Chapter 1: Introduction
Plants have developed sophisticated surveillance systems to sense the ever changing environment, including various stress conditions. After the sensing step, signals generated at the receptors/sensors are converted to cellular responses through various signal transduction pathways. The very early signaling events, such as calcium influx and protein phosphorylation/dephosphorylation, occur within minutes, which are followed by the generation of several signaling compounds/phytohormones, including reactive oxygen species, nitric oxide, ethylene, jasmonic acid, and salicylic acid. Change of global gene expression in response to these primary and secondary signals eventually alters the metabolism/physiology of plants and leads to their adaptation to the new environment.

Mitogen-activated protein kinase (MAPK) cascades are major pathways downstream of sensors/receptors that transduce extracellular stimuli into intracellular responses in eukaryotes. MAPKs consist of a family of protein kinases whose function and regulation have been conserved during evolution from yeast to complex organism like human and plants. MAPKs, a serine/threonine protein kinase (PK) itself, phosphorylates specific serine/threonine of target protein and regulate a variety of cellular activities are the last component of phosphorelay system composed of three sequentially activated PKs.

Fig. 1.1 A typical MAP Kinase cascade showing sequential phosphorylation of MAPKKK, MAPKK and MAPK.
MAPKs are activated by phosphorylation on their threonine and tyrosine (Thr-Xaa-Tyr) residues located in the activation loop (T-loop), by dual specificity kinases, MAPK kinases (MAPKKs). MAPKKs are highly specific in phosphorylating specific MAPKs. MAPKK kinases (MAPKKKs) are the first component of this phosphorelay system that activates MAPKKs by phosphorylating their serine/threonine residues (Fig. 1.1). The MAPKKKs in turn carry distinct motifs in their sequences that selectively confer their activation in response to different stimuli upon phosphorylation by other PKs. Cells receive many different stimuli from their environment and the MAPK phosphorylation system work as a link between upstream receptors and downstream targets controlling many important cellular functions.

Rice (Oryza sativa L.) is the most important food crop in the world and feeds over half of the global population. It has played a central role in human nutrition and cultured for the past 10,000 years. Cultivated rice belongs to two species, Oryza sativa (which is more widely used) and Oryza glaberrima (an African rice). The two main strains of O. sativa are japonica and indica. These two evolved in different geographical regions and cultured over thousands of years and farming groups relocated both to different ecosystems. There are thousands of varieties of japonica and indica rice grown in more than 100 countries. Some of these varieties include traits such as stiff straw stems, which prevent the plant from falling over, and upright leaves, which absorb and use solar energy more efficiently. Basmati rice occupies a prominent place in the species of Oryza sativa L. with unique quality features, pleasant aroma, long slender grains, remarkable linear elongation, and soft texture on cooking. Most of quality aromatic rice genotypes like Pusa Basmati-1 are susceptible to biotic and abiotic stresses with consequent low-yield potential and hence cannot be traded at international level. About 91 percent of the world's rice is grown and consumed in Asia. It has been estimated that world rice production must increase by 30% over the next 20 years to meet the projected demands from population increase and economic development.

In plants, the components of the MAPK cascade (MAPKKK–MAPKK–MAPK) have been identified and characterized from several species, and most of the understanding on this cascade comes from vigorous studies in dicotyledonous (dicot) species particularly the model research plant Arabidopsis (Ichimura et al., 2002; Pedley and Martin 2005). During the past decade, tremendous progress has been made towards the functional understanding of all genes in the model dicot Arabidopsis. A
total of 20 MAPKs have been identified from the *Arabidopsis* genome and are currently being analyzed using functional genomic approaches (Ichimura et al., 2002). However, little is known about the MAPK gene family and their function and regulation in rice (*Oryza sativa*) and other economically important cereal crops. In 1999, the first rice MAPK *BWMK* was isolated from an indica cultivar IR36 (He et al., 1999). Till date 10 MAPK genes have been isolated and cloned from rice, only five MAPKs (*OsWJUMK*, Agrawal et al., 2003a; *OsMAPK*, Fu et al., 2002; *OsBWMK*, He et al., 1999; *Os-MAPK*, Lieberherr et al., 2005; *OsMAPK*, Xiong and Yang 2003) have been characterized. Of these five, three (*OsBWMK*, *OsMAPK*, and *OsMAPK*) have been investigated as to how they relate to the plant defense response. The *OsMAPK* gene (also known as *OsMSRMK*, *OsMAPK2*, *OsBIMK*, or *OsMAP1*) has been isolated by several research groups and shown to be inducible by various biotic and abiotic stresses (Agrawal et al., 2002; Huang et al., 2002; Song and Goodman 2002; Wen et al., 2002; Xiong et al., 2001). Molecular, biochemical, and transgenic analyses demonstrated that *OsMAPK* is a positive regulator of abiotic stress tolerance but acts as a negative regulator of rice disease resistance (Xiong and Yang 2003). Further Lieberherr et al., (2005) reported the post translational activation of *OsMAPK* by a sphingolipid elicitor and regulation by the small GTPase *OsRacl* and heterotrimeric G protein in rice. These results clearly revealed that rice MAPKs played important roles in signal transduction in responds to differentiation, development, and a variety of biotic and abiotic stresses such as pathogen attack, hormones, wounding, cold, salt, drought, oxidative stress, ozone and sugar starvation, etc.

Now with the availability of the sequence of complete rice genome, the information available from *Arabidopsis* has been extended to rice (Hamel et al., 2006). The present study has provided the full list of rice MAPKs with unified nomenclature by analyzing the redundant MAPK database from different research groups and we have cloned all the 16 MPKs from Indian cultivar of rice. All the cloned MPK transcripts were analyzed under cold and heat stress and identified the MPKs regulating under these stress at transcript level. Further the characterization was carried out by making constitutively active and kinase inactive mutant forms of MPKs and their heterologous expression followed by *in vitro* assays. Transient transformation of rice was done with constitutively active constructs of *OsMPK3* and *OsMPK7*. We also reported and characterized the rhythmic expression of MAPK
activity in diurnal and circadian conditions. Insilico analysis of rice proteome database was carried out using different bioinformatic tools and identified 75 MAPKKKs followed by their expression in MPSS database were analyzed. Thus the overall objectives of present work are as follows

- Cloning of MAPK gene family from rice.
- Identification and characterization of temperature responsive MAPKs.
- Partial purification of heat and cold activated MAPKs using column chromatography techniques for further characterization of the protein.
- Transient transformation of rice plants with identified temperature responsive MAPKs.