CHAPTER 1
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1.1. Introduction

Rice is an important food crop, produced in many countries worldwide and feeding significant portions of the world population (IRRI, 2002; Coats, 2003). It provides about 80% of the food calorie requirements to more than half of the world’s population (FAO, 2008). It is planted on about 154 million hectares annually equivalent to about 11% of the world’s cultivated land (Khush, 2005). Asia is considered as the most significant rice producing region, accounting for ca. 94% of total world production. World’s population is projected to be 10 billion by the year 2050, and most of the population growth is expected in Asia and Africa resulting in higher demand for rice (Dawe, 2007). Besides increased demand for rice production to feed extra mouth, the limited possibility for expanding harvested area, declining rice yield growth and low returns from rice production are posing major challenges for future rice production. In recognition of the magnitude of problem and its link with food security, poverty alleviation, preservation of cultural heritage and sustainable development, in 2002, the General Assembly of the United Nations declared 2004 as the International Year of Rice. The dedication of an International Year after a single crop was unprecedented in the history of the United Nations and provided the desired impetus for developing appropriate strategies to meet the increased demands for rice production in the next century.

It has been observed that since the year 2000, the average growth rate of rice yield has declined constantly and it has been less than the world rice consumption. The factors which contribute to the decline in rice production include, decreased productivity in intensive rice production systems, involvement of abiotic and biotic stresses, increasing production costs in industrialized countries and growing public concern for the protection of environmental resources (Nguyen and Ferrero, 2006). In the above background, one of the most effective ways to address these issues is to develop concerted efforts to understand these problems in greater details and employ suitable research based approaches and innovative technologies to overcome them.

1.2. Abiotic stress in rice and its multiplicity effects

Rice is an anomaly among the domesticated cereals – a tropical C3 grass that evolved in a semi-aquatic, low-radiation habitat. It is sensitive to a variety of abiotic
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Stresses, including salinity, drought, submersion and cold (Lafitte et al., 2004). Due to these stresses, the growth and productivity of rice are significantly affected. It is estimated that around 30%–60% yield losses occur globally each year due to various kind of stresses (Seo et al., 2011). Hence, this justifies a greater need for understanding the mechanisms of responses to abiotic stresses with the hope of improving tolerance of rice plants to environmental stresses.

The process of plant responding appropriately to the environmental cues begins once the plant sense the changing environment. Due to the complex nature of stress, the likely involvement of multiple sensors for perception of signals is predicted (Xiong et al., 2002). The signal transduction pathway which is activated after the perception of signals ultimately activates the stress responsive genes and thus generating the initial stress response. These stress responses occur at different levels of organization. The cellular responses in the form of adjustments of the membrane system, modifications of the cell wall architecture and changes in cell cycle and cell division are evinced when plant is subjected to stresses. In addition, the reprogramming of metabolism by accumulation of compatible solutes such as proline, sugar alcohols and glycine betaine which are involved in stabilization of protein and cellular structure, and to maintain cell turgor by osmotic adjustment, and redox metabolism to remove excess levels of ROS and re-establish the cellular redox balance are common events during stress (Bartels and Sunkar, 2005; Valliyodan and Nguyen, 2006; Munns and Tester, 2008; Janska et al., 2010). Studies at the molecular level indicate that the environmental stress affect the expression of various genes (Chinnusamy et al., 2007; Shinozaki and Yamaguchi-Shinozaki, 2007) and modulate the epigenetic regulation of gene expression (Hauser et al., 2011; Khraiwesh et al., 2011). It has been observed that genes which are directly involved in protection from stress, including the synthesis of osmoprotectants, detoxifying enzymes, and transporters are also stress induced (Krasensky and Jonak, 2012). Other gene products such as transcription factors which regulate sets of stress specific genes and protein kinases and phosphatases are also vital components of stress responses.
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Fig. 1.1. Summary of abiotic stress induced responses in rice.

1.3. Deciphering remedial strategies for abiotic stress tolerance in rice

Abiotic stress is a major factor affecting productivity of rice crops in large areas of the world, and hence strategies for conferring abiotic stress tolerance has been an important objective for a long time. In past, there has been conscious efforts to overcome the adverse effects of stress by use of tolerant cultivars, ameliorative water management and diverse cultural practices. Other approaches adapted involve plant breeding which create useful genetic variations to withstand the environmental stress. However, the pace of progress in breeding stress tolerant rice is slow due to various factors including poor understanding of the mechanisms underlying tolerance, complexity of the traits associated with the stress, inadequate selection criteria and absence of stringent, reliable and reproducible screening methodology (Gregorio et al., 2002). Moreover, attempts to improve stress tolerance through conventional plant breeding methods are time consuming, laborious and dependent on existing genetic variability. Classical genetics suggests that stress tolerance traits are generally controlled by a large number of genes with minor effects (Barton and Turelli, 1990; Hoffmann and Parsons, 1991) and therefore, it is difficult to get the precise control on the traits that host plant will express and the guarantee of its genetic stability in
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subsequent generations. Further, the complexity of traits involved in tolerance of a particular stress and the coexistence of various type of stresses (drought and salinity, salinity and submergence, salinity and mineral toxicities/deficiencies) makes the situation more complex and significantly impedes the pace of progress in varietal development in rice (Ismail et al., 2007). The recent progress in field of molecular biology has supported researchers for identification and use of molecular markers to expedite the breeding programme for conferring abiotic stress tolerance in plants (Gregorio et al., 2013). However, it was observed that the introgression of genomic portions (Quantitative trait loci, QTLs) responsible for stress tolerance often brings along undesirable agronomic characteristics from the donor parents (Bhatnagar-Mathur et al., 2008). This could be attributed to the lack of a precise knowledge of the key genes underlying the QTLs. To overcome these problems, development of genetically engineered plants by introgression of genes that are known to be involved in stress response and putative tolerance has attracted lot of attention worldwide (Bhatnagar-Mathur et al., 2008; Oh et al., 2005; Saad et al., 2013). The exercise for precise manipulation of candidate gene(s) through gene transfer technology was considered as faster and direct way of obtaining abiotic stress tolerant plants than the conventional breeding or molecular breeding. In recent past, there are several reports of use of gene transfer technology to alter the accumulation of osmoprotectants, increase production of chaperones, enhance the superoxide radical scavenging mechanisms, exclusion, or compartmentalization of ions by efficient transporter and symporter systems to confer abiotic stress tolerance in rice (Garg et al., 2002; Liu et al., 2013; Zhao et al., 2009; Chen et al., 2007). Recently, there has been a paradigm shift from gene centric approach to genome centric approach in crop improvement programme. In this regard, engineering of the regulatory machinery involving transcription factors were given preference over the attempts to insert “single-action” genes. It is known that transcription factor encoded by a single gene regulates the expression of several other genes leading to the activation of complex adaptive mechanisms and hence represents major molecular targets to genetically improve the tolerance of crop plants against different stresses (Khong et al., 2008). However, the task of evaluating transgenic plants under stress conditions, and understanding the physiological effect of the inserted
gene(s) at the whole plant level remain as major challenges to overcome. Besides these, the transgenic approach to offer tolerance to plants faces numerous other concerns associated with their use like impact on biodiversity, associated health risks, contamination of non-transgenic crop variety and lack of public acceptance. Under these circumstances, scientists are being forced to explore innovative approaches which could be widely accepted for their role in ecologically and environmentally sound global agriculture.

Recently, the use of phytohormones for their anti-stress effects has generated a lot of interest among many researchers all over the world. These phytohormones are generally synthesized at low concentrations and they are well known to control various important biological functions in plants. Recently, with the ease of availability of biological and genomic resources, and development of novel experimental strategies driven by new molecular tools have provided a suitable impetus to explore the role of various phytohormones such as abscisic acid (ABA), indole acetic acid (IAA), cytokinins (CK), gibberellic acid (GA), brassinosteroids (BR), jasmonates (JA), salicylic acid (SA) in alleviating stress in crops (Kohli et al., 2013). The vital role of phytohormone in integrating the environmental and intrinsic cues for plants to adapt and grow under growth-limiting conditions is now being established. Recent experimental data also provides information that the fluctuation of stress-responsive phytohormones alters the cellular dynamics and thereby play critical roles in coordinately regulating the growth responses under stress. Further it has been demonstrated that the cross-talks among various phytohormones helps in integrating the diverse input signals and readjusting growth as well as acquiring stress tolerance in plants (Kohli et al., 2013).

1.4. Rationale and objectives of the work

To provide adequate global food supplies during the time of rapidly rising population, increasing food demand and declining natural resources, is a critical concern for the present century. In order to meet these challenges, it has become imperative to understand various factors resulting in the decline in crop productivity and to develop remedial strategies. Abiotic stress is one such factor which significantly imposes limitations on crop productivity and also limits land available for farming. To
counter the challenges posed by declining resources for crop cultivation and to increase the crop productivity, it has become fundamental to use modern agricultural practices. However, in recent years the use of pesticides has become a major input in modern agriculture for crop production. Though pesticides help in controlling crop pests, but they pose several unwanted side effects to the environment and biodiversity (Aktar et al., 2009; Sharma et al., 2013). Pesticides also trigger a series of morphological, physiological, biochemical and molecular changes in plants which adversely affect growth, productivity and its resistance to pests (Bhatnagar-Mathur et al., 2008; Banerjee et al., 2001; Cheng et al., 2012; Sharma et al., 2013a). Further, its superfluous application on plants raises concern towards food safety and potential hazards to quality of harvested products. Thus, in the era of sustainable agriculture, the major challenge is to evolve effective crop stress management strategies, keeping in mind the ecological concerns.

Soil salinity is another major abiotic stress which reduces agricultural productivity and effects large terrestrial areas of the world. It is reported that approximately 20% of world’s cultivated land and nearly half of all irrigated lands are affected by salinity (Zhu, 2001). In Asia alone, 21.5 million ha of land area is believed to be salt affected (Sahi et al., 2006). Salinity is particularly a major problem in coastal regions because of the intrusion of brackish water during the dry season and at the start of the wet season. Salinity stress is also major problem in inland areas owing to the buildup of salinity as a consequence of excessive irrigation water and the use of poor quality irrigation water (Ismail et al., 2010). High salinity causes hyperionic and hyperosmotic stress effects, caused by high apoplastic levels of Na\(^+\) and Cl\(^-\) concentrations in the soil, leading to altered aqueous and kinetic thermodynamic equilibria (Abogadallah, 2010). High salt stress also disrupts the integrity of cellular membranes and adversely affects the activities of various enzymes, nutrient acquisition and function of photosynthetic apparatus. This damage might be linked to the reactive oxygen species (ROS) generated by salt stress. Due to the significant impact of salt stress on growth and productivity of the rice plants, commensurate efforts towards development of crops with an inherent capacity to withstand high level of salinity stress is being pursued. Besides the practical goal of genetically improving the salt tolerance
of crop plants, salt tolerance research has lead to better understanding of subjects ranging from gene regulation, signal transduction to ion transport, and mineral nutrition, which represents an important part of basic plant biology.

In the era of sustainable agriculture, where ecological concerns are of top priority, the amelioration of abiotic stress using phytochemicals is a promising option. One of such phytochemicals is brassinosteroids (BRs) which is a class of ubiquitously present plant-specific steroid hormones. In recent years, it has attracted attention of many researchers worldwide for its wide range of applications in agriculture (Gudesblat and Russinova, 2011). It regulates various aspects of plant growth and development including cell elongation, photomorphogenesis, xylem differentiation, and seed germination (Vriet et al., 2012). Recently, the exogenous application of BRs has been implicated for a range of biotic and abiotic stress (Krishna et al., 2003; Divi and Krishna, 2009; Vriet et al., 2012, Sharma et al., 2012; Sharma et al., 2013a; Sharma et al., 2013b). BRs bind to a small family of leucine-rich repeat receptor kinases (BRI1) at the cell surface, thereby initiating an intracellular signal transduction cascade that results in the altered expression of hundreds of genes which are involved in diverse functions (Clouse, 2011). Although many studies were conducted on role of BRs in growth and development as well as on stress amelioration properties, yet surprisingly little is known about the biochemical and molecular mechanisms associated with their mode of action (Sharma et al., 2013).

Keeping in view of the above facts, the present study was designed with the following objectives;

1. To study the effect of exogenous application of brassinosteroids (Epibrassinolide and Homobrassinolide) on growth parameters in rice plants (Pusa Basmati-1) under pesticides (Imidaclorpid and Chlorpyrifos) and salt (NaCl) stress.
2. To study the effect of exogenous application of brassinosteroids (Epibrassinolide and Homobrassinolide) at physiological, biochemical and molecular level in rice plants (Pusa Basmati-1) under pesticides (Imidaclorpid and Chlorpyrifos) and salt (NaCl) stress.
3. Preparation of gene constructs for salt responsive gene (s) for localization and overexpression studies.