JGL 11118), moderate resistant (MTU 1075), Highly resistant (Pusa 1121, RNR 15048, Sheetal and Tellahamsa) and cold susceptible (Varalu, WGL 14, MTU 1010, BPT 5204, JGL11470, MTU 1001, IR-64, RNR17420, KNM 118, Krishna, RNR 27572, RNR 2354, RNR 21583, RNR 21582, Erramallelu, JGL 18047, RNR 21567, RNR 21588 and Sugandha samba) (Table 4.11).

Table 4.12 QTLs and flanking markers detected in rice genotypes

S. No	QTLs	Chr. No	Marker	Genotypes showing presence of QTLs (serial number as mentioned in table 4.10)
1	q GR-1	1	RM 9 RM 7075	3,4,5,6,7,8,9,10,11,12,13,14,16,17,18,19,20 3,4,11,14,16,17,22,25,26,27,28,29,32,35,36,39,40,41,44,45,47,48
2	q PSST-3	3	RM 231 RM 14307	5,6,7,11,17,22,28 5,6,11,16,18,19,20,22,23,25,27,32,36,40

Table 4.13 List of rice genotypes with available QTLs and cold reaction

S. No	Genotype	Confirmed QTLs	Cold reaction at genetic level	Cold reaction at phenotypic level
1	WGL 32100	q GR-1	Resistant	Resistant
2	Swarna	q GR-1	Resistant	Tolerant
3	RNR 15048	q GR-1, q PSST-3	Resistant	Resistant
4	JGL 11470	q GR-1	Resistant	Tolerant
5	JGL 17004	q GR-1	Resistant	Tolerant
6	RNR 17420	q GR-1	Resistant	Susceptible
7	KNM 110	q PSST-3	Resistant	Tolerant
8	JGL 18047	q PSST-3	Resistant	Tolerant
9	Bhadrakali	q PSST-3	Resistant	Tolerant

4.5.2 Cold reaction of rice genotypes based on genotypic data

Out of 48 genotypes only 9 genotypes confirmed the presence of QTLs for cold tolerance (plate 4.3). Among the 9 genotypes namely WGL 32100, Swarna, RNR 15048, JGL 11470, JGL 17004, RNR 17420 had one QTLs *q GR-1* and KNM 110, JGL 18047, Bhadrakali had one QTLs *q PSST-3*. The genotypes RNR 15048 has two QTLs namely *q GR-1*, *q PSST-3* and rest all genotypes has single QTLs for cold tolerance (Table 4.12). Jena *et al.* (2010) mapped QTLs for cold tolerance at reproduction stages on chromosome 3. The QTLs *q PSST-3* has flanking markers RM 569 and RM 231 with 10.3% phenotypic variation. Han *et al* (2007) mapped QTLs for cold tolerance in rice at different stages of rice on chromosome 1 explaining the phenotypic variation of 9.13% to 16.2%

Chapter V

SUMMARY AND CONCLUSIONS

The experiment titled “Studies on seedling cold tolerance and validation of identified QTLs in rice genotypes” was conducted at Department of Seed Science and Technology, PJTSAU, Rajendranagar. Thirty nine rice genotypes were screened for evaluation of cold tolerance in laboratory and field during *kharif*, 2015-2016. The confirmation of phenotypic reaction of genotypes against cold tolerance was done through genetic level using SSR markers

The rice genotypes were evaluated for cold tolerance based on seed and seedling parameters as per Cruz and Milach (2004). This experiment was laid out in Completely Randomized Design (CRD) with four hundred seed on four replication and seed were germinated under two condition *i.e* 28 °C for 7 days (control) and 15 °C for 28 days (cold), in a germinator in the laboratory.

Table 5.1 Performance of rice genotypes under control temperature

S.No.	Parameters	Genotypes significantly superior to checks
1	Germination (%)	RNR 21579, Sathya, MTU 1010 and Krishna
2	Root length (cm)	NRL 33359, MTU 1010, Bhadrakali and Taramathi
3	Shoot length(cm)	JGL 17004, Bhadrakali and WGL 14
4	Seedling length(cm)	Bhadrakali and Taramathi
5	Seedling Dry weight (g)	MTU 1010, Varalu, Sumathi, MTU 1075 and Pusa 1121
6	Seedling vigour index-I	JGL 17004, RNR 21567, RNR 21579, Bhadrakali, RNR 27572 and KNM 110
7	Seedling vigour index-II	KNM 118, NRL 33359, RNR 21564, JGL 18047 and RNR 21567

Under control analysis of variance showed highly significant difference among the genotypes for the traits *viz.*, germination, root length, shoot length, seedling dry weight, Seedling

vigour index-I and II. The superior genotypes for each parameters under control temperature are listed in (Table 5.1), against checks.

Table 5.2 Parameters under cold temperature

S.No.	Parameters	Genotypes significantly superior to checks
1	Germination (%)	Krishna
2	Germination index (%)	Pusa 1121, Krishna and KNM 110
3	PERCOL(%)	Krishna, JGL 11118, Bhadrakali and Varalu
4	REDCOL(%)	MTU 1010, Varalu and JGL 11470

Under cold, temperature analysis of variance showed highly significant difference among the genotypes for the traits *viz.*, germination, germination index (%), PERCOL(%) and REDCOL(%). The superior genotypes with request of each of these parameters against checks is given in table 5.2.

The difference between germination under control and cold temperature was low in Tellahamsa, Pusa 1121, KNM 110 and Taramathi, which have capacity to survive under cold temperature. The genotypes which recorded highest difference in germination percentage under control and cold are RNR 21579, RNR 21588, MTU 1010, RNR 17420 and MTU 1075, as they are susceptible to cold. The same set of genotypes were raised in the field during late *kharif*, so as to expose the plants to cold temperature at reproductive stage, and data were recorded on observations like field emergence (%), seedling length (cm), days to 50% flowering, days to complete flowering, pollen viability (%), days to maturity, panicle exertion (%), plant height (cm), panicle length (cm), no. of tillers plant⁻¹, no of productive tillers plant⁻¹, spikelet sterility (%), test weight - (g) and yield were recorded. Analysis of variance showed highly significant differences among the genotypes for most of the traits and mean performance of the genotypes showed significant differences for all the parameter. The genotypes which recorded significant performance over checks for each parameter are mentioned in Table 5.3.

Table 5.3 Parameters in field evaluation

S.No.	Parameters	Genotypes significantly superior to checks
1	Field emergence (%)	Nil
2	Seedling length(cm)	JGL 11118, MTU 1075, Sumathi and IR 68
3	Days to 50% flowering	BPT 5204, Swarna and JGL11470
4	Days to complete flowering	BPT 5204, Swarna, Sathya and WGL 32100
5	Pollen viability(%)	Sumathi, MTU 1001,Swarna and WGL 32100
6	Days to maturity	BPT 5204, WGL 32100, Bhadrakali,Sathya, Sumathi and RNR 21567
7	Panicle exertion (%)	Pusa 1121 and Sumathi
8	Plant height(cm)	RNR 15038, Pusa1121 and RNR 21579
9	No. of tillers plant ⁻¹	RNR 21582, Pusa 1121, Sathya, Sumathi, WGL 44, BPT 5204 and RNR 21588
10	No. of productive tillers plant ⁻¹	RNR 21582 and Pusa 1121
11	Panicle length (cm)	RNR 15038, JGL 18047 Sathya and RNR 21579
12	Spikelet fertility(%)	KNM 110, WGL 14, Varalu and Erramallelu
13	Test weight(g)	MTU 1010, IR 64 and Sathya
14	Seed yield plant ⁻¹ (g)	RNR 21582, MTU 1075, JGL 18047 and RNR 21588
15	Seed yield ha ⁻¹ (kg)	Taramthi, RNR 21582 and WGL 44

The genetic parameters like phenotypic coefficient of variation, genotypic coefficient of variation, heritability, genetic advance and genetic advance as percent of mean were studied. The highest PCV and GCV were recorded for number of tillers per plant followed by yield per hectare. Days to 50 % flowering had lowest PCV and GCV. Heritability (%) was high for seedling length and days to maturity followed by field emergence, days to complete flowering, panicle exertion and seed yield ha⁻¹.The genetic advance was high for yield ha⁻¹ followed by

number of tillers plant⁻¹ and lowest was days to maturity. The genetic advance as per cent of mean has high for yield ha⁻¹ followed by number of productive tillers plant⁻¹.

The confirmation of phenotypic responses of genotypes against cold tolerance was done at the genetic level using SSR markers (Table 5.4). Linked SSR markers were used for the polymorphism among genotypes and identified the presence of in these genotypes. Among the 48 genotypes 9 genotypes showed to be resistance at genetic level by showing QTLs. When the same genotypes were observed for resistance at phenotypic level only two genotypes viz., WGL 32100 and RNR 15048 showed resistance at both the level, whereas rest of the genotypes were found to be tolerant (Table 5.4). The only genotypes RNR 174 - 20 shown resistance at molecular level but susceptible at phenotypic level.

Table 4.15 Cold reaction at molecular level and phenotypic level

S. No	Genotype	Molecular level	Phenotypic level
1	WGL 32100	Resistant	Resistant
2	Swarna	Resistant	Tolerant
3	RNR 15048	Resistant	Resistant
4	JGL 11470	Resistant	Tolerant
5	JGL 17004	Resistant	Tolerant
6	RNR 17420	Resistant	Susceptible
7	KNM 110	Resistant	Tolerant
8	JGL 18047	Resistant	Tolerant
9	Bhadrakali	Resistant	Tolerant

The difference in cold tolerance reaction at phenotypic level and genotypic level may be due to uncontrollable weather conditions and influence of improvement on expression of characters.

FUTURE LINE OF WORK

1. More number germplasm lines must be screened continuously for three seasons over a location. Large populations must be maintained to get reliable data under field conditions for establishing correlation between laboratory and field parameters.

2. Fine Mapping of QTLs/ genes conferring to cold tolerance in rice is essential to hasten up the breeding programmes through marker assisted selection for breeding cold tolerance rice varieties.

Table 3.1 Monthly meteorological data recorded at ARI, Rajendranagar from June, 2015 to April, 2016

S. No.	Period	Temperature (⁰ C)		R.H. (1%)		Rain-Fall (mm)	Rainy Days	Rainy Shine (hrs.)	Wind Speed (Km/hr)	Evapo-Ration (mm)
		Max.	Min.	I	II					
1	JUNE, 2015	34.8	25.4	76.9	49.8	40.5	2	5.2	6.3	6.5
2	JULY, 2015	33.6	23.9	78.2	50.4	5.6	1	7.0	10.4	6.6
3	AUG, 2015	31.5	23.0	86.9	61.8	24.6	3	5.1	5.5	5.2
4	SEPT, 2015	30.8	22.3	89.3	66.	39.4	2	4.9	1.5	4.2
5	OCT, 2015	32.5	19.5	91.4	44.5	8.7	1	7.8	0.7	4.3
6	NOV, 2015	30.5	18.2	87.6	57.8	4.8	0	7.0	1.9	4.0
7	DEC, 2015	30.9	15.2	87.1	36.1	0.3	0	8.0	0.7	3.9
8	JAN, 2015	29.5	13.8	79.1	31	0	0	8.2	1.3	3.8
9	FEB, 2015	33.4	16.3	77.6	28	0	0	9.1	1.6	5.5
10	MAR, 2015	35.7	20.5	73.3	30	0	0	7.9	2.2	6.7
11	APRIL, 2015	39.8	23.6	61.8	23	1.1	0.4	9.1	2.2	8.4

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